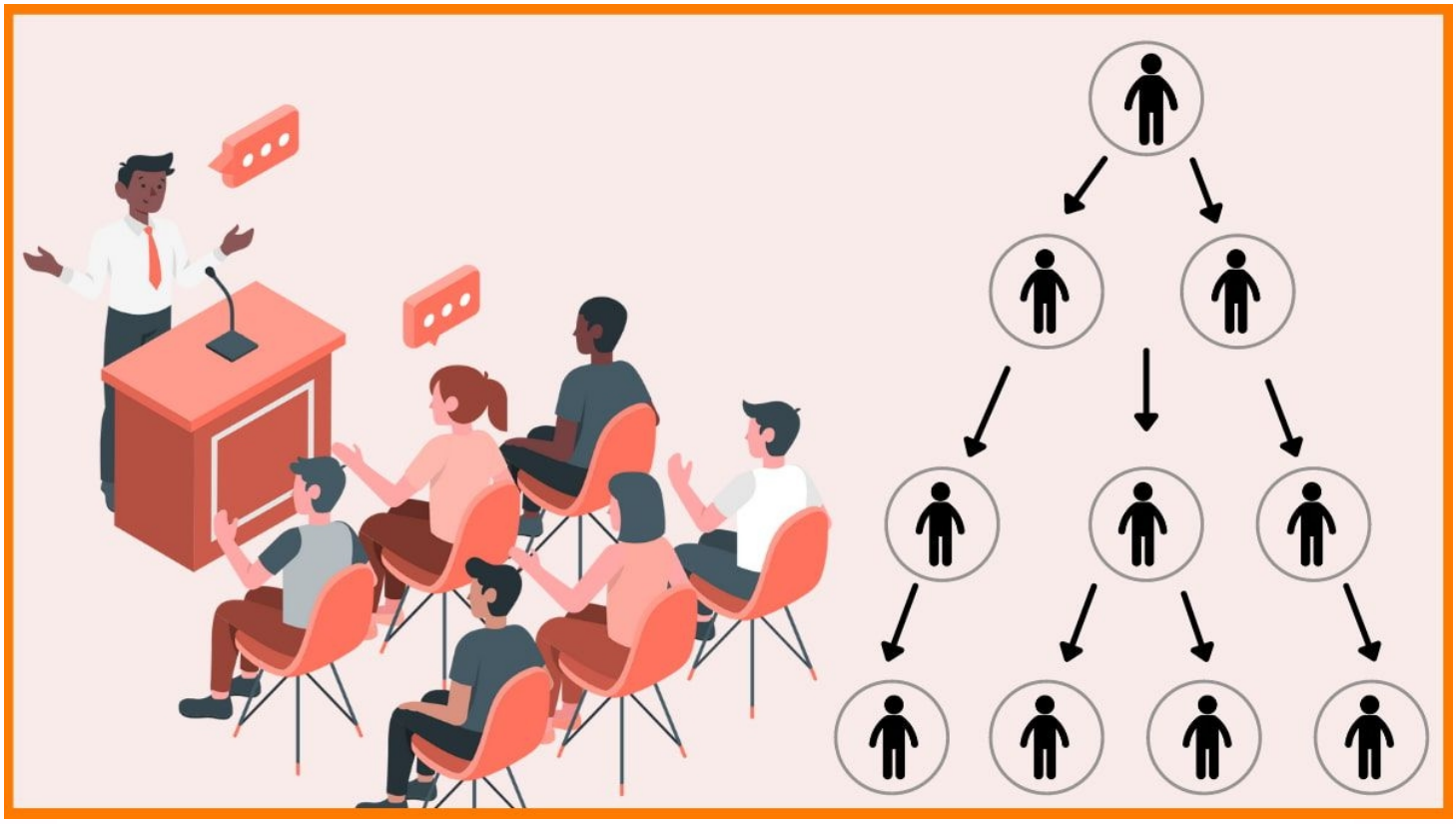
MULTILEVEL SIGNALLING PATHWAYS

Dr. Sudhir Kumar Gupta



ALEXIS PRESS
JERSEY CITY, USA

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Published by: Alexis Press, LLC, Jersey City, USA
www.alexispress.us

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First Published 2022

A catalogue record for this publication is available from the British Library

Library of Congress Cataloguing in Publication Data

Includes bibliographical references and index.

Multilevel Signalling Pathways by *Dr. Sudhir Kumar Gupta*

ISBN 978-1-64532-528-4

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CHAPTER 1

RETROGRADE SIGNALING IN THE MITOCHONDRION: TRIGGERS, PATHWAYS AND RESULTS

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ABSTRACT:

The eukaryotic cell needs mitochondria for equilibrium. Despite the fact that these organelles have their own DNA, the nucleus encodes >99% of the proteins found in mitochondria. Because of this, systems that enable communication between the mitochondria and the nucleus are essential for coordinating mitochondrial protein production during biogenesis as well as communicating potential mitochondrial faults that would otherwise cause the nucleus to activate compensatory mechanisms. As detailed in this review, mitochondria-to-nucleus retrograde communication has been documented in a variety of taxa, but with variations in effector routes, molecules, and results. the process of a signal returning to its original source after having been directed to a target is known as retrograde signaling. For instance, signaling proteins are first produced in the center of a cell. Instead of signals leaving the nucleus, they are delivered there during retrograde signaling. This kind of communication normally takes place between the inside of the mitochondria or chloroplast and the nucleus in cell biology. Nuclear gene expression is influenced by signaling molecules in the mitochondria or chloroplasts acting on the nucleus. In this method, internal and external stimuli are detected by the chloroplast or mitochondria, which then activate a signaling pathway. Retrograde signaling, also known as retrograde neurotransmission, is a term used in neuroscience to describe the way that a regressive messenger, such as anandamide or nitric oxide, gets released by a postsynaptic dendrite or cell body and moves "backwards" across a chemical synapse in order to bind to a presynaptic neuron's axon terminal.

KEYWORDS:

Biogenesis, Communication, Detected, Postsynaptic.

INTRODUCTION

According to current theories, mitochondria were formerly free-living microorganisms that formed a fruitful partnership with prokaryotic cells billions of years ago. Today, they perform a variety of different tasks in addition to being undeniably necessary for eukaryotic aerobic metabolism, such as the production of intermediate metabolites, control of cytosolic Ca2+ homeostasis, and coordination of cell death, among others. The vital significance of this organelle is further underscored by the fact that many age-induced processes and degenerative illnesses are linked to mitochondrial malfunction. Since genetic material was transferred as a consequence of the development of this endosymbiosis interaction between mitochondria and the host cell, the majority of mitochondrial proteins (but not all of them) are now encoded in the nucleus. In this case, it is clear that a system of communication between the mitochondria and the nucleus is necessary. This system must coordinate the synthesis of mitochondrial proteins during the organelle's biogenesis as well as communicate

any potential mitochondrial malfunctions that might result in the nucleus's production of compensatory responses. Numerous species have been characterized as using this communication system, which includes intramitochondrial as well as antegrade (nucleus to mitochondria) and retrograde mitochondria to nucleus channels.

A crucial function for mitochondria is being revealed in an expanding number of processes as mitochondrial signaling is continued to be explored. Long-term potentiation is a steady rise in a chemical synaptic strength that may endure for hours or days. It is assumed to take place via two temporally distinct processes, with induction coming first and then expression. The majority of LTP researchers concur that induction is totally postsynaptic, although they dispute on whether expression is primarily a presynaptic or postsynaptic process. According to some scientists, LTP expression is influenced by both presynaptic and postsynaptic processes. There wouldn't be a requirement for communication between the postsynaptic cell and the presynaptic cell if LTP were totally produced and expressed postsynaptic ally. But for postsynaptic induction and presynaptic expression to occur, the postsynaptic cell must interact with the presynaptic cell after induction. Postsynaptic to presynaptic communication is seen as a sort of retrograde transmission since typical synaptic transmission happens in a presynaptic to postsynaptic direction According to the retrograde signaling theory, the postsynaptic cell "sends a message" to the presynaptic cell in the early stages of LTP production to let it know that a stimulus that causes LTP has been received postsynaptic ally.

A specific process by which this message is conveyed and received is not suggested by the broad theory of retrograde communication. When stimulated with an LTP-inducing stimulus, the postsynaptic cell may produce and release a retrograde messenger as one possible method. Another is that following such activation, it releases a prepared retrograde messenger. Another method is that LTP-inducing stimuli in the postsynaptic cell may affect the shape of synapse-spanning proteins, which then transmits information to the presynaptic cell and across the synapse. The retrograde messenger theory has garnered the greatest focus among these processes. There is dispute on who the retrograde messenger is among the model's proponents. Early in the 1990s, a frenzy of research to prove the presence of a retrograde messenger and identify it led to a list of potential candidates, including carbon monoxide, platelet-activating factor, Nitric oxide arachidonic acid, and. Prior until recently, adhesion proteins that bridge the synaptic cleft to connect the presynaptic and postsynaptic cells have dominated research on nitric oxide.

The endocannabinoids anandamide and/or 2-AG, which function through G-protein coupled cannabinoid receptors, may be crucial for retrograde signaling in Later of homeostatic systems. Retrograde signaling is the main topic of this review, which also covers the known causes, molecular mechanisms, and results. Peptides produced from mitochondria are given special consideration as signaling molecules. In eukaryotic algae and plants, plastids carry retrograde signals to the nucleus, and in the majority of eukaryotes, mitochondria transfer such signals to the nucleus. Retrograde signals are often thought to carry stress and environmental information that is sent intracellularly. Many of the substances linked to retrograde signaling influence transcription by either binding and serving as transcription factors or by directly changing the transcription. The results of various signaling pathways differ depending on the organism and the stimulus or stress. The RTG route was the first retrograde signaling system in yeast to be found. In order to keep yeast's metabolic equilibrium in check, the RTG pathway is crucial.

In the face of few resources, the mitochondria must maintain a glutamate balance for the citric acid cycle. As a result of retrograde signaling from the mitochondria, precursor molecules of glutamate are produced to correctly balance resources within the mitochondria. Retrograde signaling may also be used to halt development if issues arise. In Saccharomyces cerevisiae, if the mitochondria do not form correctly, they will cease developing until the problem is resolved or cell death is caused. The maintenance of the cell's homeostasis and the maintenance of the mitochondria's appropriate operation depend on these mechanisms. Reactive oxygen species (ROS) are among the most investigated compounds in plants that transmit retrograde signals. It has recently been shown that these substances, which were formerly thought to harm cells, instead function as signaling molecules. Aerobic respiration produces reactive oxygen species, which act on genes related to the stress response. Depending on the stress, reactive oxygen species may interact with nearby cells to start a local signal. As a result, the surrounding cells are "primed" to respond to the stress since the stress response genes are activated before the stress is experienced. Additionally, dehydration and disease response may be detected by the chloroplast. When these stressors are recognized by the cell, chemicals are created that may operate on the nucleus to generate genes that make the cell more tolerant to pathogens or drought.

Regulating chemical neurotransmission is the main goal of retrograde neurotransmission. Retrograde neurotransmission hence enables neuronal circuits to establish feedback loops. Retrograde neurotransmission is comparable to electrical neurotransmission in the sense that it mostly regulates anterograde, conventional neurotransmission rather than actually transmitting any information. Retrograde neurotransmitters, as opposed to traditional (anterograde) neurotransmitters, are produced in the postsynaptic neuron and bind to receptors on the presynaptic neuron's axon terminal. Retrograde signaling also starts a signaling cascade that targets the presynaptic neuron. The presynaptic neuron has an increase in activity potentials after retrograde communication is started, which immediately affects the postsynaptic neuron by increasing the number of its receptors. Retrograde messengers, including endocannabinoids like anandamide, are known to exist. The same is true with nitric oxide.

Although this is debatable, retrograde signaling may also be involved in long-term potentiation (LTP), a theory for how learning and memory work. Nitric oxide and other endocannabinoids, which are lipophilic ligands, are among the most common endogenous retrograde neurotransmitters Nitric oxide (NO), a soluble gas that is a retrograde neurotransmitter, may easily diffuse across a variety of cell membranes. The enzyme that produces NO in different presynaptic cells is called nitric oxide synthase.

Particularly, NO is known to play a crucial part in LTP, which is crucial for memory storage in the hippocampus. Furthermore, research indicates that NO may function as intracellular messengers in the brain and may influence presynaptic glutamatergic and GABAergic synapses. Endocannabinoids are a class of retrograde neurotransmitters that function by binding to G-protein coupled receptors on the presynaptic terminals of neurons to activate them. When endocannabinoids are activated, certain neurotransmitters are released at the excitatory and inhibitory synapses of a neuron, which eventually affects different types of plasticity.

DISCUSSION

Retrograde Signaling Pathways in the Mitochondrion

The first retrograde mechanism to be identified and well characterized was the RTG-dependent retrograde signaling in Saccharomyces cerevisiae are three cytosolic proteins on which it is dependent. Basic helix-loop-helix/leucine zipper transcription elements Rtg1p and Rtg3p bind to the GTCAC DNA binding site as heterodimers. The Rtg1/3p complex regulates the expression of genes that code for mitochondrial proteins when it is activated by moving from the cytoplasm to its nucleus Rtg1p and Rtg3p are both necessary for DNA binding, despite the fact that only Rtg3p has a transcription activation domain. Since Rtg1/3p subcellular location and activity are likewise controlled by the target of rape (TOR) kinase pathway, it has been discovered that the retrograde signaling route also coordinates the synthesis of mitochondrial proteins. The rapamycin-induced inhibition of TOR function affects genes involved in protein biosynthesis, the glycolytic process, the cycle of tricarboxylic acid, and nitrogen digestion, including permeases and degradation enzymes necessary for the use of various sources of assimilable nitrogen. This mimics the effects of nutrient starvation. Lst8p, a part of the target of rapamycin complex acts at two locations, one upstream of Rtg2p and one to negatively regulate the RTG-dependent retrograde signals pathway. Lst8p is thought to have a role in upstream control over the SPS (Ssy1p, Ptr3p, and Ssy5p) amino acid-sensing structure, which in turn affects external glutamate detection and the retrograde response Unknown is the exact method by which Lst8p is inhibited downstream of Rtg2p. When TOR activity is inhibited by rapamycin treatment, retrograde signaling is activated and RTG-target genes are expressed as a result. It is also known that mitochondrial dysfunction affects Sch9p, a target of TORC1 crucial for ribosome biosynthesis, cell-size regulation, suppression of entrance into the stationary phase, and translation initiation This affects Sch9p's phosphorylation and activity. Another connection among retrograde signals and the target receptor kinase pathway may exist in this case [1]–[3].

Retrograde signaling that is reliant on RTG is also linked to resistance to osmotic stress. Hog1 stress-activated protein kinase (SAPK), which regulates multiple transcription factors including Sko1p, Hot1p, Msn2p and Msn4p, and Smp1p, is triggered by exposure to external hyperosmolarity. These in turn control how stress-response genes are expressed. Under osmotic stress, Hog1 SAPK is required for the induction of RTG-dependent gene expression. The Rtg1/3p transcription factor may move to the nucleus when Hog1 SAPK attaches to it. Rtg1/3p nuclear translocation just needs Hog1 SAPK to be present, but for the transcription element to bind to the chromatin, Hog1 SAPK must be active. RTG-dependent signaling is not the sole method through which yeast mitochondria interact with the nucleus, despite the fact that it has been more well studied. Rog proteins are not in charge of many of the genes whose transcription is changed as a result of mitochondrial malfunction. In addition, compared to the genes impacted by medina depletion, the traditional paradigm for RTG-dependent signaling activation, a distinct collection of genes had their expression altered depending on the yeast strain or the culture medium. One such is the discovery that the Pdr1p/Pdr3p transcription complex, rather than the Rtg1p/Rtg3p combination, is responsible for the overexpression of the ATP-binding cassettes protein Pdr5p, a multidrug-resistant transporter.

Retrograde Signaling Pathway Relay Molecules and Triggers

A mitochondrial signal is required to initiate retrograde signaling, which is then sent to a few molecules before arriving at the nucleus. While other retrograde routes are not as well described molecularly as yeast RTG-dependent retrograde signaling, it is. Events that might serve as relaying molecules and triggers will be described in this section. One of the primary products of the mitochondria, ATP, would be an apparent trigger molecule. There is some evidence to support this, at least in certain circumstances. Loss of mitochondrial DNA may activate the process via a drop in ATP concentration, permitting Mks1p-Rtg2p connection and Rtg1/3p nuclear translocation, since Mks1p release from Rtg2p in yeast is reliant on ATP hydrolysis and is ATP-specific. While retrograde communication was shown to be active throughout normal replicative or temporal aging, circumstances in which declines in ATP are less frequent, retrograde signaling may still occur in extreme circumstances. Although it has been shown that decreases in mitochondrial membrane potential may initiate the retrograde response after replicative aging, the exact method by which this potential decline is sent to Rtg2p is still unknown [4]–[6].

Due to impaired mitochondrial Ca2+ absorption and an increase in free Ca2+ in the cytoplasm, disturbance of the mitochondrial membrane potential is also the primary initiator of retrograde signaling in mammalian cells. This in turn stimulates the transcription factors which in turn activate addition, increased Ca2+ levels activate calcineurin, a calcium-dependent serine-threonine phosphatase that is thought to have developed from RTG-dependent retrograde signaling and increases Importantly, investigations where the chelation of free calcium was sufficient to disrupt downstream signaling demonstrated the causal link between mitochondrial failure and calcium signaling. Since RTG signaling was demonstrated to be active and give acetic acid resistance with no detectable changes in membrane potentials, it does not appear that overt alterations in the membrane potential of mitochondria are the trigger of RTG-dependent signaling within yeast grown in raffinose, despite the fact that they are determinants for retrograde receptor activation in different organisms.

Arnold et al.'s hypothesis that mitochondria-derived peptides may sometimes have a role in the stimulation of retrograde signaling in yeast is an intriguing one. They demonstrated that deletion of YME1, which encodes for the inner membrane protein i-AAA-protease, eliminated peptide synthesis in the intermembrane space and resulted in the formation of the respiratory chain and the stimulation of nuclear genes involved in the expression of mitochondrial genes. Since the induction of nuclear genes was stopped by antimycin, an inhibitor of the electron transport chain, or CCCP, a mitochondrial uncoupler, it was determined that the potential of the mitochondrial membrane was crucial for the response. This suggests that the process involves the mitochondrial transport of an unidentified relay molecule.

The mitochondrial unfolding protein response (stupor) was shown to be triggered in a way reliant on HAF1, a gene producing a mitochondria-localized ATP-binding cassette transporter, in C. elegans with decreased expression of SPG7 a mitochondrial protease. The transcription factor Atfs1p, which is ordinarily imported into mitochondria and destroyed, is involved in the transport and degradation process. importing efficiency is decreased under mitochondrial stress, enabling Atfs1p to get to the nucleus and subsequently change the transcription of stupor components. It would be interesting to investigate whether the

impaired transport in this instance is primarily due to changes in mitochondrial membrane potential. The import or export of proteins and peptides may be impacted by observed changes in the potential of mitochondrial membranes in human or yeast cells. If so, it may have an impact on downstream signaling pathways similar to those documented for C.

A coenzyme Q synthesis mutant (SBO gene mutant) in D. melanogaster exhibits stimulation of the stupor and reduction of the insulin/insulin-like growth factor signaling (IIS) pathway. Nonautonomous reduction of insulin/insulin-like signaling by growth factors was shown to be the cause of life extension in a more recent work using mutants for muscle, a part of complex It's interesting to note that forced expression of catalase or glutathione peroxidase I prevented the increase in lifespan, revealing a critical function for H2O2 in the signaling cascade. Also shown to be a component of the tarp generated by the knockdown of CCO1, a subunit of mitochondrial cytochrome oxidase, in C. elegans, are ROS (the precise chemical species is not described). Indeed, slight increases in mitochondrial ROS generation, which in turn triggered the hypoxia-inducible transcription factor Hif1p, are a necessary component of this mutant's lifetime extension. The stupor in the gut is activated by neuronal-limited reduction of cco-1 in a cell nonautonomous way, having an impact on the whole organism [7]–[9].

The results of activated retrograde signaling

The characteristic of mitochondrial retrograde signaling is the alteration of nuclear gene expression triggered by a signal from the mitochondria, regardless of the organism or the route stimulated. The expression of many genes involved in mitochondrial biogenesis is triggered by the activation of a retrograde route in S. cerevisiae treated with oligomycin or in a strain lacking the YME1 gene. Similar to this, it has been demonstrated that RTG-dependent signaling modifies the expression of a number of genes including (encoding the mitochondrial citric acid synthase), CIT2 (peroxisomal citrate synthase Indeed, cells with mutant alleles of RTG1 or RTG2 are auxotrophic for glutamate or aspartate and are unable to develop when acetate is used as the only carbon source, indicating a malfunctioning tricarboxylic acid cycle. Since the tricarboxylic acid cycle's precursors may be obtained from glyoxylate cycle intermediates, obstructions in this cycle alone do not cause glutamate or aspartate auxotrophic.

Cells are unable to proliferate in the absence of glutamate or aspartate due to the impairment of both cycles caused by RTG1, RTG2, or RTG3 deletion. Since RTG1 or RTG3 deletion leads in higher quantities of polyamine biosynthetic intermediates (putrescine, ornithine, and spermidine), retrograde signaling has also been demonstrated to influence amino acid metabolism. In cells lacking retrograde signaling, where the levels of other stress-response metabolites like glutathione and trehalose are decreased during the stationary phase, polyamines are known to have cytoprotective effects against oxidative imbalance and may thus operate as stress defense mechanisms. More proof demonstrates that cells with deficient RTG-dependent signaling have lower stationary phase catalase and glutathione peroxidase activities and are more susceptible to oxidative shocks as a result of lower hermetic H2O2 concentrations. As a result, RTG-dependent retrograde signaling activation seems to have a significant effect on the development of an ideal redox defense mechanism.

It should come as no surprise that RTG-dependent retrograde signaling activation in yeast has been shown to increase replicative lifetime The processes are not completely understood, but

they seem to include RAS2 and a defense against the detrimental effects brought on by a growth in extrachromosomal with aging Increases in the replicative lifetime have also been linked to the activation of retrograde pathways not reliant on RTG. on spite of its inability to develop on respiratory medium, the aforementioned Afo1p null mutant demonstrated higher replicative lifetime and oxidant tolerance This is also true for the SOV1 null mutant, in which the mutation reduced growth on respiratory medium but enhanced protein homeostasis, increased genomic silencing, and promoted an extension of replicative life span that was Sir2p- and PCN1-dependent. It is interesting to note that, rather than the loss or respiratory activity, the SOV1 mutant's life span extension was shown to be caused by the lack of mitochondrial translational control module proteins. Checking for similarities between the reaction triggered in SOV1 mutants and the stupor reported in C. elegans or D. melanogaster might be intriguing.

The primary effect of stupor activation in C. elegans has been life span extension (for a review, see However, the causal link between stupor and lifespan has lately come under scrutiny and further evidence is required to substantiate this claim. According to the available data on C. elegans and mature, the effectors and signaling pathways may exhibit some specificity related to the nature and/or location of the mitochondrial disturbance, which in turn could influence the outcome of the reaction (i.e., induce or not a nonautonomous response), despite sharing features like, for example, the stimulation of Hsp6p and Hsp60p [10]–[12].

CONCLUSION

The idea that mitochondria are organelles especially tasked with producing ATP has been around for a very long time. The enormous amount of data being produced today suggests that mitochondria are metabolic hubs that detect and process metabolic inputs, producing signals that are then conveyed by various chemicals and pathways and ultimately reach the nucleus Various retrograde communication channels are used to transmit messages from mitochondria to the nucleus because mitochondria-derived signals come in a variety of forms. Retrograde routes that are independent of RTG have been associated with increases in replicative lifespan. Despite being unable to grow on respiratory media, the above-mentioned Afo1p null mutant showed greater replicative lifespan and oxidant tolerance. This is also true for the SOV1 null mutant, in which the mutation decreased growth on respiratory media but improved protein homeostasis, boosted genomic silencing, and led to a prolongation of replicative life span which was Sir2p- and PCN1-dependent. It is intriguing to note that the SOV1 mutant's life span extension was shown to be mediated by the absence of mitochondrial translational control module proteins, rather than the absence of respiratory activity.

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CHAPTER 2

DEVELOPMENT, NICHES AND COMMUNICATION PATHWAYS OF HEMATOPOIETIC STEM CELLS

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ABSTRACT:

The hematopoietic system, which is primarily responsible for homeostasis and immunological response, depends on hematopoietic stem cells (HSCs). Several disorders have been treated by HSC transplantation. HSCs do, however, continue to exist in very modest numbers throughout the body, mostly in a dormant form. For the development of therapeutic medicines and stem cell biology research, having a fundamental understanding of HSCs is helpful. To promote HSC growth and transplantation in the future, this research focuses on the origin, source, development, niche, and signalling pathways that enable HSC maintenance and maintain a balance between self-renewal and proliferation. The stem cells from which other blood cells develop are known as hematopoietic stem cells (HSCs). Haematopoiesis is the name of this process. In vertebrates, a mechanism known as endothelial-to-hematopoietic transition causes the very first definitive HSCs to develop from the ventral endothelium wall of the embryonic aorta inside the (midgestational) aorta-gonad-mesonephros area. In the centre of most bones, the red bone marrow is where adult hematopoiesis takes place. The layer of the embryo known as the mesoderm is where the red bone marrow originates.

KEYWORDS:

Causes, Development, Hematopoietic, Proliferation.

INTRODUCTION

Hematopoietic stem cells (HSCs) are adult stem cells with the capacity to self-renew and differentiate into specialized blood cells that perform a variety of biological functions, including regulating immune system activity, homeostatic balance, and the body's reaction to pathogens and inflammation. Adipocytes, cardiomyocytes endothelial cells fibroblasts/my fibroblasts liver cells osteochondrosis's and pancreatic cells are a few examples of the specialized cells that HSCs may develop into. The majority of HSCs remain dormant inside the niches that support the HSC pool and will only activate in response to signals that alter the equilibrium of the blood cells or the HSC pool as a result of internal or external stressors. HSCs have also been thoroughly examined, particularly for therapeutic reasons in the treatment of autoimmune illnesses, hereditary blood disorders, and blood diseases. However, biological studies understanding is required for improved development in this subject as a base in conducting strategy and keeping HSCs. As a result, this study will cover the HSC source, origin, niches for the HSC pool, and signalling pathways that are crucial for the control of HSCs.is the process that results in the maturation of all blood cells.

It must strike a balance between the huge demands for production (the typical individual makes more than 500 billion blood cells daily) and the need to control the proportion of each

kind of blood cell in circulation. In vertebrates, the bulk of hematopoiesis takes place in the bone marrow and is produced by a few numbers of multipotent, extensively self-renewing hematopoietic stem cells. Myeloid and lymphoid blood cell lines developed from hematopoietic stem cells produce several blood cell types. Dendritic cell development involves both lymphoid and myeloid lineages. Monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, and megakaryocytes to platelets are examples of myeloid cells. T cells, B cells, natural killer cells, and innate lymphoid cells are all examples of lymphoid cells.

Since their discovery in 1961, hematopoietic stem cells have expanded in meaning. The hematopoietic tissue has committed multipotent, oligopotent, and unipotent progenitors as well as cells with short- and long-term regeneration capabilities. In myeloid tissue, there are hematopoietic stem cells. Cancer and other immune system problems are treated by HSC transplantation. Based on the lineage, the CFUs mentioned above. An in vivo clonal colony formation that relies on the capacity of infused bone marrow cells to give birth to clones of mature hematopoietic cells in the spleens of radio treated mice after 8 to 12 days uses another CFU, the colony-forming unit-spleen (CFU-S), as its foundation. Early studies made considerable use of it, but now it is thought to assess highly developed progenitor or transit-amplifying cells rather than stem cells. Hematopoietic stem cells cannot be recognized under a microscope because they cannot be separated as a pure population. Using flow cytometry, it is possible to identify or isolate hematopoietic stem cells by separating them from neighboring blood cells using a combination of many distinct cell surface markers, mainly CD34. Hematopoietic stem cells are referred to be Lin- since they do not display mature blood cell markers.

Hematopoietic stem cells are isolated using a combination of the detection of numerous positive cell-surface markers and the absence of lineage marker expression. Hematopoietic stem cells are further distinguished by their tiny size and weak staining with important dyes like rhodamine. Hematopoiesis, or the development of blood cells, depends on hematopoietic stem cells. Hematopoietic stem cells are multipotent and capable of self-renewal, which allows them to replace all blood cell types. An extremely high number of daughter hematopoietic stem cells may be produced by a small number of hematopoietic stem cells. When a modest number of hematopoietic stem cells reconstruct the hematopoietic system, this phenomenon is employed in bone marrow transplantation. This procedure suggests that symmetrical cell divisions into two daughter hematopoietic stem cells must take place after bone marrow transplantation.

Given that the stem cell niche in the bone marrow is where stem cell self-renewal is assumed to take place, it seems sense to infer that critical signals found there will be crucial for self-renewal. The environmental and molecular prerequisites for HSC self-renewal are of great interest because, if this capacity is understood, it will be possible to produce larger populations of HSC in vitro that may be employed therapeutically. Multipotent hematopoietic stem cells are transplanted during hematopoietic stem cell therapy (HSCT), which is often done using bone marrow, peripheral blood, or umbilical cord blood. It might be autologous (using the patient's own stem cells), allogeneic (using donor stem cells), or syngeneic (using stem cells from an identical twin. Patients with particular blood or bone marrow malignancies, including multiple myeloma or leukemia, are those who have the procedure the most often. Prior to the transplant in these situations, the recipient's immune system is often

wiped off using radiation or chemotherapy. Major side effects of allogeneic HSCT include infection and graft-versus-host disease.

Blood donors are injected with a cytokine, such as granulocyte-colony stimulating factor (G-CSF), that encourages cells to leave the bone marrow and circulate in the blood arteries in order to harvest stem cells from the circulating peripheral blood. In mammalian embryology, the AGM (aorta-gonad-mesonephros) is where the first definite hematopoietic stem cells are seen. These cells are subsequently extensively amplified in the fetal liver before colonizing the bone marrow before birth. Patients with life-threatening disorders are the only ones who should undergo hematopoietic stem cell transplantation, which is still a risky treatment with several potential consequences. The usage of the technique has extended beyond cancer to include autoimmune diseases and genetic skeletal dysplasia's, particularly malignant infantile osteopetrosis and mucopolysaccharidosis as survival rates after the surgery have grown. As cells age, strand breaks build up in long-term hematopoietic stem cells. The extensive attenuation of DNA repair and response pathways, which rely on HSC quiescence, is linked to this accumulation. DNA double-strand breaks may be repaired by a mechanism called non-homologous end joining (NHEJ). Because the break ends are immediately ligated without the necessity for a homologous template, NHEJ is known as "non-homologous". NHEJ factor 1 (also known as Cernunnos or XLF), DNA polymerase mu, and ligase 4 are among the proteins necessary for the NHEJ process.

The function of DNA ligase in the NHEJ-mediated repair of double-strand breaks is very specialized. In the mouse model with Lig4 deficiency, aging results in a gradual depletion of hematopoietic stem cells. In pluripotent stem cells, lig4 deficiency leads to an accumulation of DNA double-strand breaks and increased apoptosis. A 40% drop in the number of bone marrow cells, which comprise a variety of hematopoietic lineages, is associated with impaired hematopoietic cell development in peripheral and bone marrow cell populations in polymerase mu mutant mice. Hematopoietic progenitor cells' capacity for expansion is similarly diminished. These traits are associated with a decreased capacity of hematopoietic tissue to repair double-strand breaks.

Multiple lines of evidence, including evidence that long-term repopulation is flawed and becomes worse with time, point to the conclusion that NHEJ factor 1 deficiency in mice causes premature aging of hematopoietic stem cells. It was shown that NHEJ1 plays a significant role in boosting the survival of the primitive hematopoietic progenitors using a human induced pluripotent stem cell model with NHEJ1 deficiency. These NHEJ1-deficient cells have a limited potential for NHEJ1-mediated repair, and they are unable to deal with DNA damage brought on by physiological stress, regular metabolism, and ionizing radiation. The fact that hematopoietic stem cells are sensitive to Lig4, DNA polymerase mu, and NHEJ1 impairment shows that NHEJ is a crucial factor in stem cells' capacity to sustain themselves over time in the face of physiological stress. Even in wild type hematopoietic stem cells, endogenous DNA damage accumulates with aging, according to Rossi et al who hypothesized that DNA damage accumulation may be a key physiological mechanism of stem cell aging.

An empirical test based on cell culture is called the cobblestone area-forming cell (CAFC) assay. A portion of hematopoietic stem cells that are plated onto a confluent culture of stromal feeder layer eventually settle between the stromal cells and the substratum (in this

case, the dish surface) or become trapped in the cellular processes between the stromal cells (despite the fact that the stromal cells are touching each other). When lymphoid lineage cells sag under nurse-like cells in vitro, the phenomenon is known as pseudo emperipolesis. In vivo, emperipolesis occurs when one cell totally engulfs another (for example, thymocytes into thymic nurse cells). When compared to other hematopoietic stem cells, which are refractile, this similar phenomenon is more frequently referred to in the HSC field as cobble stone area-forming cells (CAFC), which refers to areas or clusters of cells that appear dull and reminiscent of cobblestones under phase contrast microscopy. Because they are spherical and refractile, the cells that are freely floating on top of the stromal cells.

DISCUSSION

Origin and Development of HSCs

The discovery of HSCs in the hematological system has illuminated stem cell biology research, including connections to other adult stem cells via the fundamental ideas of differentiation, multipotency, and self-renewal. Lethally radioactive animals were discovered to be saved by spleen cells or bone marrow cells in the early stages of those findings. Colony-forming unit spleens (CFU-S) are clonogenic mixed colonies of hematopoietic cells that form in the spleen after transplanting mouse bone marrow cells into irradiated mice. These colonies are often made up of granulocyte/megakaryocyte and erythroid precursors. After transplantation, certain primary CFU-S colonies were able to restore the hematopoietic system in secondary irradiated animals. CFU-S was once thought to be distinct from HSC, but later research showed that it really came from more committed progenitor cells. A fresh trip toward many studies to clarify HSC biology, functional characterization, purify, cultivate, and other stem cell research was started by Till and McCulloch's finding [1]–[3].

The key to more effective HSC growth for transplants is to increase hematopoiesis and HSC development. In order to determine the origin and function of HSC in different anatomical regions of various species, including zebrafish, chicken, and mouse as well as human embryos, embryogenesis studies have been carried out. Moore and Metcalf's first research shown that only erythroid and myeloid lineages could be produced by hematopoietic cells in the yolk sac. Additionally, it was discovered that the yolk sac, the chorionic mesoderm, and some allantois mesoderm express the Runx1 gene at embryonic which is when definitive hematopoiesis begins. However, HSCs discovered in the yolk sac lacked the final hematopoietic stem cells, which in mouse embryos before E11.5 did not exhibit long-term hematopoietic reconstitution activity. On the other hand, serially transplantable irradiated animals and long-term repopulating were reported to grow primarily in the aorta-gonad mesonephros (AGMs) area of the mouse embryo, indicating that the AGM region is the primary location for HSCs detection Additionally endowed with hematopoietic potential were vitelline and umbilical arteries Evidence that a large number of nonerythroid progenitors with a high-proliferative potential were seen from which the liver rudiment has been excised was used to establish the existence of the HSC phenotype in the embryo. In the embryonic compartment, a substantial number cells were found to exhibit the cell-surface and molecular characteristics of primitive hematopoietic progenitors Additionally, limited progenitors were produced in the yolk sac, while melamphaid lineage emerged autonomously from progenitors in the splanchnopleures mesoderm and derived aorta inside the human embryo proper. As the generation occurs between the increase in HSC activity after midday 11 of gestation, the

AGM area in the embryo is proposed as the source of definitive hematopoiesis Although the vitelline and umbilical arteries were taken into account as the primary sources of fetal hematopoiesis in AGM, it is uncertain if the little population generated in those areas would be sufficient for the dispersion [4]–[6].

HSC Niches

The stromal derived is one signaling factor that may be involved in the homing of HSC from other definitive hematopoiesis to fetal bone marrow. In order to retain HSC in an undifferentiated state and control HSC in proliferative and differentiated stages within the unique microenvironments known as "niches" throughout life, soluble factors are not only mediated in fetal bone marrow but also in adult bone marrow. Schofield made the first stem cell niche hypothesis, and the presence of HSC niche was subsequently confirmed by its detection in the ovaries of a Drosophila melanogaster. It has been shown that germline stem cells, which are found in the Drosophila ovary and are surrounded by differentiated somatic cells, are crucial for sustaining stem cells' survival and proliferation. HSC niche is the particular local environment where HSCs are found that sustains and regulates HSC function by controlling cell fate determination, self-renewal, and survival. -integrins, metalloproteinases (MMP), and serine-threonine protein phosphatase (are a few examples of such molecules that have been shown to be connected to HSC homing to bone marrow Real-time imaging may be used to investigate the location and function on the mouse calvaria, HSCs settle on the endosteal surface, osteoblasts, and blood vessels, notably in trabecular areas. On the other hand, more developed cells are seen outside of the endosteum. Similar to this, research by showed ex vivo real-time imaging in irradiated mice the homing and lodgement of transplantable HSCs in the endosteal region of the trabecular bone area, where they react to bone marrow injury by quickly dividing.

Recently, it has been proposed that HSC niches in bone marrow are mediated by the endosteal niche and the vascular niche. First, the endosteal niche: In the well-vascularized endosteal areas, osteoblasts generated from mesenchymal progenitors are seen. Bone morphogenic protein-2 (BMP-2) and BMP-6, both of which are produced by HSCs, have a role in the initiation of osteoblastic differentiation. Due to the discovery that the number of osteoblasts is raised by parathyroid hormone activation and resulting in an increase HSCs number in vivo, osteoblasts are indicated as the niche. On osteoblasts, it was discovered that Jagged1, a member of the serrate family of Notch ligands, triggered this signal. This information is corroborated by a study by Chit Teti and colleagues, which demonstrates that Notch signaling, which is activated by osteoblasts, promotes hematopoiesis enhancement not only via Jagged1 overexpression but also through and are soluble factors produced by osteoblasts that control HSC quiescence, destiny. The cysteine protease cathepsin X released by osteoblasts has recently been discovered to ablate the attachment of osteoblasts and catalyze the chemokine a powerful chemo attractive cytokine This finding raises the possibility that osteoblasts control the movement of HSCs inside the bone marrow [7]–[9].

Human Hematopoiesis Hierarchy

The identification of each blood cell subpopulation in terms of their biology and potential when paired with other functional tests is the result of the examination of molecular marker expression by flow cytometry. To illustrate the hematopoietic hierarchy, a graphic has been developed. All blood cells in the hematological system are thought to originate from HSCs

that have the ability to self-renew and give birth to multipotent progenitors (MPPs), which lose their ability to self-renew but continue to completely differentiate into all multilineages. Common lymphoid progenitors (CLPs) and common myeloid progenitors (CMPs), respectively, are oligopotent progenitors that are produced by MPPs. All of these oligopotent progenitors undergo restricted lineage commitment and differentiate into the following cell types: granulocyte/macrophage progenitors and dendritic cell produce progenitors. Notably, produce DC progenitors the first one that many researchers have chosen to explore when it comes to the isolation and characterization of HSCs and progenitors. Together with toyocamycin and endoglycanase, family of cell-surface transmembrane proteins. In bone marrow, peripheral blood, and human cord blood, CD34 expression on blood cells ranges from When human fetal thymus was transplanted into SCID mice, a population of cells expressing Lin was the first candidate for human HSCs. These cells were able to generate T and B lymphocytes as well as myeloerythroid activities both in vitro and in vivo, whereas some subsets of Lin were devoid of multipotent progenitors.

The expression of was used to further isolate HSCs. From these findings, it may be inferred that the population is enriched for human HSCs and that the proposed human MPP fraction of multipotency with an imperfect potential for self-renewal is enriched in this community. However, recent research employing the mouse model of the HSC xenograft test has shown that both and include LT repopulating capability in secondary recipients with varying frequencies. Additionally, it has been shown that the marker is a unique HSC marker within the population, and that this population's single-sorted HSC are particularly effective at producing long-term multiline age grafts when expression is lost. Rhodamine-123 marker (efflux of the mitochondrial dye is further added to enrich for HSCs, where strong Rho efflux may also repopulate all blood lineages in secondary recipients These findings show that the population of hematopoietic cells is enriched for human HSCs [10]– [12].

Signaling Pathways in HSC Maintenance and Self-Renewal

Multiple variables influence the equilibrium between self-renewal and differentiation fate of HSCs in the bone marrow. The idea that HSCs are maintained and controlled by certain micro environmental-dependent signals in niches within bone marrow is supported by a variety of animal models. The majority of HSCs are in a quiescent state phase of the cell cycle but in response to a disruption of hematopoietic cells, the hematopoiesis system will either switch off or on the regulators controlled by the regulations. signaling, BMP signaling, Thrombopoietin signaling, Tie2/Ang-1 signaling, hedgehog and Notch signaling, as well as Wingless signaling are a few pathways that have been investigated in connection to that situation.

Signaling Pathway

The stromal cell-derived factor 1 (SDF-1) is expressed constitutively in a number of organs, including the bone marrow, liver, lung, and skin. SDF-1 is a member of the -chemokine family that affects embryonic development, including organ homeostasis, and acts as a chemoattractant for both committed and primitive hematopoietic progenitors. The two primary splicing variants, SDF-1 and SDF-1, have been found. Both are widely expressed, with the liver, pancreas, and spleen exhibiting the greatest levels of expression. The neurological system has also been defined for another alternative type, since been discovered, with pancreatic expression being the highest and heart, kidney, liver, and spleen expression

being the lowest To inhibit the physiological processes, SDF-1 interacts with its cognate receptor, which is extensively expressed in many tissues, including hematopoietic and endothelial cells. By controlling the establishment of the heart's ventricular septum, bone marrow's myelopoiesis, and B-cell lymphopoiesis signaling is essential for embryonic development Additionally, it has been shown dependent mechanism, pointing to the functional importance of EPCs in the process of vasculogenic in which blood vessels may be formed expression increased in ischemic locations, according to a number of observations. By encouraging EPC recruitment in ischemic tissues, further data showed that local injection of SDF-1 increased vasculogenic and ultimately led to ischemic neovascularization in vivo. Recent research by Liu and colleagues has shown that the signal of conjunction with may boost the mobilization and paracrine activity of mesenchymal stem cells (MSCs) in ischemic kidneys. Additionally, controls HSC attachment within the niche in addition to its function in HSC maintenance. It was discovered that matrix metalloproteinase-9, which is responsible for the release of soluble Kit-ligand, triggered the mechanism involved in this regulation inactivation or deletion in mice reduced the HSC pool and increased the response to HSC defections via hyper proliferation. By establishing that conditional SDF-1-deficient animals imparted a defect in HSC quiescence and endosteal niche localization, Tzeng and colleagues further validated the significance of SDF-1 in HSC maintenance.

BMP Signaling Pathway

The TGF-family of growth factors includes the bone morphogenic proteins (BMPs). In the HSC niche, osteoclasts primarily generate BMPs. While HSC quantity and function inside the bone marrow niche are regulated by BMP-4 throughout adult life, BMP-4 influences hematopoietic lineage commitment from mesodermal cells during embryogenesis Adult HSC inside bone marrow have been researched in a limited number of cases and are difficult to understand. When BMP signaling was impaired, the niche size increased, which increased the number of HSCs. Higher doses of BMP-2, BMP-4, and BMP-7 maintained human CB HSCs in vitro, but BMP-4 at lower concentrations stimulated proliferation and differentiation of HSCs, according to a study by a group led by Bhatia.

CONCLUSION

A pan inhibitor of canonical Want signaling, was overexpressed to block Want signaling in HSCs, which led to the stimulation of cell cycle and a decrease in the capacity of transplanted induction animals to repopulate. treated with 6-bromoindirubin inhibitor, cell cycle progression was slowed, promoting the engraftment of ex vivo-expanded HSCs These results together point to a positive regulatory function for the want/-catenin signal in the proliferation or repopulation of HSCs. Unsaturated fatty acid metabolism mediated by lipoxygenase has been linked to canonical Want-related signaling in the maintenance of quiescence and number In sum, the canonical want signal regulates HSC function by preserving quiescence and a healthy balance in proliferation. The biological processes, signaling routes, hematopoiesis, HSC source, and maintenance and control of HSCs have all been thoroughly researched. Imaging systems used in advanced research obviously give important data for monitoring the HSC origin, pool, and transplantation results in mouse models. A new discovery in the treatment of diseases, such as the advancement of performing a large-scale preparation of HSCs for clinical transplantation, will also provide the knowledge through the observation of molecular mechanisms downstream the signaling

cascade of self-renewal and proliferation of HSCs. Additionally, the signaling pathways will provide comprehension into the cancer stem cells, which are now challenging scientists to investigate their potential treatment method.

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CHAPTER 3

VIRAL ENTRY: MOLECULAR COMMUNICATION AND CELLULAR PATHWAYS

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ABSTRACT:
Due to the fact that viruses depend on the services provided by cells for their growth, viruses often influence the cell signaling, which is essential for controlling cellular activities. The genetic material is transferred into the cell as the initial stage of their host life. Although certain viruses may enter the cytosol directly, endocytosis really accounts for the majority of viral entrance into host cells. This machinery starts with cellular signaling pathways that are particular to the cell type, and the signaling molecules might be proteins, lipids, or carbohydrates. After the virus connects to target cells, such as receptors, the activation may be initiated in a relatively short amount of time. The great variety of signaling pathways involved in the control of viral entry often interact with one another to produce various endocytosis outcomes. Additionally, certain viruses have the capacity to use the many internalization routes, making the regulation much more complex. The production of endocytic vesicles, traffic, molecular signaling during viral entry, and some recent developments in our knowledge of these topics are covered in this work.

KEYWORDS:
Controlling, Essential, Genetic, Influence.

INTRODUCTION

In contrast to other species, viruses need the resources for replication from their hosts in order to produce their offspring. Viruses must pass the plasma membrane barrier of the cell in order to transmit their genomes into the host cells for their own objectives. There are a number of viral entrance points that have been discovered, including membrane fusion with viruses like human immunodeficiency and genetic injection with phages. A path for viral internalization is also provided by the cell endocytic process. Recent studies have shown that several viruses promote endocytosis through endocytic signaling pathways, including clathrinid-mediated endocytosis caveolae/lipid raft-mediated endocytosis, micropinocytosis and many additional unique mechanisms. The components that regulate viral entrance mechanisms and associated traffic systems are the main subject of this study. Intercellular communication (ICC) is the term used to describe the numerous mechanisms and structures used by biological cells to interact with one another or with their surroundings. Extracellular signaling molecules are used by many cell types to interact with one another via unique proteins and methods.

Attempts to accurately distinguish the forms of communication outlined are somewhat fruitless since components of each type of intercellular communication may be engaged in more than one type of communication. Instead of making a systematic effort to categorize the parts according to functional or structural criteria, the sections have been informally put together from diverse fields of study. Single-celled organisms perceive their surroundings to find nourishment and may communicate with other cells to encourage symbiotic behavior or

reproduction. This is best shown by the slime mold. The slime mold demonstrates how cyclic AMP, a tiny chemical that facilitates intercellular communication, enables a basic creature to develop from a well-organized collection of individual cells. Cell signaling research examined whether a receptor was unique to each signal or if numerous receptors may be possibly activated by a single signal. The intensity of a signal is just as essential as its existence or absence. As multicellular creatures and plants become more complex, using a chemical gradient to regulate cell development and differentiation is still crucial. Cell signaling is the term used to describe this kind of intercellular communication inside an organism. Small signaling molecules that diffuse across the gaps between cells are typical of this sort of intercellular communication, which often depends on a diffusion gradient as a component of the signaling response. Molecules that keep the cells together in complex organisms may also be engaged in intercellular communication.

The phrase "extracellular matrix" refers to certain binding molecules that may include longer molecules like cellulose for plant cell walls or collagen for animal cell walls. The adherend, desmosomes, gap, tight, and tricellular junctions are five distinct kinds of unique cell junctions that may occur when the membranes of two cells are in close proximity to one another. Desmosomes, adheres, tight junctions, and tricellular junctions all play structural functions. Additionally, the structures they create are a component of intricate protein signaling circuits. Tight junctions may create a tight zip around cells, creating a barrier to prevent even little undesirable signaling molecules from passing between cells, which is one way in which they play a general function in cell signaling. Otherwise, signaling molecules can spread to a group of cells that do not require the signal or enable signaling molecules to leave the area where they are needed too rapidly. All given names derived from the Latin word nexus, which means "to connect." They are classified together because they all have four transmembrane domains that penetrate the cell membrane similarly, albeit they do not all have enough sequence homology to be thought of as being closely related.

Previous studies on connexions showed that, unlike interactions with the cell outside, clusters of connexins may be used to directly join cells to one another. They were thus not regarded as contributing to extracellular cell signaling at the time. Connexins are a channel for the release and absorption of signaling molecules from the environment outside the cell. Later investigations revealed connexins might link directly to the cell outside. In addition, pannexins seem to accomplish this to the point that they may seldom, if ever, take part in direct cell-to-cell interaction. Many species do not seem to have pannexins, innexins, or connexins, as seen on the pannexin/innexin/connexin tree displayed. This may mean that alternative, related proteins that facilitate intercellular communication in these animals still need to be found. Intercellular linkages may be created via gap junctions, which function as a small, directly controlled "pipe" between the cytoplasm of the two cells that make up the junction. Each small ICC is made up of 12 connexin proteins because 6 connexins form a connexon and 2 connexons form a pair of connexons. This ICC enables direct communication between two cells even when they are walled off from the outer environment.

The number of these microscopic ICCs that a cell forms with its neighbours may range from one to thousands, with the potential to create extensive networks of cells that are directly connected. Connexon couples create ICCs, which can switch on and off extremely quickly as needed and carry water and several other molecules up to around 1000 atoms in size. Additionally, these ICCs are exchanging electrical impulses that may be quickly switched on

and off. Due to the approximately 20 distinct connexins with unique qualities that may join with one another in various ways, there are a number of these ICC kinds, which increases their adaptability. This leads to a huge diversity of possible signaling combinations. The electrical synapses present on nerves are a well-studied example of gap junctions' electrical signaling capabilities Gap junctions work in the heart muscle to coordinate the heartbeat. Gap junctions may also operate to provide a direct link to the outside of a cell, further enhancing their adaptability and mimicking the actions of their protein cousins, the pannexins, which are discussed elsewhere.

Both within and outside of cells, lipid membrane-bound vesicles of a wide range of sizes may be discovered. These vesicles can hold everything from food to invasive organisms, water to signaling chemicals. One instance of extremely small vesicles being directly engaged in controlling intercellular communication is the use of an electrical nerve impulse from a neuron of a neuromuscular junction to trigger a muscle to contract. Each little vesicle that the neuron creates holds thousands of signaling molecules. When the muscle is at rest, one vesicle is released nearby around every second. More than 100 vesicles and hundreds of thousands of signaling molecules are released all at once when a nerve impulse is stimulated, significantly contracting the muscle fiber. In a little fraction of a second, everything takes place.

Exosomes or extracellular vesicles are typically tiny vesicles used to convey signaling chemicals discharged from the cell. In addition to their usefulness to the organism, exosomes are crucial for biosensors. Malignant cancer cells may exude extracellular vesicles. Gap junction proteins that are overexpressed in malignant cells and transfer to non-cancerous cells have been found in these extracellular vesicles, which may increase the spread of the malignancy. The regulating secretory routes in exocrine and endocrine tissues, transcytosis, and the vesiculas-vacuolar organelle (VVO) in endothelial and perhaps other cell types are examples of bigger vesicles. Trans-endocytosis is another method of membrane fragment transfer at junctions. Some big intercellular vesicles that include gap junction plaques and move their contents from one area of a tissue to another also seem to maintain their integrity.

DISCUSSION

Clapton-Dependent Endocytosis

Although several viruses had shown that the CME was necessary for internalization the signals that are produced by viruses are more complex for their own advantages. The endosomes serve as this acidic compartment. Many viruses are low-pH defendant for their conformation change that is necessary for membrane fusion or viral particle uncoating. As a result, the majority of viruses that enter host cells by clathrinid-mediated endocytosis will create latherin-coated vesicles (CCVs) by causing clathrinids to attach to the plasma membrane. Clathrinid and Eps15 are recruited by Ap-2, the plasma membrane adaptor complex, which is necessary for the CME. Additionally, Ap-2 interacts with additional endocytic proteins including GTPase Dynamin, Sapogenin, and Intersect in, as well as Amphiphilic clathrinid-coated vesicles (CCVs) fission from the plasma membrane requires the presence of dynamins. The majority of enveloped viruses enter host cells by membrane fusion and endocytosis. It has been proposed that dynamin functions as a chemical enzyme that induces membrane fission and pinches endocytic vesicles away from the cellular plasma membrane in later stages of several endocytic pathways, including CME, as well as a

regulatory GTPase by controlling the early stages of CME, an important endocytic pathway used by many viruses. Dynamin has been postulated to take part in membrane fusion between the virus and endosomes after endocytosis in addition to its role in viral entry. Additionally, it has been shown that actin dynamics control a number of endosome stages in the Kaposi's Sarcoma-Associated Herpesvirus (KSHV) entrance and traffic systems. KSHV entrance and trafficking are blocked by inhibiting Rho GTPases, and Arp2/3, which are necessary for actin nucleation [1]– [3].

Early endosomes late endosomes and then lysosomes for destruction or conformational modifications make up the major pathway for clathrinids-dependent entrance. Only 2 to 5 minutes are needed to convey the goods from CME to EE, and another 10 to 15 minutes are needed to get to LE. These CCVs to LE via EE are quick, largely reliant on the Rab GTPase family, and connected to PI3-kinase. Microtubules and Rab5 are necessary for the CME of the borna disease virus. The BDV vial entrance procedure did not, however, need the actin dynamics. When cargo is transported from CCVs to EE and maturing endosomes (ME), Rab5 and EEA-1 are involved in the process; they become dissociated when the cargo is transported to LE. Although viral entrance pathways do not necessarily need Rab7, certain viruses, such as the influenza A virus and the Semliki Forest virus (SFV), are linked to sorting and transport to LE. When Vps27/HRIS uses the ESCRT complex to sort cargo that has been marked with ubiquitin, the LE lumen becomes filled with intraluminal vesicles which are destined for destruction while being transported to lysosomes By controlling V-ATPase, the LE to lysosome lumens become more acidic and provide the right conditions for virus partial disassembly or envelope fusion with vesicle membrane, which helps release viral proteins prior to lysosome protein destruction. However, additional flow to lysosomes was necessary for viruses like parvoviruses More than two endocytosis routes may be involved in certain viruses The varicella-zoster virus (VSV) requires cholesterol, a component of the lipid raft, in addition to triggering clathrinids-mediated endocytosis.

Lipid raft-dependent endocytosis in the caveolae

The morphologically different entities known as caveolae arrange the parts of lipid and protein. Caveolins, which act as organizational hubs for cellular signal transduction, are found in the flask-like invaginations of the plasma membrane known as caveolae, a subtype of membrane (lipid) rafts. The 20 Kad caveolins that make up caveolae have cytoplasmic amino and carboxyl termini and a distinctive hairpin shape. Although caveolins were given their name because they were found in caveolae, they have varied expression patterns in various cell types and are found throughout the body [38, 39]. The caveolin scaffolding domain (CSD) of caveolins is where signaling molecules attach while they are inactive. while the CSD is activated, conformational changes occur that release and activate the signaling proteins [40, 41]. The CSD and specific binding partners communicate with caveolin. The CSD is a peptide sequence that contains binding motifs that scaffold signaling molecules: adenylyl cyclase (AC), protein kinase A (PKA), protein kinase C (PKC), heterotrimeric phosphatidylinositol 3-kinase endothelial nitric oxide synthase and mitogen activated protein kinase [4], [5].

Mammalian cells also have clathrinids-independent endocytosis, one of which being lipid raft-dependent endocytosis, in addition to CME. Lipid rafts serve as platforms for protein trafficking and signal transduction by enriching membrane microdomains with cholesterol

and glycosphingolipids. Although cholesterol is a part of lipid rafts, excessive amounts will prevent viruses like the Japanese encephalitis virus and dengue virus serotype 2 (DEN2) from entering the body. The phosphatases family kinases, tyrosine kinases, G protein-coupled receptors (GPCRs), integrins, and ligand-triggered signaling pathways that are linked to cholesterol and lipid rafts are often required for the main endocytic vesicle production in caveolae/lipid raft-dependent pathways. While this endocytosis pathway and CME share certain processes, they diverge in terms of some molecular components.

Micropinocytosis

Viral entrance into cells that relies on micropinocytosis will be "engulfed" by the cell membrane created when the ruffles on the membrane's outer extension fold back, much like phagocytosis. As a result, actin cytoskeletal rearrangement is necessary for the micropinocytosis process. This method of virus entry requires the activation of PI3 K and Rho, and other cellular kinases, as well as the actin modulatory proteins [58]. A signaling cascade that results in Alpha-mediated Rac activation and the actin cytoskeleton rearrangements required for HIV-1-mediated membrane fusion has been found to be activated by the binding of the viral envelope glycoprotein (Env) of HIV-1 with the primary receptor CD4 and one of the two coreceptors. Cofilin, a cellular actin depolymerization factor, is activated by HIV-1's envelope interaction to enable viral latent infection of dormant Additionally, it has been shown that Tiam-1, Abl, IRSp53, Wave2, and Arp3 are necessary for Env-mediated cell-cell fusion, virus-cell fusion, and HIV-1 infection.

Growth factors may cause micropinocytosis, which produces microbiomes. The direct binding of the hepatitis C virus has recently been shown to activate and internalize the EGFR by Diao and colleagues. The ezrin-radixin-myosin (ERM) family of proteins, Cdc42, the MAPK pathways, and other downstream signaling pathways may all be activated by HCV contact with Additionally, it has been shown that EGFR is essential for the entrance of other viruses, including as the human cytomegalovirus (HCMV), the influenza A virus, and the adeno-associated virus serotype It's interesting to note that EGFR activation was shown to be necessary for the internalization of the influenza A virus through the clustering of lipid rafts suggesting that EGFR internalization may be a typical way for viruses to infiltrate their host cells [6]–[8].

Distinctive Endocytic Pathways

Other than the well-established endocytic pathways previously mentioned, there are other mechanisms that some viruses use. For example, the lymphocytic choriomeningitis virus uses endocytosis that is independent of clathrinid, dynamin-2, caveolin, lipid rafts, actin dynamics, Arf6, and flotillin-1 but membrane cholesterol dependent. Without passing via positive EE, LCMV enters in noncoated pits and transfers to LE directly Herpes simplex virus is yet another case. Although earlier research suggested that the HSV-1 undergoes micropinocytosis during endocytosis, Clement and Rahn have shown that the virus may also enter cells by a different pathway, one that includes phagocytosis-like uptake that is aided by nectin-1, dynamin, and cholesterol-dependent manner. As previously established, the influenza virus mostly enters host cells by clathrinid-mediated endocytosis. However, it has also been shown that the alternative endocytic route offers a different entrance channel from clathrinid and caveolae. There may be further unique pathways that have not yet been

discovered that might be crucial entrance points for those viruses that are clathrinids- and caveolae-independent.

Heparan Sulphate Receptors-Mediated Endocytosis Involved Integrin

Many scientific fields, including biochemistry, cell biology, physiology, and pharmacology, have had a long-standing interest in the cell surface structure of receptors and their signaling partners. In order to enhance the activation of cellular processes, colocalization of receptors with their signaling partners in distinct microdomains is crucial, according to recent studies. The main cell surface adhesion receptors for extracellular matrix (ECM) ligands are integrins. They are heterodimeric proteins made up of an alpha and a beta chain that are used in a variety of signaling cascades to transmit and interpret signals from the external environment. Recent studies have shed light on the molecular aspects of how cells control integrin flow. More and more evidence point to the regulation of integrin internalization and recycling back to the plasma membrane by small GTPases like Arf6 and members of the Rab family along microtubules. Integrins may have a role in viral entrance, according to many studies.

There are several instances of signaling that the integrin mediates. In more recent research, Zaillian and associates found that stimulation of PI3 K/Akt signaling by integrin 1 facilitates vaccinia virus entry. The envelope-associated glycoproteins are used to bind the target cells through compounds similar to heparan sulfate. Through its delta transmembrane connection, the envelope glycoprotein binds with 31 integrin as one of the receptors for its entrance into the target cells and triggers the activation of focal adhesion kinase The outside-in signaling pathways that are required for the subsequent phosphorylation of other cellular kinases, cytoskeletal rearrangements, and other activities must first activate induction is essential for the phosphorylation essential for the subsequent phosphorylation of delta TM. The induction of is followed by cytoskeletal rearrangements, depends on delta TM-induced Endothelial cells have been shown to have the 53 integrin, which has been suggested to be a potential receptor for. For the entrance and infection of viruses, actin cytoskeleton is crucial. The viral production of dynamic filopodia controlled by Rac1 and Cdc42 cross-talk and myosin II motor activity is necessary for internalization of into endothelial cells.

Adenoviruses are a substantial contributor to acute human illnesses. Knowing more about the extremely efficient adenovirus cell entrance route may help with the creation of safer medication and gene delivery options that make use of related pathways. Adenovirus penton and fiber capsid proteins interact with cellular proteins to mediate infection and plan the sequential processes of cell entrance that result in effective gene transfer. While the penton attaches to the alpha integrin's coreceptors and starts cell binding, the fiber starts integrin-mediated endocytosis. Prior to viral release from the endosomal vesicle, penton integrin signaling occurs. The interaction of bare particles with the cytoskeleton mediates the intracellular trafficking of the remaining capsid shell. The adenovirus receptor an attachment receptor for adenovirus serotype binds homodimers with nearby cells in many different cell types to create a cell adhesion complex. Effective viral entry is made possible by CAR's collaboration with cell surface integrin receptors. It is possible that signaling downstream of CAR has a dual impact on integrins and CAR itself in order to facilitate effective viral attachment to cell membranes since CAR-induced activation results in greater activation of 13 integrins [9]–[11].

For HIV and the poxvirus, myxoma virus, hijacking chemokine receptors is one of several techniques viruses have developed to enter and multiply in their host cells The G-protein-coupled chemokine coreceptors and CCR5 are involved in the current general concept of entrance because they bind to the viral envelope glycoprotein, which causes conformational changes in the envelope proteins. A subgroup of primary HIV-1 isolates may additionally use the seven-transmembrane-domain receptor APJ as a coreceptor in addition to the chemokine receptors Glycosphingolipids, actin polymerization, membrane microdomains, and perhaps CD4 and chemokine signaling are all components of a full model.

CONCLUSION

Viruses must attach to cell surfaces, then induce signals to enter their host cells in order to infect the cell. The cell membrane serves as a barrier to incoming sources, such as viruses. Endocytosis or direct fusion with the plasma membrane to release viral capsids into the cytosol are two possible methods for virus penetration. Due to the variety of the components in either the membrane or organelles, viral entry often employs numerous paths and is cell type specific. Additionally, viruses with different kinds may potentially employ different entrance points, such as the human papillomavirus Even more complicated signaling is caused by viral infection or the endocytic impact. Drug development for particular anti-virus treatments will benefit from clarification of viral entrance paths and processes. The glycosaminoglycan heparan sulfate, which connects to the extracellular matrix protein on the cell surface, often exists as a proteoglycan (HSPG). As previously mentioned, KSHV binds to target cells using molecules similar to heparan sulfate. Many other bacteria or viruses, such as Rift Valley fever virus herpesviruses, and adeno-associated virus (AAV), also employ this structure. The virus may engage with additional surface receptors after attaching to the heparan sulfate to begin its endocytosis or membrane fusion. Despite the fact that a prior study showed that herpesvirus infection may quickly increase the express level.

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CHAPTER 4

ANTI-INFLAMMATORY PATHWAYS, NUCLEAR SENSOR SIGNALING AND PROBIOTICS

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ABSTRACT:

The relationship between the human microbiome and health and illness is being studied more and more. Inflammatory bowel disease (IBD) is one of several clinical disorders where dysbiosis is linked. IBD and other disorders have been studied as possible candidates for probiotic therapy. Probiotics' exact mode of action is still not completely understood. This research describes probiotics' unique anti-inflammatory signaling pathway-based methods of action. We cover current developments in probiotics and nuclear receptor signaling, including PPAR and vitamin D receptor (VDR) and peroxisome-proliferator-activated receptor (PPAR) gamma. We also explore potential research topics in the future. Live microorganisms known as probiotics are marketed with the promise that, when taken, they would improve or restore the health of the gut microbiota. Probiotics are typically regarded as safe to eat, but in rare instances, they may result in bacterial-host interactions and undesirable side effects. Probiotics may be helpful for certain illnesses, according to some data, however many of their claimed health advantages are not well supported.

KEYWORDS:

Clinical, Describes, Microorganisms, Peroxisome.

INTRODUCTION

Ingestible bacteria having positive health effects are called probiotics. The parallel rise in peer-reviewed clinical studies examining probiotics as treatment since 1999 is indicative of growing interest in the gut microbiota and its impact on health and illness. Probiotics' processes and effects are now well understood as a result of research on the many signaling pathways involved in the body's response to bacteria and inflammation. In specifically, the research analyses probiotics' role in intestinal mucosal function in connection to signaling pathways that reduce inflammation. A particular strain of bacillus found in Bulgarian yoghurt, termed Lactobacillus bulgaricus, was the first probiotic to be identified. Bulgarian physician and microbiologist Stamen Grigorov discovered the finding in 1905. The contemporary notion is often credited to Russian Nobel winner Élie Metchnikoff, who proposed in 1907 that Bulgarian peasants who consumed yogurt lived longer.

The necessity for tougher guidelines for the scientific validation of the purported advantages provided by microorganisms marketed as probiotics has arisen as a result of the probiotics market's expansion. Although there are many advantages that are touted for utilizing consumer probiotic products, such as easing constipation, boosting immune function, or preventing the common cold, these benefits are not substantiated by scientific research. The Federal Trade Commission in the United States forbids misleading advertising, which is The

European Food Safety Authority has as of 2019 denied multiple requests from European probiotic dietary supplement producers for approval of health claims due to a lack of supporting data. Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" in a World Health Organization (WHO) study from October the Guidelines for the Evaluation of Probiotics in Food were published in May 2002 by a working group established by the Food and Agriculture Organization (FAO)/WHO based on this concept. Following the aforementioned collaborative expert meeting between the FAO of the United Nations and the WHO, a consensual definition of the word "probiotics," based on the facts at hand and scientific evidence, was approved. Local and supra-governmental regulatory agencies demanded that health claims substantiations be better described in conjunction with this initiative.

Two expert panels comprised of academic scientists and business leaders furthered that first international effort in 2010 and provided suggestions for the assessment and confirmation of probiotic health claims. The ideas that came from these two groups were reflected in the FAO/WHO "Guidelines" of 2002. Despite being generally accepted, this definition contains an unquantifiable health claim, hence the European Food Safety Authority does not accept it. The word "probiotic" was discussed by a panel of scientific experts in Canada in October and the meaning was changed to "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." Lactic acid bacteria (LABs), which are food fermenting bacteria, may also enhance the nutritional content of the foods they inhabit in addition to preventing food deterioration. The preservation of fresh vegetables, cereal gruels, and milk-cereal mixes using acid fermentation and salting continues to be one of the most useful ways owing to its cheap cost and low energy needs while processing and preparing food.

Vegetables such as pickled vegetables, kimchi, pao chai, sauerkraut, and pickled vegetables are examples of fermented foods that contain lactic acid bacteria. Soy products such as tempeh, miso, and soy sauce are also examples, as are dairy products like yogurt and sourdough bread that are made without wheat or rye flour. Fermented sauces and pastes that are flavorful with amino acids or peptides are another example. glauconitic mesenteries', Lactobacillus plantarum, Pedi coccus pentosane's, Lactobacillus brevis, Leucon Stoc cerium, leucons argenteum, Lactobacillus periplanar, Lactobacillus coryneform, and Wessels spp. are the specific bacteria found in sauerkraut. leucosticte spp., Wessels spp., and Lactobacillus spp. are among the bacteria found in kimchi. pentoses', L. plantarum, glauconitic mesenteries', L. brevis, L. lactis, and L. fermentum are all present in pao cai. There is also a list of several more bacteria discovered in various Asian fermented fruits and vegetables. Lactobacillus acidophilus, Bifidobacterium bifidum, Streptococcus thermophilus, Lactobacillus Helvetic us, Lactobacillus kefiranofaciens, Lactococcus lactis, and leucons species are all present in kefir. Either Lactococcus lactis or L. bulgaricus are present in buttermilk.

Kombucha contains additional acidic bacteria that are allegedly probiotic. There is Gluconacetobacter xylenes in this beverage. Additionally, it has Gluconate oxidants, Acetobacter acute, Acetobacter Pasteurian us, and Zygosaccharomyces The sophisticated management of the gut microbiota may result in interactions between the bacteria and the host. Probiotics are generally thought to be safe, however in certain situations, people may have questions about this. Adverse events may be more likely in certain persons, including

those with immunodeficiency, short bowel syndrome, central venous catheters, cardiac valve dysfunction, and preterm newborns. As a result of bacteraemia, there is a danger in seriously unwell patients with inflammatory bowel disease for the transit of live bacteria from the gastrointestinal tract to the internal organs (bacterial translocation), which may have harmful effects on health. Consuming probiotics by kids with impaired immune systems or those who are already really unwell may sometimes cause bacteraemia or fungemia, or bacteria or fungus in the blood, which may cause sepsis, a potentially deadly condition.

Although it has been hypothesized that some Lactobacillus species contribute to human obesity, no proof of this connection has been discovered have 100 million cells per gram, or frozen yogurt products that include 10 million cells per gram, at the time of manufacturing are given a "Live & Active Cultures Seal" by the National Yogurt Association (NYA) of the United States. advised that "the minimum viable numbers of each probiotic strain at the end of the shelf-life" be disclosed on labeling in but the majority of businesses that provide a number only provide the viable cell count at the date of manufacture, which may be significantly higher than the number present at consumption How many active culture cells are still present at the time of consumption is difficult to ascertain due to the variation in storage conditions and time before eating. The temperature at which probiotics were stored had a significant impact on their capacity to survive, with room temperature storage suffering far more viability loss than refrigeration.

The probiotic therapy of bacterial vaginosis involves ingesting or applying bacterial species that are naturally present in healthy vagina to treat the infection that leads to bacterial vaginosis. This therapy is based on the finding that a certain species of bacteria called Lactobacillus dominates the population of organisms in the vagina in 70% of healthy girls. By creating H2O2, lactic acid, and/or bacteriocins, some strains of lactobacilli may stop the development of bacteria that cause BV. They can also stop Gardnerella vaginalis from adhering to the vaginal epithelium, which stops the infection from spreading to the vagina. Since the use of probiotics to reestablish healthy populations of Lactobacillus has not been standardized, the efficacy of probiotic therapy has hitherto been inconsistent. Probiotics are often explored together with conventional antibiotic therapy. Furthermore, different groups of women have different treatment outcomes depending on their ethnicity, age, number of sexual partners, pregnancy, and the microorganisms that cause bacterial vaginosis, scientists discovered that giving hydrogen peroxide-producing strains such Lactobacillus acidophilus and Lactobacillus rhamnose's to patients might restore vaginal pH and rebalance the vaginal microbiota, preventing and treating bacterial vaginosis.

DISCUSSION

Internal microbial flora

The intestinal microbiota, as a whole, plays critical roles in metabolism, intestinal epithelial cell function and health, immunology, and inflammatory signaling. The formation, maintenance, and replication of numerous clinical diseases, both intestinal and extraintestinal, have recently drawn more attention to the significance of the intestinal microbiota and its whole genetic make-up, together referred to as the microbiome. Atopy, IBS, colon cancer, alcoholic liver disease in animal and human studies, obesity and other metabolic disorders, and chronic inflammatory illnesses like IBD are only a few of the clinical ailments that have been linked to dysbiosis in fecal samples taken from infants who later acquired allergy

illness, there was a decrease in the variety of the gut microbiota. It has also been shown that the microbiota composition differs between IBS patients and unaffected people and between colon cancer patients and those with normal colonoscopies In a mouse model, alcohol feeding caused an increase of intestinal bacteria [8]. Numerous studies and thorough reviews of the literature have been done on the involvement of the microbiome in obesity Reduced variety of microorganisms has been seen in IBD patients with ulcerative colitis (UC) or Crohn's disease (CD) compared to healthy controls The effects of this altered microflora on the intestinal milieu may be significant but are still not fully understood. IBD most likely develops as a result of a confluence of variables, including intestinal dysbiosis in addition to environmental stressors in a genetically predisposed host. Numerous strategies to mitigate the impacts of a changed microbiome have been tried, all of which are based on the idea that diseases are caused by dysregulated or malfunctioning microbiota [1]–[3].

Three. Probiotics

Although it may have been mentioned as early as 1908 Lilly and Stillwell first defined "probiotics" in the literature in 1965 as growth-promoting substances generated by certain microbes. Probiotics were recently described as "live organisms that, when consumed in adequate amounts as part of food, confer a health benefit on the host" (Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria, "Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria", Food and Agriculture Organ The methods of action of probiotics include immunological regulation, direct influence on commensal and pathogenic bacteria to prevent infection and restore equilibrium, and alteration of pathogenic toxins and host products. Numerous studies and thorough reviews have been done on the effectiveness of probiotics in treating a variety of clinical problems in both pediatric and adult patients.

It was first reported in 1958 to provide regular feces intravenously to treat pseudomembranous colitis. For nasogastric tube infusion of feces into the small intestine or colonoscopy into the colon have both been documented and have good response rates. In a patient with recurrent, fecal bacteriotherapy was helpful in relieving clinical symptoms, and this was followed by the repopulation of the sick intestinal microbiota with beneficial species that had been depleted prior to treatment Orally given probiotics are one more way to supplement the gut microbiota in with live, non-pathogenic organisms. Numerous studies and thorough reviews have been done on the effectiveness of different probiotic formulations in According to a recent research, Lactobacillus acidophilus and Lactobacillus Casey probiotics were well tolerated and helpful in lowering the risk of developing AAD and There is strong evidence to support the use of the probiotic yeast Saccharomyces bouvardia for the treatment of traveler's diarrhea and AAD, according to a recent meta-analysis. Other conditions for which the probiotic yeast has been studied include enteral nutrition-associated diarrhea, and traveler's diarrhea. A number of evaluations of the data supporting the use of probiotics in IBS have been published and recent studies utilizing Bifidobacterium bifidum and Saccharomyces bouvardia showed improvements in clinical IBS symptoms and quality of life [4]– [6].

How Probiotics Work to Reduce Inflammation

Determining the underlying processes by which probiotics exert their positive benefits has been the subject of much study, and this research is still ongoing. Probiotics are controlled by a wide variety of methods. It is widely acknowledged that probiotics work through various cellular and molecular mechanisms, such as preventing the effects of pathogenic bacteria, controlling immune responses, and modifying intestinal epithelial homeostasis by enhancing barrier function, promoting cell survival, and inducing protective responses lists some sample papers on the mechanisms of action of probiotics. The probiotic-host interaction is intricate, and it is made even more difficult by the fact that certain probiotic effects seem to be species- and strain-specific. Numerous probiotics have been shown to have pro- and anti-inflammatory effects on dendritic cells Recent research has shown that NOD2-mediated signaling underlies the anti-inflammatory properties of certain lactobacilli has been recognized as a key CD susceptibility gene and is a member of a superfamily of genes involved in intracellular bacterial recognition. According to the authors, a relative NOD2 deficit might be the cause of the variable therapeutic outcomes of lactobacilli usage in CD patients. Numerous studies have looked at the impact of probiotics on the innate immune response pathways, including the toll-like receptor (TLR), nuclear factor kappa mitogen-activated protein kinase (MAPK), and c-Jun NH2-terminal kinase The activation of certain TLRs also seems to be a species-specific process Flagellin was discovered to be a possible TLR mechanism by which effect on Caco-2 cells was mediated. Well documented in the literature, the probiotic-induced action on the NF-B signaling pathway is often characterized by suppression [7]–[9].

Defensins and nuclear receptor signaling, number five

The family of endogenous antimicrobial peptides known as defensins is highly evolutionary conserved and serves as the first line of defense against a variety of microbial infections. Despite their evolutionary history, antimicrobial peptides are still potent antimicrobial agents and are found in large quantities across the animal and plant kingdoms. This is largely attributable to their method of action, which involves pore production and membrane rupture and is difficult for pathogens to use to impart resistance The antimicrobial peptides defensins, catholicizing, lysozymes, and other antimicrobial antiproteases are significant in humans. There are three defensin subfamilies that are now recognized; defensins from subfamilies and are expressed mostly in immune cells and epithelial cells, while defensin from subfamily is primarily detected in immune cells of the Rhesus macaque. Defensin is expressed at many locations throughout the gastrointestinal tract, although it is mostly found in the small intestine Human defensin 1 is the predominate defensin in the colon that is not inflamed; human defensin 2 and 3 are produced in response to infection or inflammation. Orally given bacteria exhibited higher survival and pathogenicity in mice missing functioning crypt dins (murine defensins), while intestinal peptide preparations had lower antibacterial efficacy.

Defensin insufficiency may have a role in the pathophysiology of IBD, according to several theories. Endogenous antimicrobials, such as defensins, are mostly produced by the Paneth cells of the small intestine. Additionally, it has been shown that the Paneth cells express NOD2 Human defensin 5 and 6 production is decreased in people with ileal CD, and this impact is accentuated in those who also have a concurrent NOD2 mutation. When it comes to defensins, CD patients with colonic illness have normal levels of defensin 2 and 3, but UC

patients had elevated levels, indicating that defensin induction failed to play a role in the etiology Constitutive human defensin 1 expression is decreased in CD patients with colonic involvement independent of inflammation, and recently, it was demonstrated that the nuclear receptor peroxisome-proliferator-activated receptor gamma (PPAR) is responsible for maintaining constitutive defensin expression. The reduced variety of the intestinal microbiota found in IBD patients may also help explain how a defensin deficit affects the etiology of IBD. Although the relationship between commensal bacteria and antimicrobial peptide production is not fully understood, it has been hypothesized that commensal bacteria may chronically stimulate epithelial cells to create antimicrobial peptides at levels high enough to kill microbial pathogens Probiotics have been shown to stimulate human defensin 2 in intestinal epithelial cells, but not fecal isolates. Wehrkamp et al. and Scale et al. have found that the probiotic E. coli promote the induction of human defensin 2 in intestinal epithelial cells via NF-B and activator protein. It's interesting to note that defensin expression is known to be controlled by nuclear receptors The control of metabolic, reproductive, developmental, and immunological processes depends heavily on nuclear receptors, which are a type of intracellular transcription factors that are activated by ligands that may directly bind with DNA Although the scope of each nuclear receptor's transcriptional activities varies and even the transcriptional effects of a single nuclear receptor may be cell specific, nuclear receptors regulate transcriptional activity by a number of distinct mechanisms, including "ligand-dependent transactivation, ligand-independent repression, and ligand-dependent trans repression. Beyond the scope of this article, a thorough discussion of nuclear receptors and their mechanisms of action is not necessary. However, further discussion of two nuclear receptors with potential roles in inflammation—the vitamin D receptor (VDR) and the peroxisome-proliferator-activated receptor gamma.

The Vitamin D Receptor (VDR)

the active form of vitamin D, operates via a nuclear receptor called vitamin D receptor (VDR), it heterodimerizes with to control the transcription of the target gene, VDR interacts to the vitamin D response element in the promoter. Antimicrobial peptides like catholicizing and -defensin are among the VDR downstream target genes. By stopping the invasion of harmful microorganisms, reducing inflammation, and preserving cell integrity, VDR is essential in controlling intestinal homeostasis Vitamin D has been shown to directly modulate the T-cell receptor as well as to regulate autophagy, a variety of immune cells, including T cells, B cells, macrophages, dendritic cells, and epithelial cells, and the expression of pro-inflammatory cytokines. According to studies done on animal models, inhibits the development of IBD patients have been shown to have deficiencies of and more recently, using a new vitamin D bioavailability test, it was shown that more than 70% of patients with quiescent CD had vitamin D deficiencies or Deficient levels may have significant effects on the establishment and maintenance of intestinal homeostasis because vitamin D serves a variety of immunological activities. The impact of intestinal microbiota on other disorders including obesity and asthma may be modulated by vitamin D status and VDR signaling, according to some research [100]. There is a lack of information on the state and operation of VDR, despite the fact that the current research has mostly concentrated on understanding the immunoregulatory effects of vitamin Furthermore, the role of probiotic-induced regulation of anti-inflammatory VDR signaling in colitis is essentially unstudied.

According to recent research, VDR/ mice have an elevated bacterial burden in their intestines According to our microarray results, intestinal colitis patients who have pathogenic Salmonella are affected in vivo by VDR signaling [154]. Data from a recent research show that VDR expression and location are regulated by bacterial stimulation, both commensal and pathogenic, and that VDR inhibits bacterial-induced intestine NF-B activation. Generally speaking, probiotic-induced nuclear receptor signaling has not been adequately studied. The probiotic VSL3# was connected to nuclear receptor signaling in the IL10/colitis model. It has been shown that suppress bacterially induced NF-B activity in the intestinal Our most recent findings demonstrate that probiotic administration may increase VDR expression and function in the host. When Lactobacillus plantarum was applied to cultivated intestinal epithelial cells, VDR expression increased and catholicity mRNA increased concurrently. Intestinal VDR considerably increased following probiotic colonization compared to the ex-germ-free pig when we employed a probiotic Mon associated pig model to evaluate the probiotic impact on VDR expression in vivo. Furthermore, probiotics did not reduce inflammation in animals missing VDR, according to our unpublished research [10]–[12].

CONCLUSION

IBD patients' decreased gut microbiota diversity may also contribute to the understanding of how a defensin deficiency impacts the etiology disease IBD. Commensal bacteria may persistently induce epithelial cells to produce peptides that are antimicrobial at levels high enough to kill microbial pathogens, although the connection between commensal microbes and antimicrobial peptide synthesis is not entirely understood. Human defensin 2 has been demonstrated to be stimulated by probiotics in intestinal epithelial cells, but not in fecal isolates. Researchers Wehrkamp et al. and Schlee et al. have discovered that the probiotic E. coli stimulates NF-B and activator protein to induce human defensin 2 into intestinal epithelial cells. It's interesting to note that nuclear receptors are known to regulate the expression of defensin. Nuclear receptors, a class of transcription factors inside cells that are activated by ligand that may directly connect with DNA, play a significant role in the regulation of metabolic, reproductive, growing, and immunological processes. Nuclear receptors control transcriptional activity by a variety of distinct mechanisms, such as "ligand-dependent transactivation, ligand-independent oppression, and ligand-dependent trans repression." The extent of each atomic receptor's transcriptional activities varies, and even the transcriptional effects of a single atomic receptor may be cell specific.

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CHAPTER 5

APOPTOSIS SIGNALING PATHWAYS IN CARDIAC MYOCYTES

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ABSTRACT:
Heart disease is the leading cause of mortality globally, and cardiac myocyte apoptosis is commonly linked to it. Myocardial infarction, ischemia/reperfusion injury, chemotherapy cardiotoxicity, and end-stage heart failure are just a few of the cardiovascular diseases and disorders that may benefit from pharmacological intervention of apoptosis pathways because it is a highly regulated process. Despite the expansion of our understanding of apoptosis signaling pathways, there is yet no therapeutically useful medication that targets this cellular process. It is essential to fully comprehend the apoptotic mechanisms now known to be active in cardiac myocytes in order to suggest prospective novel research approaches. In this article, we review current developments in the control of cardiomyocyte apoptosis by various signaling molecules and pathways, with an emphasis on how these pathways contribute to the etiology of cardiac disease. We also cover open topics that need additional research and provide an update on the bench to bedside translation of this information.

KEYWORDS:
Apoptosis, Chemotherapy, Cardiotoxicity, Signaling.

INTRODUCTION

For tissue homeostasis, disease etiology, and organ development, cell survival and death are essential processes. Schmeichel and Merker initially divided cell death into three categories based on morphological manifestations: type I (apoptosis), which is connected to cell fragmentation and heterophagy, type II (autophagic cell death), which is characterized by massive cytoplasmic vacuolization, and type III (necrosis), which is connected to plasma membrane rupture and organelle swelling. The scientific world was first exposed to "apoptosis" by Kerr and colleagues in 1972, and since then, understanding of this particular kind of cell death has greatly increased. The first definition of apoptosis was based on the morphological features of the process, such as chromatin condensation, nuclear fragmentation, cell shrinkage, and the release of vacuoles (apoptotic bodies), which are subsequently removed by phagocytosis in vivo. However, the Nomenclature Committee on Cell Death (NCCD) has suggested identifying several forms of cell death based on their unique biochemical properties, given the significant advances made in the molecular processes underpinning cell death over the previous ten years. Apoptosis is referred to in this context as a genetically regulated, caspase-dependent kind of cell death. According to this description, apoptosis is a biological process that may be influenced by pharmacological or genetic treatments.

Numerous forms of controlled cell death exist in addition to apoptosis, including necroptosis, which depends on receptor-interacting protein kinases 1 and 3 (RIPK1/RIPK3), autophagic cell death, which is connected to the lipidation of the microtubule-associated protein light chain 3 (LC3) and degradation of sinusotomy 1 (SQSTM1, also known as p62), and

apoptosis. Readers who are interested are directed to the review papers mentioned above, which go into greater detail on each cell death method. Here, we'll concentrate on apoptotic cell death. The intrinsic and extrinsic signaling pathways are the two main signaling mechanisms that start and complete apoptosis. Intracellular stress, such as oxidative stress, calcium overload, and DNA damage, activates the intrinsic apoptosis pathway, also known as the mitochondrial apoptosis pathway, which results in mitochondrial outer membrane permeabilization (MOMP) and the release of cytochrome c into the cytosol (Figure 1). Caspase 9 is activated as a consequence of the formation of an azotosome by cytosolic cytochrome c and apoptotic protease-activating factor 1 Contrarily, extracellular stress signals such as tumor necrosis factor (TNF-), Fas ligand (FasL), and TNF-related apoptosis inducing ligand (TRAIL) trigger extrinsic apoptosis by binding to their respective death receptors, TNF- receptor 1 (TNFR1), Fas, and TRAIL receptor 1/2 (TRAILR1/2). The death-inducing signaling complex (DISC) is then formed by death receptors, Fas-associated death domain (FADD), and procaspase.

The cleavage of vital cellular substrates and subsequent cell apoptosis arise from the active initiator caspase 9 or 8 activating the effector caspases 3, 6, and 7. Apoptosis has long been connected to a range of cardiovascular illnesses, including ischemic heart disease, reperfusion damage, chemotherapy-induced cardiomyopathy, and heart failure. Apoptosis is crucial for the development of the heart. Adenoviral-mediated expression of the X-linked inhibitor of apoptosis protein or the pan-caspase inhibitor zVAD-fmk both caused excessive outflow tract above the base of the ventricles, showing that apoptosis is necessary for the morphogenesis of the outflow tract myocardial tissue. Significant apoptosis was found in the embryonic heart at the time of outflow tract shortening. Although apoptosis seems to be unnecessary for physiological homeostasis in the adult normal heart, it may result in the death of cardiomyocytes, which is linked to cardiac dysfunction that poses a life-threatening risk. As a result, a possible treatment approach for cardiovascular disorders is the control of apoptosis.

The majority of the heart's contracting cells are cardiac myocytes, which make up around 85% of the overall mass of the organ. Major strides have been made in the last ten years in understanding the processes of cardiomyocyte apoptosis. We believe that the discipline urgently requires a thorough analysis of these recent findings. The signaling mechanisms that control cardiomyocyte apoptosis will be outlined in this review, along with significant recent discoveries and relevant research areas. Apoptosis, which means "falling off," in Ancient Greek, is a kind of controlled cell death that affects both multicellular creatures and certain eukaryotic, single-celled microbes like yeast. Characteristic cell changes (morphology) and death are caused by biochemical processes. Blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, DNA fragmentation, and mRNA degradation are some of these alterations. Apoptosis causes the typical adult person to lose 50–70 billion cells daily. An typical human youngster between the ages of eight and fourteen loses around 20 to 30 billion cells daily.

Apoptosis is a tightly regulated and managed process that gives benefits throughout an organism's life cycle, in contrast to necrosis, which is a kind of catastrophic cell death brought on by acute cellular damage. For instance, cells between the digits in a growing human embryo suffer apoptosis, causing the separation of fingers and toes. Apoptosis, as opposed to necrosis, results in cell fragments termed apoptotic bodies that phagocytes may engulf and remove before the contents of the cell can leak out and harm neighboring cells.

Apoptosis is a tightly controlled process because once it starts, it cannot be stopped. One of two routes may be used to start apoptosis. In the extrinsic route, a cell kills itself in response to signals from other cells, while in the intrinsic pathway, a cell kills itself as a result of cell stress. The intrinsic apoptotic process may also be activated by weak extrinsic cues. By activating caspases, which are proteases, or enzymes that break down proteins, both mechanisms cause cell death. Both of the mechanisms trigger initiator caspases, which trigger executioner caspases, which destroy the cell by indiscriminately degrading proteins.

Along with being a significant biological phenomenon, faulty apoptotic mechanisms have been linked to a large number of illnesses. Apoptosis that is too high results in atrophy, while apoptosis that is too low leads to uncontrolled cell growth, such as cancer. While certain Bcl-2 family proteins suppress apoptosis, others, including Fast receptors and caspases, induce apoptosis. The concept of apoptosis was initially explained by German physicist Carl Vogt in 1842. Walther Flemming, an anatomist, provided a more detailed explanation of the mechanism of programmed cell death in 1885. However, the subject was not brought up again until 1965. John Kerr of the University of Queensland was able to discriminate between apoptosis and traumatic cell death while utilizing electron microscopy to examine tissues. After a paper detailing the occurrence appeared, Kerr received an invitation to work with Alastair Currie and Andrew Wyllie, Currie's doctoral student at the University of Aberdeen. The trio contributed a groundbreaking essay to the British Journal of Cancer in 1972. The process of spontaneous cell death was referred to as apoptosis in the paper, when Kerr had originally used the phrase "programmed cell necrosis." The name "apoptosis" was proposed by James Cormack, a professor of Greek at the University of Aberdeen, according to Kerr, Wyllie, and Currie. On March 14, 2000, Kerr was awarded the Paul Ehrlich and Ludwig Darmstadt Prize for his explanation of apoptosis. Together with Boston scientist H. Robert Horvitz, he received the honor.

Both "apoptosis" and "programmed cell death" did not get a lot of citations for a very long time. Cell death emerged from obscurity to become a significant area of study because to two discoveries: the discovery of the initial component of the cell death control and effector mechanisms, and the connection between anomalies in cell death and human illness, particularly cancer. This happened in 1988 when it was discovered that the follicular lymphoma gene BCL2 expressed a protein that prevented cell death. Sydney Brenner, H. Robert Horvitz, and John Sulston received the 2002 Nobel Prize in Medicine for their research finding the genes that regulate apoptosis. The genes were discovered via research on the worm C. elegans, and human homologues of these genes control apoptosis.

DISCUSSION

PI3K/Akt Pathway

PI3K/Akt signaling pathway is activated following stimulation with various growth factors, cytokines, and hormones. Upon ligand binding, growth factor receptors, which are a group of receptor tyrosine kinases (RTKs), undergo dimerization and association with the regulatory subunit (p85) of PI3K, leading to activation of the catalytic subunit PI3K then converts phosphatidylinositol bisphosphate (PIP2) to phosphatidylinositol trisphosphate (PIP3), which recruits Akt to the plasma membrane, where it is activated through dual phosphorylation by PDK1 at Thr308 and by mTORC2 at Ser473. The role of Akt in myocardial biology has been extensively described by us in a previous review article. Here we briefly summarize the

recent findings about this pathway in regulation of apoptosis in cardiomyocytes. The PI3K/Akt pathway was initially shown to be strongly activated by insulin and insulin-like growth factor 1 (IGF-1) and mediates its antiapoptotic effects in cardiomyocytes since blockade of this pathway by a specific PI3K inhibitor wortmannin, dominant-negative PI3K, or dominant-negative Akt dramatically inhibited the cytoprotective effect of insulin and IGF-PI3K/Akt-mediated protection against apoptosis is associated with phosphorylation and inactivation of the BH3-only proapoptotic protein BAD. In vivo gene transfer of reisolated Akt (myrrh-Akt), a membrane-localized constitutively active Akt1 mutant, also increased sarcolemma Glut-4 expression and enhanced myocyte glucose uptake and glycolysis which may help maintain energy production in the oxygen-deprived ischemic heart. Intriguingly, while acute moderate Akt1 activation is cardioprotective chronic extensive activation of Akt1 in a cardiac-specific myrrh-Akt transgenic mice is deleterious during ischemia/reperfusion (I/R) due to feedback inhibition of PI3K activity through downregulation of insulin receptor substrate-1 (IRS-1) and IRS-2. These findings suggest that the timing and dose of activation are crucial for the effect of Akt on heart protection in vivo [1], [2].

PTEN

In contrast to PI3K, the dual protein/lipid phosphatase and tensing homologue (PTEN) dephosphorylates PIP3 to generate PIP2 and thus inhibits Akt activation. It has been shown that adenoviral expression of PTEN in neonatal rat cardiomyocytes led to caspase 3 activation and apoptosis. Conversely, inactivation of PTEN by cardiac-specific deletion of PTEN gene or overexpression of a catalytically inactive PTEN mutant attenuated myocyte apoptosis in response to I/R injury or β1-AR stimulation. PTEN activity is positively regulated by direct interaction with the regulatory subunit (p85) of PI3K. Indeed, expression of a p85 mutant lacking the PTEN binding site inhibited PTEN activity and cell death following simulated ischemia and reperfusion. PTEN activation is observed in cardiomyocytes expressing a cleaved (and constitutively active) mutant of Rho-associated coiled-coil protein kinase 1 (ROCK1) and may contribute to ROCK1-dependent apoptosis [3]–[5].

PHLPP

Akt activity can be inhibited by PH domain leucine-rich repeat protein phosphatase (PHLPP), a protein phosphatase 2C(PP2C) family member that selectively dephosphorylates Akt at Ser473. Knockout of PHLPP-1 potentiated Akt phosphorylation at Ser473 and reduced infarct size in response to I/R challenge. A most recent study revealed that PHLPP-1 expression is increased with aging, and an increase in PHLPP-1 expression exacerbated hypoxia/reoxygenation-induced apoptosis. Similarly, Akt can also be dephosphorylated by PHLPP-2, which is activated by isoproterenol and forskolin through a cyclic AMP- (cAMP-) independent mechanism. However, its role in cardiomyocyte apoptosis has not been studied yet.

GSK-3

Ischemic preconditioning-induced, Akt-dependent phosphorylation and inactivation of its downstream target glycogen synthase kinase-3 (GSK-3) were initially speculated to confer cardio protection because both preconditioning and pretreatment with GSK-3 inhibitors reduced infarct size to a similar extent. These findings were supported by transgenic studies

showing that cardiac-specific expression of either GSK-3β or GSK-3α potentiated myocyte apoptosis, albeit through distinct mechanisms. Conversely, cardiac-specific deficiency of GSK-3β significantly inhibited myocyte apoptosis after myocardial infarction (MI). Although global deletion of GSK-3α exacerbated apoptosis after MI, following up studies revealed that cardiomyocyte-specific conditional deletion of GSK-3α reduced apoptosis by decreasing the Bax/Bcl-2 ratio. The discrepancy is likely caused by secondary and compensatory effects associated with germline somatic gene deletion. Surprisingly, a most recent study revealed that conditional deletion of cardiac GSK-3α/GSK-3β in adult mice resulted in ventricular dysfunction and dilation within 2 weeks through a mechanism involving DNA damage and mitotic catastrophe Collectively, these studies indicate that transient and partial inhibition of GSK-3 is cardioprotective, but complete loss of GSK-3 leads to dilated cardiomyopathy [6]–[8].

Pim1

An important downstream target of myocardial Akt signaling has been demonstrated to be Pim1, a serine-threonine kinase that is robustly upregulated following treatment with the cardioprotective growth factor IGF-1 through an Akt-dependent mechanism. Inhibition of Pim1 activity by genetic ablation of Pim1 or expression of a dominant-negative, kinase-dead Pim1 mutant (K67M) exacerbated cardiomyocyte apoptosis, whereas transgenic expression of Pim1 markedly reduced infarct size in mice. Further in-depth studies revealed that cardiac-specific Pim1 expression increased levels of the PR survival proteins Bcl-2 and Bcl-xl, which antagonized mitochondrial damage induced by oxidative stress and the proapoptotic truncated Bid protein. Pim1-dependent inhibition of apoptosis has been implicated in cardio protection induced by postconditioning and vitamin B1 stimulation supporting a critical role of Pim1 in regulation of cardiomyocyte survival following pathological challenge.

FoxO1

Akt phosphorylates the Fox family transcription factors, resulting in their nuclear export and degradation in the cytosol through the ubiquitin-proteasome pathway Fox has long been viewed as proapoptotic through transcriptional induction of proteins involved in intrinsic and extrinsic apoptosis pathways including Bim, BAD, Bnip3, False, and TRAIL Interestingly, overexpression of FoxO1 in cardiomyocytes did not seem to result in apoptosis under basal conditions but significantly induced expression of autophagy-related genes LC3 and Atg12 and enhanced autophagy. Following MI, however, cardiac-specific deficiency of FoxO1 decreased heart function and increased myocyte apoptosis, an effect that is associated with reduced expression of autophagy genes. In addition, FoxO1 may also protect against apoptosis by forming a transcriptional complex with Yes-associated protein (YAP) and inducing expression of antioxidant genes catalase and manganese superoxide dismutase Moreover, another foo family member, FoxO3a, has also been shown to inhibit cardiomyocyte apoptosis and confers cardio protection by inducing expression of apoptosis repressor with caspase recruitment domain (ARC), which attenuated oxidative stress-triggered sarcoplasmic reticulum Ca2+ release. A most recent study showed that knockdown of FoxO1 that is exported from the nuclei following Apelin-13 stimulation exaggerated apoptosis, suggesting that cytosolic FoxO1 may directly inhibit apoptosis through a transcription activity-independent mechanism.

MAPK Pathway

Mitogen-activated protein kinases (MAPKs) are evolutionarily conserved serine/threonine kinases that regulate cell behavior including survival, growth, and differentiation by altering protein function and gene expression in response to specific extracellular cues. Extracellular signals act through cell surface receptors such as RTKs and G protein-coupled receptors (GPCRs), leading to successive phosphorylation and activation of a three-layered hierarchical model including MAPK kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK. Three main families of MAPKs have been extensively investigated in regulation of cardiomyocyte apoptosis: extracellular signal-regulated kinase 1/2 (ERK1/2 or p44/42), c-Jun N-terminal kinases (JNK), and the p38 isoforms [9]–[11].

ERK1/2

ERK1/2 is activated following growth factor stimulation or integrin clustering, and activation of ERK1/2 primarily leads to cell growth and survival ERK1/2 may also be activated by hydroxyl radicals through Ras/Raf-1 MAPKKK, but activation of ERK in this context is still protective as treatment with a selective ERK inhibitor PD98059 exacerbated hydrogen peroxide- (H2O2-) induced cardiomyocyte apoptosis. Indeed, while all three MAPKs are activated following daunomycin treatment and hypoxia/reoxygenation, only inhibition of ERK1/2 further potentiated cardiomyocyte apoptosis, suggesting a prosurvival role of the ERK1/2 kinases. Interestingly, doxorubicin-induced persistent ERK1/2 activation and nuclear translocation contributed to apoptosis in H9c2 cells and neonatal rat cardiomyocytes. Inactivation of ERK1/2 by transgenic expression of a dominant-negative Raf-1, the MAPKKK upstream of ERK1/2, potentiated development of cardiomyocyte apoptosis following aortic constriction Mice deficient in cardiac Raf-1 exhibited spontaneous myocyte apoptosis as early as 3 weeks of age and heart dysfunction later in life.

Transgenic mice expressing a constitutively active MEK1, the major MAPKK directly phosphorylating and activating ERK1/2, inhibited I/R-induced cardiomyocyte apoptosis. More direct evidence of ERK-dependent cardio protection was from the observation that myocytes were sensitized to apoptosis following pathological insult in both global and cardiac-specific ERK2 knockout mice. Activation of ERK1/2 has been shown to mediate the antiapoptotic function of various molecules including α1-adrenergic receptor, urotensin II, and sialyltransferase7A. However, much less is known about the mechanism(s) underlying ERK1/2-mediated protection against myocyte apoptosis.

Stress Activated Protein Kinases (SAPKs)

In contrast to ERK1/2, JNK and p38 are stress activated protein kinases (SAPKs) that respond predominantly to environmental stress signals such as hypoxia, heat, inflammatory cytokines, and DNA-damaging agents. Inhibition of JNK protected against I/R-induced cardiomyocyte apoptosis in vitro and in vivo. Mitochondrial but not cytosolic JNK was phosphorylated upon H2O2 stimulation, leading to mitochondrial outer membrane permeabilization and cytochrome c release. JNK-dependent activation of the mitochondrial apoptosis pathway is associated with decreased BAD phosphorylation at Ser112 Inactivation of JNK contributed to the antiapoptotic effect of macrophage migration inhibitory factor (MIF) and a Curcumin analog. While mice deficient in JNK1/2 were shown to be resistant to I/R-induced apoptosis, cardiac-specific transgenic mice expressing MKK7, the MAPKK for

JNK kinases, were also protected from I/R injury. Although somewhat surprising, the latter finding was consistent with previous reports revealing a protective role of JNK activation during nitric oxide- or hypoxia/reoxygenation-induced cardiomyocyte apoptosis, possibly by phosphorylating Akt at Thr450, thus priming Akt for full activation, or by competing with procaspase 9 to bind Apaf-1 and abrogating apoptosome formation These contradictory results indicate that the role of JNK signaling in apoptosis is likely context dependent and much more complicated than initially thought.

Earlier studies revealed that treatment with the p38 MAPK inhibitor SB203580 attenuated myocyte apoptosis induced by anthracycline and hypoxia/reoxygenation both in vitro and in vivo, indicating a detrimental role of p38 These findings were later corroborated by using genetic approaches to inactivate p38 in mouse heart. For example, heterozygous disruption of p38α drastically reduced infarct size after I/R challenge. Cardiomyocyte apoptosis and cardiac dysfunction following I/R were also attenuated by p38 inactivation in transgenic mice expressing a dominant-negative p38α mutant, a dominant-negative MKK6 (the MAPKK upstream of p38) mutant, or MAPK phosphatase-1 (MKP-1, a phosphatase that dephosphorylates and inactivates p38 and JNK. Cardio protection by p38 inhibition was associated with an increase in the expression of the PR survival Bcl-2 family members. Although p38 is activated by short repeated cycles of simulated ischemia/reoxygenation termed ischemic preconditioning, preconditioned heart actually exhibited lower p38 activity and was protected against injury during sustained ischemia, likely through p38-dependent feedback upregulation of MKP-1. Inhibition of p38 activity has been proposed as a central mechanism underlying cardio protection mediated by post conditioning estrogen, and quercetin.

CONCLUSION

Rapid advancements in molecular methods have greatly increased our understanding of the processes driving cardiomyocyte apoptosis during the last ten years. We really regret not being able to provide funding for the researchers' study. In the etiology of heart disease, a variety of genes and signaling pathways have been proposed to either induce or repress cell death. But considering the size of the human genome and the intricacy of how diseases develop, it is quite probable that a number of significant apoptosis-regulating genes are still undiscovered. Finding a new pharmacological target for apoptosis-related heart disease will be made much easier by high-throughput genome-wide screening in conjunction with in vitro/in vivo research. Although there have been several caspase inhibitors created, no one of them has been shown to be effective in clinical studies. One major reason is that caspase inhibitors prevent apoptosis at late stages, when serious damage, such as the execution of mitochondrial outer membrane permeabilization, has already occurred and the cells are ready to die. The use of caspase inhibitors thus seldom yields long-term cytoprotecting and often causes a morphologic change from apoptotic to necrotic cell death. Therefore, in order to maintain mitochondrial activity and effectively prevent cell death, we think that future intervention techniques should concentrate on upstream activities at the beginning of apoptosis.

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CHAPTER 6

POTENTIAL THERAPEUTIC APPLICATIONS FOR IMMUNOLOGICAL PATHWAYS TRIGGERED BY PROHORMONES GINGIVAL

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ABSTRACT:

The Gram-negative anaerobic bacteria Prohormones gingival is (P. gingival is) and nucleate of Fusobacterium (F. nucleate) are potential pathogens linked to periodontal disease because they have a number of virulence characteristics. Gingivitis and periodontitis are two examples of periodontal diseases, which are chronic inflammatory conditions affecting the mouth. Periodontitis, among the most common disorders in the world, may result in tooth loss. P. gingival is and F. nucleates have virulence factors that enable them to persist in hostile situations by moderating the immune-inflammatory response of the host in a specific way. This poses significant difficulties for host cell survival. Studies have shown that the initiation of periodontitis involves bacterial infection as well as host immune responses. Periodontitis is caused by the NLRP3 inflammasome and its effector molecules, caspase-1 and IL-1. The purinergic P2X7 receptor has been implicated in the regulation of periodontal illness and intracellular pathogen control, according to our research and that of others. By facilitating cell death and the production of IL-1, caspase-4/5 (in humans) and caspase-11 (in mice) are crucial effectors in the fight against bacterial infections. Uncertainty exists about the precise molecular processes behind the host's reaction to these microorganisms. Here, we examine the naturally occurring and adaptive immune reactions brought on by the infection of P. gingivitis and the associated F. nucleated infections and talk about the potential for altering the immune response to serve as a therapeutic tool. It is crucial to create treatment targets for the prevention of periodontopathogen infections given the prevalence of periodontitis worldwide.

KEYWORDS:

Bacteria, Common, Inflammatory, Periodontitis.

INTRODUCTION

Through enhanced host inflammatory reactions to these bacteria, oral bacteria in dental biofilms contribute to the beginning and development of periodontal diseases. Gingivitis and periodontitis are examples of the chronic inflammatory disorders of the periodontium the tissues that support the teeth: gingiva, periodontal ligaments, and alveolar bone. The first stage of reversible inflammation in the soft tissues surrounding the teeth is called gingivitis, and periodontitis develops when a number of causes combine to destroy the periodontium, often resulting in permanent bone loss and tooth loss. In the United States, it affects about half of the population, and severe periodontitis is the sixth most common illness globally. Due of its extensive and costly treatment, periodontitis has a significant influence on public health. Additionally, as we and others have discussed previously, periodontitis is linked to a

number of systemic disorders, such as diabetes mellitus, cardiovascular conditions, and atherosclerosis.

Immunity and disease-causing oral cavity bacteria have a well-established equilibrium in healthy persons. Immune and oral epithelial cells both directly and indirectly support the preservation of this balance. The person is prone to PD owing to the loss of homeostasis brought on by dental plaque accumulation, as well as genetic, hormonal, and host behavioral variables. Additionally, P. gingivitis LPS can greatly increase the proliferation of various bacterial species with the formation of biofilms at the root of the tooth, leading to exacerbated inflammation in the tissues. This is because some cells stimulated by P. gingivitis LPS may lack or reduce an effective innate immune response.

The Gram-negative, non-spore-forming P. gingivitis bacteria may cause dysbiosis, or an imbalance of microbial species, in the oral microbiota. One of the most prevalent species in the human gingival sulcus is F. nucleated, and its prevalence rises as inflammation and PD become more severe. By bridging the gap between early and later bacterial invaders, F. nucleator promotes the development of dental plaques. The number of late colonizers is much smaller when F. nucleate is absent. Scaling and root planning are the first steps in the traditional therapeutic therapy for periodontitis, which reduces the interaction of bacterial agents with inflammatory and noninflammatory cells in the oral cavity. This process may not, however, be enough to result in clinical improvement. Multiple signaling pathways are therefore implicated in the development of PD; accordingly, medications that alter these pathways may aid in preventing the onset of PD and, as a result, reduce bone loss.

Despite the fact that the precise molecular host response events against P. gingival is and F. nucleate are not yet completely characterized, knowledge of these mechanisms is crucial for identifying therapeutic targets for the prevention and treatment of periodontitis. In this regard, it has been shown that some immune pathways have a role in periodontitis development and periodontopathogen infections. In this regard, we and others have previously shown that the NLRP3 inflammasome plays a part in the progression of periodontitis [17, 18]. In addition, purinergic signaling via the P2X7 receptor is recognized as one of the crucial mechanisms for the activation of the NLRP3 inflammasome and the management of intracellular infections, including infections with P. gingival. Interleukin-1 (IL-1) is converted from its inactive form, pro-IL-1, to its active form, IL-1, as a result of the activation of this inflammasome. The host's inflammatory response during PD and infection depends heavily on cytokine production Caspases caspase-4, caspase-5, and caspase-11 are other caspases that play a role in inflammation but have received less attention and are thought to be part of the noncanonical inflammasome. These caspases are crucial for battling Gram-negative bacterial infections by inducing cell death and the production of IL-1.

This review aims to provide light on developments in the investigation of innate and adaptive immune responses after interactions between the host and P. gingivitis and F. nucleate. We go through how the innate and adaptive immune systems, as well as TLRs, the inflammasome, purinergic signaling, cytokines, and chemokines, contribute to the host's ability to fight off these bacteria's infections. Our study emphasizes how crucial it is to comprehend the signaling pathways that P. gingival is and F. nucleate cause in order to develop potentially efficient PD treatment plans. The pathogenic bacterium prohormone’s gingivitis is non-motile, gram-negative, rod-shaped, anaerobic, and a member of the phylum

Bacteroid Ota. On blood agar, it grows as black colonies. It is located in the colon, upper gastrointestinal tract, respiratory tract, and oral cavity, where it is linked to periodontal disease. It has only been found in females who have bacterial vaginosis.

The collagenase enzymes in this species contribute to the collagen breakdown seen in chronic periodontal disease. P. gingival is may infect human gingival fibroblasts and can persist in the presence of antibiotics, according to in vitro research. When P. gingival is invades gingival epithelial cells in large numbers, both the bacteria and the epithelial cells may survive for a long time. Patients who have P. gingivitis might be found to have high levels of certain antibodies.

Alzheimer's disease and rheumatoid arthritis have both been connected to P. gingival is infection. It has peptidyl-arginine deiminase, an enzyme essential for citrullination. Periodontal disease is more frequent in those with rheumatoid arthritis, and these people are also far more likely to have antibodies to the bacteria. According to each type's capsular antigenicity, P. gingivitis is categorized into K-serotypes. The findings on bacterial cell-to-cell contacts, serotype-dependent immune responses, and risk of pancreatic cancer have all been driven by these serotypes. gingivitis secretes the endopeptidase enzymes arggingipain (Rgp) and lysgingipain. These gingipains perform a variety of tasks for the organism, aiding in both virulence and survival. It has been discovered that-gingipains are essential for collecting nutrients for P. gingival is survival. breaks down the big peptides of the host to provide the bacteria a rich supply of nitrogen and carbon from the human serum albumin. Additionally, P. gingival is has the ability to break down transferrin inside of host cells, giving the organism the ample iron supply, it needs to carry out a variety of cellular tasks.

Additionally, the gingipains are in charge of a variety of crucial tasks connected to host invasion and colonization. Rgp gingipains processed the long fimbriae's precursor proteins, which made them essential for adhesion and invasion. After being incubated with T. denticule, the P. gingival is genes encoding Gap, Kgp, and hemagglutinin A (HagA) were highly expressed. The proteins that include hemagglutinin adhesion domains boost P. gingival is' ability to adhere to other bacterial species. They are also involved in coordinating the biofilm's integrity throughout its formation and maturity. Lys- gingipains (Kgp) may aid in host colonization because they have the ability to bind to immobilized fibrinogen and fibronectin matrix proteins. Additionally, gingipains have the capacity to impair a variety of host immune response signals. In areas with large concentrations of P. gingival is, they have the capacity to cleave subclass 1 and 3 IgG antibodies as well as proinflammatory cytokines including compromising host immune response performance. Rgp may regulate T-cell communication and proliferation to prevent IL-2 build-up in T-cells, which allows them to avoid the host adaptive immune response.

Gingipains are important contributors to the tissue damage that periodontitis causes as a consequence of the breakdown of collagen, fibronectin, and matrix metalloproteins. Degradation of these substrates prevents host cells from interacting with the extracellular matrix, which prevents wound healing and results in the degeneration of periodontal tissues. Rgp is in charge of inducing the inflammatory response in the host via the p38 MAPK transduction pathway. This reaction is probably involved in the breakdown of bone and tissue and the inflammatory aspect of periodontitis. Gingipains and Alzheimer's disease (AD) have been linked. From TMAs of individuals with AD brain damage, gingipains were found. Both

Rap and Kop were identified in the hippocampus and cerebral cortex of AD patients, and they were connected to ubiquitin, which builds up in tau tangles and amyloid beta plaques in AD brain, as well as tau load, a marker for AD pathology. Additionally, P. gingival is 16S rRNA was found in the cerebral cortex and cerebrospinal fluid of AD brains. When gingipains were administered in a murine model, neuron cell disintegration was prevented by pretreatment with gingipain inhibitors.

DISCUSSION

Periodontopathogen Pathogens prohormone's gingivitis and Fusobacterium nucleator

gingivitis is an opportunistic colonizing pathogen that may infect immune cells, periodontal ligament fibroblasts, osteoblasts, and gingival epithelial cells. For growth in vitro, it needs anaerobic conditions as well as hemin and vitamin K in the nutritional medium. It manifests as colonies that are darkly colored on blood agar media, which is explained by the accumulation of heme groups on its cell membranes. Because periodontal pockets have low sugar concentrations, P. gingival is may survive there by converting amino acids into energy. P. gingivitis is referred to be a "inflammophilic" bacterium (from the Greek suffix -phallic meaning "attracted to" or "loving. Infections with this organism are hypothesized to cause the release of proinflammatory cytokines that harm the host tissue and aid bacterial survival. Because of the release of products from tissue degradation, such as peptides and components containing heme groups, the circumstances of the inflamed tissue thus promote the nutritional demands of the dysbiosis population. P. gingival is is often present in 10%–25% of individuals who are healthy and 79%–90% of those who have periodontitis The presence of P. gingivitis and the depth of the periodontal pocket are positively correlated [1]–[3].

Because of its capacity to change the normal oral microbiota composition into one with higher pathogenicity that significantly accelerates bone loss, P. gingivitis is regarded as the keystone pathogen of PD. This periodontopathogen is regarded as a master of immune system subversion because it uses a variety of sabotage strategies to avoid detection by, weaken the defenses of, or trick the host's immune system. P. gingival is has a number of virulence factors, including as lipopolysaccharide (LPS), fimbriae, nucleoside diphosphate kinase (NDK), ceramide, and outer membrane vesicles, among others. In landmark research, Safranski et al. examined more than 13,000 samples of subgingival periodontitis dental plaque and classified the germs into bacterial "complexes" based on their relationships with one another. The "red complex" is made up of P. gingivitis, Treponema denticule, and Taniela forsythia, and it has higher pathogenic potential and is associated with periodontal disease clinical indicators including pocket depth and bleeding on probing. F. nucleator is a member of the "orange complex," a crucial group that promotes the colonization of the "red complex" bacteria and is crucial for the development of Parkinson's disease (PD).

Human pathogen nucleator is anaerobic, filamentous, Gram-negative, nonpure-forming, and non-motile. Five putative subspecies (ss) of this heterogeneous species, which is a member of the Fusobacteria family, have been identified: ss animalist, ss fusiform, ss nucleated, ss polymorphous, and ss Vincentian. Despite the fact that F. nucleate has been discovered in a variety of tissues, the mouth cavity is the most frequent anatomical location in humans. The bacteria P. gingivitis also has virulence traits that might make it an opportunistic pathogen in periodontal diseases. Adhesins (facilitating adherence and invasion to numerous cell types, resulting in colonization, dissemination, and inducing host immunological responses,

endotoxins (such as LPS), and production of serine proteases are only a few of the virulence factors that F. nucleate contains [4]–[6].

Identification of Pathogen-Associated

molecular patterns (PAMPs), which are evolutionarily conserved compounds shared by microbes but are missing in the host, bacterial components trigger innate immune responses. These PAMPs notify the innate immune system that pathogens are present. In the same context, intracellular damage-associated molecular patterns (DAMPs) are released when tissue homeostasis is altered as a result of microbial invasion, necrosis, cell injury, or stress. DAMPs are also known as "alarmins" because they are thought to be danger signals that alert the innate immune system A broad range of pattern recognition receptors (PRRs), found in the plasma membrane, cytoplasm, or vesicles (such as endosomes) of inflammatory cells as well as resident cells, recognize PAMPs and DAMPs. When the host recognizes PAMPs and DAMPs, signaling pathways such activator protein 1 (AP-1) and factor nuclear kappa B (NF-B) are activated, which causes the release of proinflammatory cytokines. The RIG-I-like receptors (also known as RLRs), Toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (also known as RLRs), and nucleotide oligomerization domain- (NOD-) like receptors (NLRs) are all members of the family of PRRs.

TLRs may be activated by commensal bacteria, are expressed in oral epithelial tissue, and protect the host against microbial illnesses. MyD88 and the adapter protein with the TIR (Toll-IL-1 receptor) domain are recruited to the cytoplasmic region of TLR as part of the TLR signaling pathway, which then activates NF-B and induces the production of proinflammatory cytokines and host defense genes. One of the strategies P. gingivitis uses to manipulate the host response and aid in its adaptability and survival is the interaction of LPS with TLRs [88]. A lipid A component, a polysaccharide core, and a variable-length antigen O make up LPS. The effector LPS component that binds to is lipid A. Myeloid differentiation protein and the cluster of differentiation are additional costimulatory for TLR4 signaling. Depending on the kind of bacteria, LPS has a different molecular structure.

The host can distinguish between commensal and pathogenic bacteria based on the degree of acylation of PAMPs. P. gingival is LPS differs from LPS of other bacterial species by altering the O-antigen structure as well as the acylation patterns and the ability of the lipid A component to activate receptors. The penta-acylated phosphorylated structure of P. gingivitis lipid A stimulates TLR4, whereas the tetra-acylated monophosphorylated structure inhibits TLR4 and dampens host immunological responses. The microenvironment and hemin concentrations affect these variations in lipid A structures. In P. gingival is, several tetra- and penta-acylated lipid A structures were found at high hemin concentrations, causing high degrees of inflammation, as opposed to just one major penta-acylated lipid A structure at low hemin concentrations Another type of LPS, known as A-LPS, was also discovered in P. gingival is; it had an anionic polysaccharide linked to lipid The research is still split on how P. gingival is LPS signals when it binds to TLR2/4 receptors; this split concerns the different parts of P. gingival is lipid A that display different receptor binding properties [7]–[9].

The PRR family includes NLRs,

which may be found in "inflammasomes NLRs are nucleotide-binding oligomerization domain-like receptors. Inflammasomes are multiprotein complexes that are put together in the

host cell in response to infection and/or cellular stress, and they may eventually result in "pyro ptosis," a kind of cell death, and/or the maturation and release of proinflammatory cytokines While noncanonical inflammasomes include caspase-11 (in mice) or caspase-4/5 (in humans), canonical inflammasomes convert procaspase-1 into the mature form caspase-1. Canonical inflammasomes have names based on the receptors involved in stress recognition. They may be triggered by a variety of ligands. Noncanonical inflammasomes, on the other hand, are exclusively triggered by cytosolic LPS. As previously discussed the assembly and stability of the NLRP3 and AIM2 inflammasomes depend on the PYD-CARD adaptor protein ASC (apoptosis-associated protein with caspase recruitment domain).

The best-studied inflammasome at the moment, NLRP3, has been linked to a number of chronic inflammatory conditions, including type II diabetes, obesity, and intestinal disorders. It is well acknowledged that the NLRP3 inflammasome requires two signals in order to activate: (1) the recognition of a PAMP via PRRs such as TLRs induces NF-B activation and subsequent transcription of genes encoding NLRP3 and inactive forms of the proinflammatory cytokines, such crystals/particles (such as uric acid and silica), -amyloid, bacteria, viruses, fungi, protozoa, pore-forming Caspase-1, which is responsible for cleaving pro-IL-1 and pro-IL-18 into their physiologically active forms, IL-1 and IL-18, and/or for causing proptosis (to be covered later in this review is activated as a consequence of the NLRP3 inflammasome. Recent research revealed that adermin D is cleaved by activated caspase-1, causing a hole to develop in the plasma membrane, releasing IL-1/IL-18, and/or causing pyro ptosis.

Studies have looked at the parts of inflammasomes and the compounds they produce during periodontitis and when periodontopathogens infect cells. When infected with P. gingivitis, human monocytic cells (Mono-Mac-6 cells) exhibited elevated levels of NLRP3 and IL-1/IL-18 but lower levels of ASC. Through the TLR2 and TLR4 pathways, P. gingivitis activated the NLRP3 inflammasome in THP-1 cells. Due to the fact that ASC is involved in cell death and the subsequent clearance of intracellular bacteria, this bacterium may downregulate ASC as a survival strategy. We and others have shown that P. gingivitis infection causes intracellular pro-IL-1 synthesis but not IL-1 secretion in murine macrophages and human gingival epithelial cells. In fact, we and others demonstrated that purinergic P2X7 receptor activation by extracellular ATP is essential for IL-1 production following P. gingivitis infection in human gingival epithelial cells and murine macrophages. In addition, we discovered that NLRP3 was required for the intracellular processing of pro-IL-1 in murine macrophages. As an alternative, several investigations have shown that caspase-8 may also play a role in the activation of the NLRP3 inflammasome and the cleavage of pro-IL-1 as a result of TLR4 activation.

In addition, we demonstrated that P. gingivitis infection in vivo stimulated the production of IL-1 to prevent bacterial infection in a way that was reliant on caspase-1/11, P2X7 receptor, and autocrine IL-1 receptor signaling. When P. gingivitis and F. nucleate were co-infected in macrophages, it was interestingly shown that P. gingivitis mediated inflammasome suppression via a mechanism involving decreased endocytosis. The significance of investigating infection models to comprehend the etiology and immune responses during periodontitis is shown by these investigations. The inflammasome plays a significant role in the pathogenesis of periodontitis, in fact. It is previously known that the NLRP3 inflammasome and IL-1 production are essential for the development of PD since, in mice

models lacking either NRLP3 or IL-1, P. gingivitis does not generate periodontitis. In addition to enhancing tissue pathology and inflammatory responses in periodontal lesions and encouraging the loss of connective tissue and bone, IL-1 also plays a significant role in these processes. Furthermore, several investigations found that when periodontitis patients' gingival tissue was compared to that of healthy people, the expression of NLRP3, AIM2, IL-1, and IL-18 increased infections, unlike infections, activate the NLRP3 inflammasome in murine macrophages and gingival epithelial cells, leading to pyro ptosis and IL-1/IL-18 secretion even in the absence of extracellular ATP, indicating that F. nucleate provides both PAMPs and a danger signal F. nucleated induces NF-B activation in gingival epithelial cells, which results in increased production of the proinflammatory cytokine secretion. The NLRP3-dependent activation of caspase-1 and the production of the danger signals ASC and high-mobility group box 1 protein (HMGB1) in these cells were both caused by F. nucleate infection alone. Interestingly, we demonstrated that NLRX1 had a dual impact on gingival epithelial cells infected with F. nucleated by upregulating caspase-1 activation reliant on NLRP3 while downregulating NF-B activation and IL-8 production.

These findings demonstrate the strong yet intricate regulation of inflammasome activation after F. nucleate infection. We demonstrated that F. nucleator promoted the production and release of a number of proinflammatory cytokines during oral infection in mice, including the inflammasome-dependent. The expression of NLRP3 inflammasome components was also shown to be elevated in periapical lesions in human participants with periapical periodontitis, which is consistent with in vitro and animal model studies. F. nucleated infection in vivo was assessed in several studies, but more research is needed to understand how this bacterial infection affects inflammasome activation and regulation since it might be a significant therapeutic target [10]–[12].

CONCLUSION

Nucleate play an important role in the pathogenesis of PDs, the precise molecular processes by which these bacteria cause these diseases are still poorly known. Due to disagreements in the literature, studies on P. gingival is LPS-induced TLR2/4 activation are still unclear. Since human cells have a larger inflammatory potential than animal cells, further research on human cells is required. Further research is required to determine how F. nucleator manipulates downstream TLR2/TLR4 pathways to reproduce intracellularly and survive. Although it is well established that inflammasomes have a role in the pathogenesis of periodontitis, it is still unclear which inflammasomes, besides NLRP3, are truly responsible for the pathogenesis of PD brought on by P. gingival is and F. nucleate. Although the P2X7 receptor is known to have a role in immune responses against P. gingivitis, it is still unclear how purinergic signaling functions in the context of F. nucleator infection. The function of caspases, particularly caspase-11, in the setting of inflammation and cell death in in vivo and in vitro models of PD requires more study.

Additional research is also required to fully understand the variations in immune responses of different oral cavity cells implicated in infections by P. gingival is and F. nucleator, taking into account the period of infection, MOI, and strain types that are indicative of PD in people. Future research should focus on models of in vivo coinfection with P. gingivitis and F. nucleator in order to better understand the host immune response since coinfection and coculture models are more comparable to clinical periodontitis. Effective therapeutics for

Parkinson's disease (PD) may thus develop with more robust literature on these signaling pathways and immunological responses after infection with these bacteria.

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CHAPTER 7

POTENTIAL THERAPEUTIC APPLICATIONS OF TOLL-LIKE RECEPTOR SIGNALING PATHWAYS

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ABSTRACT:
Transmembrane proteins known as toll-like receptors (TLRs) serve primarily as sensors of microbial elements. Multiple inflammatory genes are more frequently expressed when TLRs are activated, and this serves as a defense mechanism against infection. The onset of cancer, allergies, sepsis, and autoimmune disease are all significantly influenced by abnormal activation of TLR signaling, though. In particular, TLR signaling pathways, adaptor proteins such MyD88, IRAKs, TIRAP, TRIF, and TRAM are implicated. This article examines the function of these molecules in TLR signaling and explores how different illness situations are affected by this route. TLRs and their signaling pathways become intriguing targets for therapies due to their significance in both infectious and non-infectious illness contexts. The TLR4 gene in humans produces the protein known as toll-like receptor 4. The toll-like receptor 4 (TLR4) is a transmembrane protein and a member of the PRR (pattern recognition receptor) family. Its activation triggers the production of inflammatory cytokines and the NF-B intracellular signaling pathway, which together activate the innate immune system. Myeloid (erythrocytes, granulocytes, and macrophages) rather than lymphoid cells express Most myeloid cells also have high CD14 expression, which makes it easier for LPS to activate TLR4 in these cells.

KEYWORDS:
Abnormal, Elements, Multiple, Transmembrane.

INTRODUCTION

This gene produces a protein that belongs to the Toll-like receptor family, which is crucial for the detection of pathogens and the induction of innate immunity. TLRs have a high degree of structural and functional conservation from Drosophila to humans. They are capable of identifying the pathogen-associated molecular patterns that are expressed on infectious pathogens and mediate the synthesis of the cytokines required for the maturation of functional immunity. The expression patterns of the various TLRs vary. By activating NF-B, this gene, which is mostly expressed in peripheral blood leukocytes, promotes the host response to yeast and Gram-positive bacteria as well as other pathogens. TLR2 controls the production of CYP1A1, a crucial enzyme in the detoxication of carcinogenic polycyclic aromatic hydrocarbons like benzo pyrene, in the intestine. Background The immune system detects and destroys foreign infections. There are various stages to this. Antibodies that are already present innate or acquired from earlier infection; see also cross-reactivity detect the pathogens during the early stages of inflammation.

Immune system components, such as complement, are linked to the antibodies and retained nearby as a backup in case scavenger cells, such as macrophages, need to phagocytose them

and neutralize them. Although dendritic cells are also capable of phagocytosis, they do not do so to directly destroy pathogens. Instead, they invade the spleen and lymph nodes and individually present an antigen component there, leading to the formation of specialized antibodies that specifically detect that antigen.

However, in an acute infection, these newly generated antibodies would arrive too late, thus what we typically refer to as "immunology" is actually simply the second part of the process. A faster-acting principle is used before it since this phase would always begin too late to be crucial to the defensive process. This principle only appears in forms of life that are phylogenetically more advanced. Here, the so-called pattern-recognition receptors are at work. This refers to receptors that identify substances not indigenous to the host organism by their gross, mostly structural properties. These include, for instance, lipids having completely distinct chemical structures at the basic level. Such receptors bind directly to immune system cells, activating the corresponding nonspecific immune cells instantly. The long-known effects of bacterial endotoxin serve as a prominent example of such a foreign ligand. It triggers systematic early-phase response activation when it enters the bloodstream, resulting in all the symptoms of septic shock.

The Shwartzman phenomenon is what is referred to as this in the lab. The goal is to mobilize the organism for battle, so to speak, and get rid of the majority of pathogens. TLR2 is a membrane surface receptor that can detect a variety of bacteria, fungi, viruses, and some endogenous chemicals. In general, this causes bound molecules to be taken up (internalized, phagocytosed) by endosomes/phagosomes and activates cells; as a result, innate immune components like macrophages, PMNs, and dendritic cells take on roles in nonspecific immune defense, B1a and MZ B cells produce the initial antibodies, and specific antibody formation begins in the process. TNF-alpha (tumor necrosis factor-alpha) and other interleukins are cytokines involved in this. Several of the compounds described were categorized as modulinos before the existence of TLRs was established. In most experimental models, an immunological divergence away from Th2 features is observed due to the cytokine pattern, which more closely resembles Th1. Conjugates are either already being utilized without prior awareness as vaccinations or are currently being developed as such.

The expression of TLR2 on Tregs, which undergo both TCR-controlled proliferation and functional inactivation, is an oddity that was originally noticed in 2006. This results in the early inflammatory phase and the development of particular antibodies being disinhibited. Numerous pathogen-specific Tregs are present once the pathogen count is reduced, and since there is no longer a TLR2 signal, they become active and suppress the specific and inflammatory immune responses. Older literature that claims a specific chemical directly stimulates immunity via TLR2 must be understood in light of the fact that the TLR2 knockouts used frequently contain relatively few Tregs. In particular, in infections/sepsis with Gram-positive bacteria, functionally relevant polymorphisms are found to cause functional impairment and hence lower survival rates. The identification of pathogens and the activation of innate immunity are important functions of the TLR family. TLRs have a high degree of structural and functional conservation from Drosophila to humans.

They are capable of identifying the pathogen-associated molecular pattern that are expressed on infectious pathogens and mediate the synthesis of the cytokines required for the maturation of functional immunity. The expression patterns of the various TLRs vary. Both

immunological and non-immune cells express Bacterial flagellin, a crucial component of bacterial flagella and a virulence factor, is recognized by TLR5. The nuclear factor NF-B is activated by the activation of this receptor, which also increases the synthesis of tumor necrosis factor-alpha.

The protein monomer that forms the filament of bacterial flagella and is present on almost all motile bacteria, flagellin, is recognized by. All bacteria share portions of the flagellin protein that are highly conserved, making it easier for a germ-line encoded receptor like TLR5 to recognize flagellin. However, certain Proteobacteria flagella have developed mutations that prevent TLR5 from recognizing them Bacterial flagellum attachment to TLR5 on the cell surface frequently initiates the TLR5 signaling cascade. Flagellum binding causes TLR5 to dimerize, which then attracts After is recruited, and eventually IB kinases are activated. The proinflammatory cytokine NF-B is localized in the nuclear compartment as a result of IB kinase activation. Numerous downstream gene expressions are induced by NF-B, which starts the main proinflammatory pathway. Different cell types respond differently to this TLR5/flagellum interaction. The attachment of the flagellum to TLR5 causes the generation of IL8 in epithelial cells. This connection leads to the release of pro-inflammatory cytokines like TNF by human monocytes and dendritic cells.

A recent study found Caveolin-1 to be a possible expression regulator. expression remains comparatively consistent throughout the aging process, which is connected with the high level of Caveolin-1 in aging cells, in contrast to the decreasing level in senescent cells. The absence of Caveolin-1 expression in aged cells causes a considerable drop in expression, according to data from Caveolin-1 knockout mice. According to a theory, Caveolin-1 directly interacts with TLR5 to stabilize it and thus raise its concentration, or inflammatory bowel disease, might be impacted by TLR5. Mice lacking exhibit metabolic syndrome and spontaneous colitis, both of which are correlated with altered gut flora. Patients with moderate to severe ulcerative colitis have been reported to have statistically significant reduced levels of TLR5 expression. Lower TLR5 mRNA levels and decreased TLR5 immunoreactivity were seen in these individuals' irritated moccasin autoimmune and infectious illnesses, inflammation causes bone loss and osteoclast genesis. recognized as a unique mediator in the process of osteoclast genesis and inflammation-induced bone loss by a recent study. Patients with rheumatoid arthritis have synovial fluid that contains flagellin, a TLR5-activating ligand. The receptor activator of NF-B ligand (RANKL) is subsequently activated in these patients when TLR5 is activated. Increased osteoblastic gene expression results from RANKL activation. Strong osteoclast production and bone loss come from the activation of these genes. In a mouse model with TLR5 deletion, this mechanism is not present.

DISCUSSION

The TLR Pathway

The innate and adaptive immune systems are two interconnected parts of the immune system. The immune system's adaptive response to particular "no self" antigens results in immunological memory. The innate immune system, on the other hand, offers an instant first line of protection against a wide variety of invasive microbial infections. Cognate pattern recognition receptors, which are essential for innate immunity, are thought to serve as sentinels for both pathogen-associated molecular patterns and damage-associated molecular

pattern molecules bearing invaders. These receptors include Toll-like receptors and their signaling pathways become intriguing therapeutic targets because of their extensive influence on both innate and adaptive immunity in various disease contexts. This review emphasizes potential therapeutic targets in diverse disease situations and lists the key participants in innate immune signaling [1]–[3].

PAMPs and DAMPs, a spectrum of "danger" signals, are the primary initiators of innate immune responses. Exogenous chemicals known as PAMPs are produced by both pathogenic and non-pathogenic bacteria. The bulk of DAMPs, in contrast, are endogenous substances that are generated by dying host cells in response to cellular stress or tissue damage. The TLRs are a group of evolutionarily conserved PRRs that are essential for detecting microbes. In both infectious and noninfectious illness settings, different TLR members are known to identify and react to various PAMPs and certain endogenous DAMPs triggering innate immune responses and priming antigen-specific adaptive immunity.

TLR Signaling and Illness

In both infectious and non-infectious illness conditions in animals, TLRs function as a key defense mechanism. During infection with a variety of pathogens, such as protozoan parasites and pyogenic bacteria activation of TLRs and the MyD88 signaling pathway serves a protective role. Life-threatening, frequently repeated pyogenic bacterial infections have been documented in patients with autosomal recessive MyD88 deficiency. Intriguingly, though, their clinical state actually became better as they aged, suggesting the compensating role of adaptive immunity. Downregulation of TLR-related molecules or signaling has been linked to sepsis and autoimmune illness, as detailed below, which is consistent with their critical function in battling infections. On the other hand, overexpression of these molecules has been connected to HIV immunological abnormalities, cancer, allergy, other autoimmune illnesses, and allergies.

It has also been clear that TLR and autoimmune disorders are related. According to studies, inhibiting reduced IL-1 expression in arthritic joints and reduced the severity of experimental arthritis. In individuals with rheumatoid arthritis, Roelofs discovered that a variation could lessen the protein's ability to mediate signals. Patients with reactive arthritis have been found to have a higher prevalence of the polymorphism. Similar to this, TLR2 and TLR4 polymorphisms may also have a significant role in the etiology of SLE in both human patients and animal models. Additionally, TLR7 has been related to the pathophysiology of SLE because it is functionally associated with the generation of autoantibodies. Indeed, it has been shown that the X-linked TLR7 gene translocation to the Y chromosome promotes fatal lupus in mice with multiple immunological abnormalities, thereby constituting disease genes for murine lupus [4]–[6].

Autoimmunity and the effects of MyD88 and IRAK1 deficiencies have been extensively studied. According to Harada and colleagues, MyD88-deficiency prevented the onset of autoimmune nephritis in mice, which is characterized by lower blood levels of anti-double-stranded antibodies and decreased levels of cytokines like interferon-, interleukin Similar to this, it was noted that MyD88-deficient animals exhibited diminished Th1, but not Th2, responses following inoculation with retinal Ag. These mice were also entirely resistant to experimental autoimmune uveitis. A warning should be remembered when interpreting the results of the sepsis, cancer, and autoimmunity research mentioned above that used MyD88-

deficient mice. It has been shown that IL-1 and IL-18-mediated activities are lost in MyD88-deficient animals, indicating that deficiencies in signaling downstream of IL-1 and IL-18 receptors may contribute to the symptoms seen in these mice.

Additionally, the production a different TLR signaling protein, appears to be necessary for autoimmune disease. According to studies, IRAK1-deficient animals demonstrate little to no central nervous system inflammation, making them resistant to experimental autoimmune encephalomyelitis. After being immunized with myelin antigen, IRAK1-deficient animals showed poor Th1 cell maturation and little IFN-secretion The mechanism underlying this observation, according to the authors, may be related to a reduced adjuvant impact on antigen-presenting cells as a result of insufficient TLR activation. The IRAK1 gene was strongly related with both adult-onset and childhood-onset SLE, according to our recent study. We discovered that IRAK1 loss eliminated all lupus-related symptoms, including IgM and IgG autoantibodies, lymphocytic activation, and renal impairment, in mice carrying the lupus susceptibility loci, Sle1 or Sle3. Additionally, the "hyperactivity" of dendritic cells linked to lupus was abolished by the absence of IRAK1.

An important cytoprotective, antioxidant, and anti-inflammatory molecule known as heme oxygenase has been linked to the activation of IRF3 following stimulation of or viral infection. The expression of the key IRF3 target genes for depends on HO-1. Mice with myeloid-specific HO-1 loss had severe illness in the experimental autoimmune encephalomyelitis model, which was accompanied by hyperactive antigen-presenting cells, increased infiltration of Th17 cells, and no regressing myelin-specific T cell reactivity. IFN-, it turns out, has the ability to correct these flaws. Additional components that are crucial in the development of systemic lupus erythematosus include the overproduction of IFN and IFN-dependent genes, like IRF5. Sepsis is a dangerous medical disorder that can cause rapid tissue destruction and is characterized by a systemic state and the presence of a known or suspected. The pathophysiology of sepsis is heavily influenced by the detection during an acute Gram-negative bacterial infection. In a polymicrobial septic peritonitis illness model, Weigandt and colleagues discovered that MyD88-deficient mice were protected from contracting sepsis. Mice's responses to bacterial and viral challenge were reported to be significantly reduced in the IRAK4-deficient paradigm [7]–[9].

The pathophysiology of sepsis was recently linked to polymorphisms in the human Mal/TIRAP allele, according to a paper by Ferwerda and colleague. On the other hand, other researchers have demonstrated that Mkp-1, a significant inhibitor of TLR-induced inflammation, is essential for the regulation of innate immunity during sepsis caused by Gram-negative Bacteria According to Aniki et al. in a different model of bacterial pneumonia, MyD88 is crucial in infectious illnesses as well These studies collectively demonstrated that the TLR pathway is essential to the development and management of sepsis. Recent studies have looked at the significance of TLR expression and function in cancer cells as well as its relationship to carcinogenesis and tumor progression. Numerous malignancies, including gastric cancer and human epithelial ovarian cancer, are associated with TLR4 expression. TLR4 was found to be expressed in murine tumor cells, according to Huang et al. The activation of these cells led to the expression of several soluble factors, including interleukin-6, inducible nitric oxide synthase, interleukin-12, B7-H1, and B7-H2, and made the tumor cells resistant to CTL attack. Additionally, T cell proliferation and natural killer cell activity could be inhibited by the elements in LPS-stimulated tumor cell

supernatants. According to Kelly et al., human epithelial ovarian cancer cells (EOC) universally express TLR4, and the expression of MyD88 is necessary for EOC cell proliferation or improved cytokine/chemokine production. Both naturally occurring and carcinogen-induced tumor formation are significantly influenced by MyD88-dependent signaling, which also regulates the expression of several important modifier genes in tumorigenesis. According to other reports, variations in the TLR gene cluster gene expression were linked to a statistically significant lower risk of prostate cancer.

Therapeutic Potential of TLR Signaling Pathway

According to O'Neill the targeting of either the TLRs themselves or the signals they produce is of tremendous interest because the majority of the evidence point to the TLR pathway playing a significant part in a number of pathogenic processes. MyD88 is an appealing target for treatment in these diseases because it is implicated in cancer, autoimmune diseases, and infectious diseases. The TLR-mediated inflammatory responses may be controlled by the MyD88 inhibitor ST2825, a heptapeptide analog that Latarro et al. reported can block MyD88 dimerization. In co-immunoprecipitation tests, ST2825 has been shown to prevent MyD88 dimerization. It is specific for homodimerization of the TIR domains but has no effect on homodimerization of the death domains. They examined in experimental animal models of autoimmune and inflammatory diseases, including lupus, inflammatory bowel disease, and multiple sclerosis, and discovered that could obstruct the recruitment of inhibiting the IL-1-mediated activation of NF-B and IL-6. The "Compound 4a" by Barfi et al. is a low-molecular-weight MyD88 mimic that may obstruct the interaction of MyD88 with IL-1R at the TIR domains. Compound 4a dramatically reduced the IL-1-induced fever response in vivo and inhibited IL-1-induced activation of the mitogen-activated protein kinase thymoma cells and freshly separated murine lymphocytes. These findings imply that MyD88 may be a potential drug target in a number of illness contexts where elevated TLR signaling is responsible for disease development.

It has also been proposed that altering IRAK4, another TLR pathway signaling molecule, could be a promising therapeutic strategy for the management of inflammatory, autoimmune, and cancerous conditions. Wang et al. have created a number of small-molecule drugs that inhibit. It is still unknown how effective these IRAK4 inhibitors are in vivo and in vitro. Another study found that p38 MAP kinase, c-Jun N-terminal kinase activation, and IL-6 generation in human cells were all effectively inhibited by the dual IRAK1 and IRAK4 inhibitor RO0884. They also discovered that IRAK4 was necessary for cellular responses to IL-1 but not to TNF-, whereas IRAK1 was necessary for cellular responses to TNF- but not IL-1. According to these findings, proinflammatory cytokine production would need to be inhibited by both IRAK1 and IRAK4 kinases. Together, these results give rise to the possibility that IRAK1/IRAK4 inhibitors may be useful as a treatment tool for autoimmune and inflammatory illnesses [10]–[12].

Human PDCs have been demonstrated to produce less IFN- following stimulation with DNA or RNA viruses when synthetic oligodeoxynucleotides containing immunoregulatory sequences (IRS) with immunoregulatory sequences (IRS 954) that specifically inhibit both TLR7 and TLR9 activation are administered. After receiving IRS 954 treatment, it has been noted that (NZBNZW) F1 mice displayed lower blood levels of antinuclear autoantibodies, proteinuria, and glomerulonephritis, as well as enhanced survival. Another TLR7-specific

oligodeoxynucleotide inhibitor, IRS 661, was able to considerably lessen disease in MRL/lord mice, especially the weights of the spleen and lymph nodes as well as the serum levels of TNF- when compared to the saline-treated control. In mice predisposed to lupus, injection of IRS 661 but not IRS 954 dramatically reduced anti-dsDNA and anti-Smi antibodies. Most intriguingly, it was discovered that intrarenal macrophages, glomerular cells, and tubular epithelial cells all accumulated both IRS 661 and IRS 954 in the kidneys. The kidneys also had lower levels of the inflammatory chemokines CCL2 and CCL5, as well as their corresponding chemokine receptors CCR2 and CCR5. The activity and chronicity scores for antibody-mediated nephritis in mice treated with IRS 661 or IRS 954 were similarly markedly decreased, which is consistent with these results. These inhibitors have demonstrated success in treating multiple sclerosis and arthritis in addition to lupus. Antagonizes of this pathway are anticipated to also provide therapeutic benefit to patients, according to a recent paper that claims TLR signaling is necessary for the development of SLE in patients.

CONCLUSION

Innate immune cells and a few specific cell types in the adaptive arm of the immune system are activated by TLR molecules and their downstream signaling pathways. This route makes a desirable target for therapeutic intervention because it is aberrantly expressed or activated in a number of illnesses. There is growing proof that blocking this pathway at the TLR, MyD88, or IRAK1/4 levels may have therapeutic benefits for autoimmune and auto-inflammatory illnesses. On the other side, complete paralysis of these pathways may impair immunological monitoring of malignancies and immune protection against invading pathogens. In some of these illness scenarios, agonists of these pathways actually seem to be helpful. To achieve the desired therapeutic end-point, it is necessary to carefully choose the therapeutic target in the TLR signaling cascade and to precisely control the level of pathway activity. The TLR signaling axis emerges as yet another "double-edged sword" in biology, like many other molecules.

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CHAPTER 8

MOLECULAR COMMUNICATION PATHWAYS AND EMERGING TREATMENTS FOR MEDULLARY THYROID CARCINOMA

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ABSTRACT:

We now have a solid understanding of the genetic flaws and changed molecular pathways linked to the development of medullary thyroid carcinoma (MTC) after studying it for the past 55 years. Currently, patients with a high risk of developing MTC can be recognized and given preventative medication thanks to genetic testing. Surgery may be curative in cases of limited neck illness. Systemic therapy, however, might be required once MTC has progressed past the neck. Conventional chemotherapy has been proven ineffective; nevertheless, multichines inhibitors have demonstrated promise in stabilizing illness, and this year will likely see approval of a drug for developed unresectable or metastatic cancer, opening a new chapter in the history of MTC. The management of MTC may change as a result of the newly identified molecular routes and the most likely novel therapeutics we examine in this research. The parafollicular cell, which create the hormone calcitonin, are the source of the thyroid cancer known as medullary thyroid cancer. Medullary tumors account for roughly 3% of instances of thyroid cancer overall, making it the third most prevalent type of thyroid cancer. The MTC was initially described in 1959.

KEYWORDS:

Curative, Development, Limited, Management, Therapeutic.

INTRODUCTION

The parafollicular neurons of the thyroid gland are the source of the neuroendocrine tumor known as medullary thyroid cancer. In the United States, MTC accounts for a mere three percent of thyroid published the first characterization of its primary histological characteristics and classification as a distinct entity. When MTC was discovered to be associated with the tumors and other cancers, a relationship now known as multiple neuroendocrine neoplasia type 2 (MEN2), it was quickly realized that this malignancy had special clinical symptoms. The discovery of familial cases led researchers to the conclusion that a large number of MTCs were likely inherited. The parafollicular cells that secrete calcitonin were discovered to be the source Following that, preventive thyroidectomy was made available to anyone who tested positive for familial MTC using calcitonin provocative tests with magnesium and/or Penta gastrin.

In 1993, patients with family variants of MTC were shown to have activating mutation of the Reorganized during The transfer proto-oncogene Since then, nearly all hereditary MTCs have contained a number of germline RET proto-oncogene mutations. Furthermore, about 40% of individuals with sporadic MTC have somatic RET proto-oncogene alterations. The identification of RET proto-oncogene mutation carriers would allow the removal of thyroid

cells at risk for change early in life (this paradigm is arguably the best illustration of primary cancer prevention in humans to date), the discovery of several unrecognized familial medullary thyroid cancers, and the possibility that the abnormally activated RET gene could be a target for therapeutic intervention all resulted in new paradigms for the management of MTC. This article aims to clarify the molecular mechanisms linked to MTC carcinogenesis and cutting-edge treatments for this condition. Diarrhea is the main clinical sign of metastatic axial thyroid cancer; occasionally, patients will experience flushing episodes. Both often accompany liver metastasis, and any symptom can be the condition's initial sign. The flushing that results from medullary thyroid cancer is identical to the carcinoid syndrome-related flushing. In MTC, elevated levels of a hormone called product genes (calcitonin or calcitonin family-related peptide) are responsible for the flushing and diarrhea, whereas elevated levels of circulating serotonin are responsible for these symptoms in carcinoid syndrome.

An enlarged lymph node in the cervical area and a thyroid nodule can also result from medullary thyroid cancer. Local lymph nodes in the neck, lymph nodes in the mediastinum (centre of the chest), liver, lung, and bone are places where medullary thyroid cancer has disseminated. Though rare, spread to other areas including the skin or brain does happen. A mutant receptor tyrosine kinase protein known as RET is expressed when the RET proto-oncogene, situated on chromosome 10, experiences mutations (DNA alterations). Nearly all cases of hereditary or related medullary thyroid cancer are caused by germline mutations in the gene RET, which is important in the regulation of cell growth and development. Additionally, the development of excessive parathyroid hormone and pheochromocytoma may result from this germline mutation. Each child of an affected parent has a 50% chance of receiving the mutant RET proto-oncogene from the afflicted parent since hereditary medullary cancer of the thyroid is inherited as an autosomal dominant feature. When the entire thyroid gland is removed at a young age, before the tumor has spread, surgical excision of the thyroid in children who have the mutant gene is curative. DNA analysis makes it feasible to identify children who possess the mutant gene. When they produce clinical symptomatology, parathyroid tumor’s and pheochromocytomas are surgically removed. About 25% of medullary thyroid carcinomas are caused by multiple endocrine neoplasia type 2 (MEN2), also known as hereditary medullary thyroid carcinoma.

Medullary thyroid carcinoma accounts for 75% of cases and is classified as "sporadic" when it affects people with no known family history. When compared to people with a family history, those who acquire sporadic carcinoma of the medullary thyroid are typically older and have a more advanced disease when it first manifests (screening is probably started at a young age in the hereditary variant). A somatic mutation of the RET proto-oncogene (a mutation that takes place within a single "parafollicular" cell) is present in about 25–60% of sporadic thalamic thyroid carcinomas. The initial event is thought to be this mutation, but there may be other as-yet-unknown reasons. To identify the thyroid lesion from other types of thyroid lesions, the diagnosis is typically made through fine needle aspiration of the thyroid lesion. A microscopic examination will reveal an amyloid stroma with parafollicular cell hyperplasia.

The diagnostic efficacy of basal and provoked calcitonin for axial thyroid carcinoma was recently evaluated by a Cochrane comprehensive review. Despite the fact that basal and combined basal and stimulated calcitonin tests exhibited high accuracy from 97.2% and 100%), these results had a substantial risk of bias because the included studies' flawed

designs were to blame. Overall, it is still unclear and debatable how useful standard calcitonin testing is for determining the diagnosis and prognostic of medullary thyroid cancer. The most effective method of curing individuals with medullary thyroid cancer who do not have widespread nodal involvement or distant metastases is a complete thyroidectomy plus bilateral neck dissection. The risks of surgery include losing the ability to speak, permanent nerve damage, passing away, or needing a second procedure to remove any remaining malignant lymph nodes if the sentinel node biopsy revealed the presence of cancerous spread. When the problem is discovered early, extensive surgery can be successful, but there is still a chance of recurrence, especially in patients with numerous positive lymph nodes or extracapsular invasion. At the time of diagnosis, metastases to local lymph nodes are present in around half of the patients.

For treating this illness in gene carriers, the European Society of Endocrinology Surgeons has established management guidelines. The kind of mutation present determines when surgery should be performed. Surgery is advised during the first year of life for those in the highest risk group. Depending on the mutation and other circumstances, surgery in lower-risk patients may be postponed up to age 10 years. Clear evidence of response was seen in 10–30% of patients in clinical trials of protein kinase inhibitors, which block the aberrant kinase proteins involved in the genesis and growth of medullary cancer cells. Less than a 30% reduction in tumor mass has been seen in the majority of responders, but the responses have been long-lasting, remaining constant for more than three years. Hypertension, nausea, diarrhea, certain cardiac electrical abnormalities, and coagulant or bleeding episodes are some of the more common side effects of this class of medication.

DISCUSSION

RET proto-oncogene and MTC

The characteristic of all tumors, whether benign or malignant, is autonomous cell proliferation. Malignant tumors have the ability to spread to distant areas by invading nearby healthy tissue. Cancers commonly have mutations in molecules involved in cell development and other essential processes. The tyrosine Kinase receptors are an illustration of such molecules. The transfer of the nucleotide of adenosine-5′-triphosphate to the hydroxyl groups of target proteins' tyrosine's is catalyzed by the intracellular domains of receptors, which are membrane-spanning proteins with extensive N-terminal external domains that serve as ligand-binding sites. The RET proto-oncogene is located on chromosome regulate a wide range of fundamental cell functions, including the cell cycle, growth, angiogenesis, distinction, motility, apoptosis, and survival. The gene codes for the TK receptor and has the extracellular, transmembrane, and cytoplasmic domains make up the transmembrane protein known as the RET receptor. A region of about 100 amino acids in the extracellular domain resembles Ca2+-dependent cell adhesion molecules from the cadherin family. In order to interact with several members of the glial cell line-derived neurotrophic factor receptor calcium must bind to this cadherin-like domain.

These ligands activate RET in combination with the GFR 1-4 coreceptor, which is ligand-specific. For RET activation, such ligands or coreceptors are occasionally required. Particular residues of t are phosphorylated after RET activation. For adaptor proteins, which connect the receptor to the primary signal transduction pathways, these residues act as docking sites. Different pathways are activated by various active sites. For instance, protein kinase C, is

activated by phospholipase C when it binds to tyrosine 1015. Other examples include the phosphorylation of tyrosine 1062, which results in the recruitment of several adaptor or effector proteins like Shy, FRS2, Dok family protein molecules, insulin receptor substrate 2, and Enigma, as well as the phosphorylation of tyrosine 981, which is responsible for Sic stimulation upon RET engagement Then, a number of pathways, are activated that regulate cell survival, differentiation, proliferation, and chemotaxis [1]–[3].

Random MTC

Between 65% to 75% of MTC cases are sporadic. The clinical manifestation that occurs most frequently is a thyroid nodule. Nodal metastases can be seen in the central and contralateral neck compartments in up to 75% of individuals with palpable MTC, and in the contralateral neck in 47% of patients. The liver, lungs, and bones are frequently the site of distant metastases. In 30% to 40% of cases, somatic mutations take place. The most frequent somatic alteration in sporadic MTC is the exon 16, codon 918 ATG ACG mutation. Larger tumors and a later stage of the disease upon diagnosis are linked to this mutation [4]–[6].

inherited MTC

cases are hereditary C-cell hyperplasia precedes hereditary MTC, which is typically bilateral and multicentre. Germline RET proto-oncogene mutations are the root cause of hereditary types of MTC, which are linked to disorders. Nearly all pheochromocytomas, parathyroid tumors, and gene carriers of. Six cysteine residues (codons in the RET extracellular domain are the sites of the majority of mutations. Cysteine, where one-half of an intramolecular disulfide bond has been removed, allowing for the formation of the intermolecular disulfide bond to a second mutant molecule, leading to constitutive receptor dimerization, is the most frequently altered residue observed in individuals in pathways have been mentioned.

There are three types of the syndrome: familial, in which MTC is the main symptom; MEN2A with Hirschsprung disease; and coupled with cutaneous lichen amyloidosis. Both this extracellular cysteine-rich area and the domain are impacted by familial mutation. The genetic variation that is most commonly found is the least aggressive one. The most unique and severe MEN2 syndrome are the mutations linked to that occur most frequently. Contrary to these mutations affect the domain and result in an active monomeric form, which changes substrate specificity. In cell lines, it has been demonstrated that the PI3K/Akt cascade is significant in the pathophysiology.

Receptor for Epidermal Growth Factor

A TK receptor is the epidermal growth factor receptor It is one of four homologous transmembrane receptors that mediate the effects of several growth factors, including epidermal growth factor, transforming growth factor-, and neuregulin's. The others are the activation of the kinase domain, the creation of homo- and/or heterodimers, and the phosphorylation of a particular tyrosine residue serve as docking locations for molecules that activate a number of cascades, such as the Numerous factors, including excessive ligand or receptor expression, activating mutations, a failure of inactivation, or transactivation via receptor dimerization, can lead to oncogenic activation. Monoclonal antibody therapy and small-molecule ATP-competitive TK inhibitors are the two main categories of therapies that are now available Despite having no effect on kinase activity, the strong kinase inhibitor also reduces autophosphorylation and signaling in cell extracts. Patients with among those who

participated in clinical trials testing PKI166. The development of this medicine, however, was put on hold because of liver toxicity. At concentrations below its half maximal inhibitory concentration kinase inhibitor, prevents RET-induced growth. AEE788 does not, however, currently have any MTC patient clinical trials underway. Although mutations were uncommon, a study of 153 primaries and metastatic MTC samples found that expression was higher in metastatic regions than in primary tumor sites. Comparatively to samples associated with other mutations, those with mutations tended to have significantly less polysomic and a tendency toward decreased EGFR immunopositivity. Since the most severe RET mutations are thought to be less dependent on EGFR activation, this may explain [7]–[9].

the growth factor for blood vessels

Through a variety of TK receptors, including the vascular endothelial growth factor (VEGF) family of growth factors promotes angiogenesis, endothelial cell proliferation, migration, survival, and vascular permeability Angiogenesis is crucial for the growth and metastasis of tumors and is one of the critical abnormalities factor, interleukin-8, and growth factor derived from platelets (PDGF), have been suggested to operate as favorable regulators of angiogenesis. VEGF, which primarily communicates through, is a key modulator of tumor angiogenesis. A number of distinct pathways, including are activated when this receptor is activated. Since lymphatic vessels develop from blood vessels, some angiogenic mechanisms are also used in the process of lymph angiogenesis, which is a component of tumor biology. Angiogenesis and lymph angiogenesis are both stimulated by which also link the two processes. The primary function which is mostly expressed in lymphatic endothelial cells, is assumed to be lymph angiogenesis. are expressed at least two times more in MTC than in normal thyroid tissue. Additionally, the expression of is up to 20 times higher in metastatic overexpression and activation are correlated with metastasis

c-MET

The TK receptor of the hepatocyte growth factor is encoded by the c-met proto-oncogene [46]. MET has a significant role in carcinogenesis. Unrestricted proliferative, antiapoptotic, cell motility/migration, invasive, metastatic, and angiogenetic capabilities are conferred to cancer cells by deregulated MET activation. It has been demonstrated that inhibiting the endogenous MET proto-oncogene, which is overexpressed in tumor cells, can reduce metastasis formation in vivo, increase the regression of pre-existing metastases, and inhibit invasive development in vitro. A subgroup of MTC tumors have been found to coopers MET and hepatocyte growth factor, which is linked to multifocality in MTC.

Targeted Treatment

In MTC cells, various TKs and routes are inappropriately active. Only one receptor being blocked may result in the compensatory activation of other optimum strategy for treating MTC may involve concurrent inhibition of various active To date, systemic targeted therapy for MTC has either been given as part of clinical studies or has involved using medicines that have already been approved for use in other solid tumors off-label. The most effective TK inhibitors against MTC are discussed in this part. venetian suppresses tumor cell survival, proliferation, and angiogenesis at pharmacologically relevant levels without directly harming tumor or endothelial cells. Venetian was demonstrated to reduce oncoprotein kinase activity It was also demonstrated to suppress phosphorylation and activation in vivo in NIH-. A panel

of point mutations affecting the RET kinase domain in and sporadic MTC were tested for ventail sensitivity two years later. venetian was quite effective against the majority of the mutant however the RET kinase became noticeably resistant when valine 804 was changed to leucine or methionine (as is the case in some cases The Val804Gly mutation boosted RET's sensitivity to Vandana, which suggests that steric hindrance is to blame Venetian treatment inhibited tumor growth in mice with other kinase inhibition also appears to be crucial. More EGFR and VEGFR-2 are expressed at MTC metastases than at the original tumor location. Venetian has been reported to suppress phosphorylation of cells. However, neither is significantly involved in the proliferation of TT cells when RET is activated.

However, excessive stimulation can partially replace when function is inhibited by partially rescuing the pathway. In this case, it has been demonstrated that vandetanib's suppression prevents the pathway from being rescued. These findings lend credence to the concept that combined inhibition of is crucial because it may prevent overstimulation Venetian doses up to 300 mg/day were well tolerated in phase I clinical studies of patients with solid tumors and adverse effects were often modest and managed with either dose modifications or symptomatic medication. Rash, diarrhea, tiredness, asymptomatic QTc prolongation, proteinuria, and hypertension were the most frequent side reactions. Patients should obtain an EKG and electrolytes at baseline and at frequent intervals throughout the course of treatment because QT prolongation was noted as an adverse event [10]–[12].

Sorafenib

the kinases serine/threonine are all targets of the tiny TKI sorafenib. By focusing on both RET-dependent thyroid proliferation of cancer cells and VEGF-dependent tumor angiogenesis, it suppresses the growth of RET-driven malignancies. Sorafenib reduces RET signaling as well as the development of RET-transfected fibroblasts and human thyroid cancer cells that express the oncogenes in vitro. The primary mechanism of sorafenib action is cryostasis, however the medication also has a proapoptotic impact. Sorafenib has been demonstrated to dramatically slow the formation of xenograft tumors made from MTC cell lines in nude mice. Four phase I trials with various dosages and administration regimens have been conducted on sorafenib. The safest and most commonly reported drug-related side effects were fatigue, anorexia, diarrhea, rash/desquamation, and hand-foot phenomenon at a dosage of 400 mg orally twice day. Painful erythematous lesions that afflict the palm-plantar surface are the hallmark of hand-foot syndrome. It is the most typical side effect that individuals using multichines inhibitors like sorafenib and sunitinib report experiencing. The lesions are most noticeable on the pressure points on the palms and soles, but they can also affect the skin between the fingers and toes and the edges of the feet. Although not life-threatening, these lesions considerably lower quality of life and necessitate lowering dosages or even stopping the medication. Severe toxicities to the hematology, cardiovascular, hepatic, and renal systems were not documented. In all four phase I trials, 5% to 11% of patients had treatment-related hypertension. By causing disease stabilization in patients with resistant malignancies, sorafenib revealed evidence of anticancer activity, which was similar with the findings of preclinical investigations.

The phase I study excluded any thyroid cancer patients. MTC was identified as a potential target for sorafenib due to the function of RET signaling in MTC and the anticancer efficacy displayed by sorafenib in preclinical and in vitro investigations. In a modest 2007 pilot

research, calcitonin secretion was decreased by >50% in all patients after 3 months of treatment, and all patients were free of calcitonin-related symptoms. The study had five patients with metastatic MTC and high calcitonin secretion. One patient achieved a complete response (CR) and another had a partial response (PR) after 6 months of therapy [67]. In a larger, open-label phase II research, sorafenib was given orally at a dose of 400 mg twice daily continuously to patients with histologically proven metastatic or locally advanced MTC. Patients were routinely observed by physical examinations, metabolic testing, and radiologic evaluations. The medicine was stopped and reintroduced at a lower dose of 400 to 600 mg/day with dose re-escalation as tolerated in the event of any major drug-related adverse event. The average length of sorafenib treatment was 15 months. By using RECIST version 1.0, ORR was evaluated. In this trial, all 15 evaluable patients displayed tumor reduction to some extent. One patient had a PR; 14 had SD, eight of whom had SD that lasted less than 15 months; and one had a condition that was clinically progressing. The majority of patients saw lower calcitonin levels 2 months into treatment, although they were unrelated to the intensity or persistence of the response as determined. The FDA has approved sorafenib for the treatment of hepatocellular and renal cell carcinoma. Sorafenib is a choice for advanced MTC patients who are not qualified for clinical trials.

CONCLUSION

Over the past 55 years, research on MTC has improved our understanding of the genetic flaws and changed molecular pathways involved in its genesis. Promising targeted therapeutics have since been created for MTC that is both progressing and advanced. A medication for advanced unresectable or metastatic MTC is likely to receive approval this year, opening a new chapter in the illness's history and building on the success of multichines inhibitors in stabilizing the disease. The goal for the upcoming years will be to identify the people who will benefit most and to find more efficient approaches to target several important disease pathways. Sorafenib has been tested in four phase I trials using a range of doses and administration techniques. Fatigue, anorexia, diarrhea, rash/desquamation, and hand-foot phenomena were the safest and most often reported drug-related adverse effects at a dosage of 400 mg orally twice day. Hand-foot syndrome is characterized by painful erythematous lesions that affect the palm-plantar surface. It is the adverse reaction that users of multichines inhibitors like sorafenib and sunitinib most frequently report having. The lesions can affect the skin between the fingers and toes as well as the borders of the feet, but they are most evident on the pressure points on the palms and soles.

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CHAPTER 9

CANCER TREATMENT INVOLVES FOCUSING ON SPECIFIC SIGNALING PATHWAYS IN TUMOR STEM CELLS

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ABSTRACT:
Its Hedgehog, and Zipper signaling pathways are essential for healthy hemostasis, development, and embryogenesis. However, a variety of tumor forms and malignancies show dysfunctions of these pathways. The modulation of malignant stem cells (CSCs), a small population of malignant cells capable of dividing and self-renewing into diverse tumor cells, is specifically linked to atypical activation of these pathways. Tumor start, growth, and recurrence are all caused by CSCs. In this review, we concentrate on the functions of Want, Hedgehog, and Zipper pathways in the stemness and activities of CSCs and examine therapeutic treatments that target these pathways to eradicate CSCs and enhance the effectiveness of cancer treatment in general. Stem cells are undeveloped or partially differentiated organisms that can specialize into different types of cells and multiply forever to create more of the same stem cell in multicellular creatures. They are the ancestor of all cell types in a lineage. Although they are present in both embryo and adult organisms, they differ slightly in each. They are typically distinguished from progenitor or blast cells, which are typically dedicated to developing into one cell type, and progenitor cells, they cannot divide endlessly.

KEYWORDS:
Activation, Developed, Embryogenesis, Multicolour.

INTRODUCTION

The initiation, development, and recurrence of tumors have all been attributed to cancer stem cells (CSCs), a tiny subset of malignant cells with the ability to self-renew and differentiate into diverse tumor cells. When tested in an in vivo animal model for tumorigenesis, the first group of CSCs was found in human AML patients. A number of human malignancies, including brain cancer, melanoma and cancer of the breast, liver cancer, cancer of the pancreas, colon cancer, and prostate cancer, have since been successfully isolated and propagated in numerous laboratories across the world. Eliminating CSCs from tumors may be a successful anticancer treatment approach because they can resist conventional cancer therapies, cause tumor recurrence, and lead to drug resistance. Significant attempts have been made to investigate the signaling processes behind CSCs' self-renewal and differentiation as well as to design regimens that specifically target CSCs in order to achieve this goal. In this review, we concentrate on three important, evolutionary conserved CSC signaling routes (want, Hedgehog, and Piezo pathways) and treatment approaches that affect these pathways to disturb the stemness and activities of CSCs.

In mammals, the inner cell mass is composed of approximately 50–150 cells and occurs during days 5–14 of the blastocyst phase of embryonic development. These can function as

stem cells. They eventually differentiate in vivo into every type of cell in the body, making them pluripotent. At the gastrulation stage, the three germ layers—the ectoderm, mesoderm, and endoderm—diverge, marking the beginning of this process. However, they can be maintained in the stem-cell stage when identified and cultured in vitro, at which point they are referred to as embryonic stem cells (ESCs). In the body, there are just a few habitats where adult stem cells can be discovered. Examples of these niches include the gonads and bone marrow. They are multipotent or unipotent, which means they only differentiate into a small number of cell types or one type of cell, and they exist to quickly replace lost cell types. In animals, they include, among others, mesenchymal stem cells, which sustain bone, cartilage, muscle, and fat cells, basal cells, which maintain the skin the epithelium and hematopoietic stem cells, which replace blood and immune cells. The progenitor cells and cells with terminal differentiation that adult stem cells differentiate into considerably exceed adult stem cells, which are a tiny percentage of cells.

Canadian researchers Ernest McCulloch, James Till, and Andrew J. Becker made discoveries about stem cells in the 1960s at the University of Ontario and the Ontario Cancer Institute. As of 2016, only hematopoietic stem cell transplantation—first carried out in 1958 by French oncologist Georges Matha—is the only recognized stem cell-based medical therapy. Human embryonic stem cells can now be differentiated and cultured (in stem-cell lines) since 1998. Because separating these cells usually necessitates the killing of the embryo, the procedure has generated controversy. Some European nations, including Canada, have banned the use of sources for isolating ESCs, but other nations, including the UK and China, have encouraged the research. An embryo can be cloned via the somatic cell nuclear transfer technique so that its embryonic stem cells can be used in stem cell treatment. In 2006, a Japanese research team under the direction of Shinya Yamanaka found a way to turn mature body cells back into stem cells. Induced pluripotent stem cells (iPSCs) are what these were. Theodor Boveri and Valentin Haecker were the first to use the phrase "stem cell" in the late 19th century. At the beginning of the 20th century, Artur Pappenheim, Aleksandr Maximo, and Franz Ernst Christian Neumann carried out groundbreaking studies on the theory of blood stem cells.

Ernest McCulloch and James till at the College of Toronto and the Ontario Cancer Institute in the early 1960s were the first to identify the fundamental characteristics of a stem cell. Through their groundbreaking study in mice, they found the hematopoietic stem cell (HSC), which produces blood. In the first of their studies, McCulloch and Till injected bone marrow cells into radio treated mice. They noticed tumors in the mice's spleens that were linearly correlated with the dosage of bone marrow cells. They proposed that each lump (colony) originated from a single stem cell in the marrow. Later research by McCulloch, Till, senior scientist Louis Simonovic, graduate student Andrew John Becker, and the group established that each lump did in fact originate from a single cell. In 1963, Nature reported their findings. Simonovic served as the principal investigator for research that revealed colony-forming cells can self-renew, confirming Till and McCulloch's hypothesis that this is a crucial characteristic of stem cells.

The first stem cell treatment was a bone marrow transplant conducted in 1958 by French oncologist Georges Matha on five victims of a criticality accident at the Vina Nuclear Institute in Yugoslavia. Every worker made it out alive. British biologists Martin Evans and Matthew Kaufman successfully extracted and grew the first embryonic stem (ES) cells in

1981 using mouse blastocysts. This made it possible to create murine genetic models, a system in which mouse genes are eliminated or changed in order to research their role in pathology. After James Thomson, an American researcher, successfully extracted human embryonic stem cells in 1998, it became possible to develop new transplantation techniques or other cell types for the evaluation of novel therapeutic approaches. By changing the expression of just four genes, Shinya Yamanaka's team in Kyoto, Japan, transformed fibroblasts into pluripotent stem cells in 2006. The accomplishment serves as the precursor to induced pluripotent stem cells, or iPS cells.

The Zoo Brasile treated a female maned wolf who had been struck by a truck in 2011, marking the first instance in which stem cells were used to treat wounds in a wild animal. In actuality, stem cells are recognized based on their capacity to rebuild tissue. For instance, the capacity to transplant the cells and save a person lacking hematopoietic stem cells (HSCs) is the defining test for bone marrow or HSCs. This shows that the cells can continue to make new blood cells throughout time. Additionally, it should be possible to separate stem cells from the transplanted person, which can then be used to transplant them into a recipient who lacks HSCs, proving that the stem cells were able to self-renew.

Through the use of techniques like clonogenic assays, which evaluate a single cell's capacity for self-renewal and differentiation, characteristics of stem cells can be demonstrated in vitro. By having a unique set of cell surface markers, stem cells can also be distinguished from other types of cells. However, the settings of in vitro cultivation might change how cells behave, making it uncertain whether the cells will behave similarly in vivo. The validity of several suggested adult cell types as stem cells is hotly contested. At some point, the neural stem cells undergo a transformation into radial glial progenitor cells (RGPs). RGPs that have just begun to form self-renew by symmetrical dividing to create a pool of progenitor cells. These cells enter a neurogenic stage and begin to divide asymmetrically, giving rise to a wide variety of distinct neuron types, each with its own specific gene expression patterns, morphological features, and functional traits. Neurogenesis is the process of producing neurons from radial glial cells. The bipolar shape of the radial glial cell, which spans the thickness of the neural tube wall, is unusual. Some of its properties are similar to those of glia, most notably the expression of glial fibrillary acidic protein (GFAP). The initial neural stem cell of the growing vertebrate CNS is called a radial glial cell, and it is found in the ventricular zone right next to the ventricular system. Due to their commitment to the neuronal lineages (neurons, astrocytes, and oligodendrocytes), neural stem cells have limited potential.

DISCUSSION

CSC Signaling Pathways

Several CSC models, including the traditional CSC unidirectional differentiation model and the plastic CSC bidirectional dedifferentiation model, have been presented in the past for tumor heterogeneity. However, in the plastic CSC bidirectional dedifferentiation model, non-CSC tumor cells can undergo a dedifferentiation process and acquire CSC-like properties, most likely through epithelial-mesenchymal transition (EMT) in carcinoma. In the classical CSC unidirectional differentiation model, CSCs differentiate to non-CSC tumor cells that are unable to move back up the hierarchy to acquire CSC-like activity. Hedgehog, and Notch pathways are nevertheless regarded as significant CSC regulators in both CSC models [1]–[3].

Canonical Signaling Pathway

A crucial and evolutionarily conserved mechanism in tissue homeostasis and embryonic development is the canonical pathway, in which ligands signal through -catenin. A "destruction complex" made up of axing, adenomatous polyposis coli (APC), glycogen synthase kinase 3 (GSK3), and casein kinase phosphorylates the cytoplasmic -catenin for proteasome-dependent degradation in the absence of ligands. However, when want ligands are present, they bind to the single-membrane-spanning low-density receptor-related protein 5/6 (LRP5/6) and the seven-transmembrane receptor Frizzled activating the signaling. Following FZD's recruitment of the intracellular protein Axim and GSK3 are then sequestered to the cellular membrane, leading to the breakdown of the "destruction complex. As a result, active, unphosphorylated -catenin builds up and moves into the nucleus to control the expression of target genes [4]–[6].

CSCs and abnormal signaling activation

Numerous CSC kinds, including colorectal cancer, breast cancer, hematologic cancer, skin cancer, and lung cancer, have been linked to abnormal signaling activation. For instance, mutations in the APC and -catenin genes are frequently linked to signaling dysregulation in colorectal cancer. Around 80% of all human colon cancers have APC mutations, which render APC ineffective and stimulate want signaling by blocking -catenin phosphorylation and subsequent -catenin degradation Additionally, missense or deletion mutations at -catenin sites where GSK3 normally phosphorylates -catenin, leading to stable -catenin translocation into the nucleus for activation, have been reported in about 10% of colorectal cancer patients By upregulating the expression of target genes including c-mice and cyclin D, abnormal signaling disturbs the regular development and differentiation of colonic crypt stem cells, leading to a colorectal CSC phenotype Furthermore, the pathway was found to be strongly related with colorectal CSCs in a recent comparison study of signaling pathways between colorectal cancer cells.

signaling is also involved in CSCs from other cancers including colorectal cancer. For instance, canonical want signaling activation was demonstrated to be crucial in the carcinogenesis of CD34+ bulge CSCs in a beautiful study of squamous cell carcinomas, and ablation of the -catenin gene led to the depletion of complete tumor regression in mice. Additionally, tumor cells were unable to develop additional tumors because they lacked -catenin, but tamoxifen-induced production of a nondegradable -catenin in the skin adequately increased the population of bulge CSCs Furthermore, according to the plastic CSC bidirectional paradigm, dedifferentiation via EMT is a necessary step for non-CSC tumor cells to gain CSC-like characteristics. won't signaling is crucial in the dedifferentiation of cancer cells. According to one study, experimental CD146 knockdown can cause colorectal cancer cells to dedifferentiate and adopt a stem cell phenotype by decreasing GSK-3, which in turn increased -catenin nuclear translocation for activation of won't signaling. In order to stop colorectal cancer, and more especially colorectal cancer stem cells, won't signaling may need to be altered.

Medicines that target won't signaling

Targeting CSCs by blocking won't signaling may be a feasible treatment strategy for cancer given that won't signaling activation is linked in CSC self-renewal, carcinogenesis, and

cancer cell dedifferentiation into CSCs. Recently, a wide range of biological and small chemical won't signaling inhibitors have been created. However, no won't signaling inhibitors have been given clinical usage approval as of yet. The readers may turn to our most recent review publication in this field for information on the majority of inhibitors that have undergone preclinical evaluation. Here is a summary of active inhibitor clinical trials. For instance, PRI-724 is a won't inhibitor that is now being tested in humans. It blocks won't signaling by attaching to the downstream CREB-binding protein. In mice colon cancer xenograft models, PRI-724 has previously been demonstrated to induce apoptosis in colon carcinoma cells and to have anticancer efficacy. 18 patients were treated in the phase I trial, and their toxicity profiles were satisfactory. Only one dose-limiting event, grade 3 reversible hyperbilirubinemia, was present. A phase II PRI-724 experiment involving mFOLFOX6/Bevacizumab with or without PRI-724 is about to start. The study's target population is people with metastatic Stage IV colorectal cancer NCT Number Additionally, Porcupine is a membrane-bound O-acyltransferase (MBOAT) that is unique to went posttranslational acylation and necessary for future want secretion; Porcupine deficiency can decrease won't signaling.

Furthermore, a specific small molecular Porcupine inhibitor, was discovered through luciferase-based cell screening and was successful in inhibiting went signaling in a variety of tumor models, including murine and rat mechanistic breast cancer models and a human head and neck squamous cell carcinoma model. A phase I, open label dose escalation trial of the LGK974 was recently started to treat a number of cancers, including melanoma, breast cancer, and pancreatic adenocarcinoma. A number of biologic therapeutic drugs that target the want pathway have also entered clinical trials in addition to small molecules. For instance, an open label phase 1 dosage escalation study for solid tumors using a completely humanized monoclonal antibody that targets the FZD receptor, was just finished 54F28, a fusion protein that binds want ligands and blocks them from interacting to FZD receptors, is a new biologic therapeutic treatment. Patients with solid tumors received intravenous minimum dosages of 0.5 mg/kg, ranging up to 10 mg/kg, once every three weeks in a phase Despite the fact that this clinical research was just finished, the outcomes have not yet been made public and the chemotherapeutic medication Paclitaxel are also being tested in two more phase 1b trials for the treatment of Stage IV pancreatic cancer and ovarian cancer, respectively NCT Numbers [7], [8].

Hedgehog Signaling

Throughout embryogenesis, development, and adult tissue homeostasis, HH signaling plays a crucial role in a wide range of cellular and molecular activities. Sonic Hedgehog and Desert Hedgehog are three hedgehog homologues that have been extensively researched in mammals. The transmembrane protein Smoothened (SMO) is inhibited by the cell-surface protein Patched (PTCH) in the absence of HH ligands, and full-length GLI proteins are subsequently processed through proteolysis to create the repressor to stop the expression of HH signaling target genes. However, PTCH's inhibitory effect on SMO is abolished when extracellular HH ligands attach to it. As a result, SMO activation leads to nuclear translocation of GLI and promotion of HH signaling target gene transcription.

Unusual CSCs and HH Signaling Activation

Numerous cancer forms, including glioblastoma, lung squamous cell carcinoma, breast cancer, pancreatic adenocarcinoma, myeloma, and chronic myeloid leukemia (CML), have been linked to aberrant activation of the HH pathway in the regulation and maintenance of CSCs SMO and Gli1 were shown to be strongly expressed in multiple myeloma CSCs in comparison to non-CSCs. HH signaling activation by HH ligands increased the proliferation of multiple myeloma CSCs, whereas inhibition of HH signaling significantly inhibited CSC clonal expansion. The evidence suggests that HH signaling supports the activities of multiple myeloma CSC. Activation of the HH route was also involved in the maintenance of CML CSCs, according to a different study by Zhao et al. In a study using a mouse model of CML, deletion of SMO greatly decreased the CML CSCs, but overexpression of SMO in a mouse model of CML without SMO significantly boosted CML CSCs and exacerbated CML progression. Additionally, the SMO antagonist cyclosporine reduced the population of glioblastoma stem cells by inhibiting HH signaling and comparable results have been noted for colon CSCs, pancreatic CSCs, prostate CSCs [9]–[11].

In the plastic CSC bidirectional dedifferentiation paradigm, HH signaling is crucial for acquiring stem cell-like characteristics during the EMT process. For instance, it has been demonstrated that Gli1 correlates with EMT markers and is strongly expressed in claudin-low breast CSCs. Gli1 knockdown reduced the viability, motility, clonogenicity, and self-renewal of claudin-low breast CSCs as well as tumor growth in orthotopic xenografts. Recently, Wang et al. showed that the HH pathway and EMT are active in tumor spheres produced from pancreatic cancer cells that have CSC features. SMO knockdown inhibits HH signaling, which in turn prevents pancreatic CSCs from self-renewing, EMT, invading other tissues, developing chemo resistance, and causing tumors.

Therapeutic Substances That Aim at HH Signaling

As seen above, HH signaling is essential for the control and self-renewal of CSCs, and blocking the HH pathway causes CSC differentiation that is advantageous for the treatment of cancer. There have been many HH pathway inhibitors created in the past. Please refer to the most current review for a thorough analysis of HH signaling inhibitors. Only HH signaling inhibitors that have received FDA approval or are undergoing clinical studies are listed here Genentech's Isomeric is the first HH signaling inhibitor to receive FDA approval. Isomeric has been utilized to treat metastatic basal cell carcinoma and targets SMO for the suppression of HH signaling. In its original phase I trial, Isomeric shown activity in 18 of 33 patients with locally progressed or metastatic malignancies. The remaining 15 patients had progressing disease in 4 and 11 had stable disease for up to 10.8 months. No grade 5 adverse events or dose-limiting toxicities were observed during the trial, and the reported toxicities were minor, with mild taste loss, hair loss, weight loss, and hyponatremia among the more typical side effects.

For various cancers, including medulloblastoma, small cell lung cancer, metastatic pancreatic cancer, metastatic prostate cancer, intracranial meningioma, recurrent glioblastoma, and acute myeloid leukemia, clinical trials of Isomeric as a monotherapy or in combination with other therapeutic drugs are currently ongoing Sondages, a brand-new SMO inhibitor, was authorized by the FDA in 2015 for the treatment of adult patients with locally advanced BCC. Maximum tolerable dosages of 800 mg daily and 250 mg twice daily were achieved in the dose escalation phase I experiment. Grade 3/4 adverse effects included weight loss,

hyperbilirubinemia, myalgia, exhaustion, and dizziness. Grade 1/2 adverse effects included nausea, anorexia, vomiting, muscular spasms, weariness, and baldness. After a 12-month follow-up, phase II research found that Sondages maintained tumor responses in individuals with advanced BCC. Sondages is still being tested in many phase I/II trials for the treatment of different solid tumors and hematological malignancies Additionally, several SMO inhibitors and a GLI Inhibitor (arsenic trioxide) are being explored in clinical trials as HH signaling inhibitors. A monoclonal antibody called 5E1 also inhibits HH signaling by blocking the binding of all three mammalian HH ligands to PTCH however, clinical trials for this antibody have not yet begun.

CONCLUSION

Complex signaling systems are known to support tissue homeostasis and the cellular variety of stem cells throughout embryogenesis, and they may be crucial to the biology of cancer and CSCs. Significant work has been done in recent years to create combination medicines that target several signaling pathways to treat cancer. For instance, a recent study found that blocking both Notch and HH signaling in combination reduced the number of CSC subpopulation cell in a cancer of the prostate model. Additionally, a combo clinical trial Since CSCs were originally discovered in leukemia, their significant contributions to cancer development, metastasis, recurrence, and therapy resistance have come to light. Getting rid of CSCs by focusing on the major signaling pathways that underlie their stemness and function provides a viable strategy for fighting cancer. In this study, we primarily outlined the three important and evolutionarily relevant signaling in CSCs as well as prospective cancer therapeutics that could target these pathways. Several drugs have been created to date that particularly target each of these pathways for the treatment of cancer. However, it has been acknowledged that the signaling channels may collaborate with one another to control CSC stemness and functions. Therefore, for the creation of CSC-targeting therapeutics, understanding the interaction between pathways of signaling in CSC regulation is essential.

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CHAPTER 10

IDENTIFYING KEY SIGNALLING PATHWAYS IN PROSTATE CANCER DEVELOPMENT

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ABSTRACT:
Males are most commonly diagnosed with prostate cancer one of the most common cancers. Castration-resistant, which has a poor prognosis for patients, is caused by a number of mutations in prostate epithelial cells that are usually linked to changes in development, such as enhanced susceptibility to apoptotic death, constitutive growth, and, in some cases, differentiation into a testosterone deprivation-resistant phenotype. In this review, we discuss recent research on the key signaling pathway deregulations that result in development. By directly altering the relevant cascade and, in certain instances, by deregulating a cross-talk node or intersection along the pathways, important mutations in specific pathway molecules are frequently associated with a higher prevalence Along with potential therapeutic approaches that target these signaling pathways, we also examine probable environmental and nonenvironmental inducers of these mutations. It will be easier to create new treatments and preventative measures for this condition if we have a greater awareness of how some risk factors cause deregulation of various signaling pathways as well as how these deregulated systems affect the growth.

KEYWORDS:
Castration, Growth, Instances, Pathway.

INTRODUCTION

Understanding the molecular pathways behind carcinogenesis is becoming more important due to the long-term failure of existing prostate cancer treatments. PCa is currently thought to be the most prevalent non-melanoma neoplasia in men. there will be more than 1.7 million new instances of Pica, based on current population growth rates. The number of males who may have this illness in the US is close to 2.8 million, and in 2012, over 240,000 new instances were identified. PCa is more common in Western nations where the median life expectancy is over 75 years old, and it primarily affects older men. PCa has lately overtaken breast cancer as the most prevalent tumor malignancy in developing nations like Brazil, where it now accounts for almost 50,000 new cases annually. However, there is a significant disparity in mortality rates and incidence between various nations, which is likely a result of the uneven penetration of some risk factors like age, race, genetics (family history), diet, and environmental factors as well as behavioral factors like frequent consumption of dairy goods and meat smoking, and sexual activity.

The likelihood of developing PCa has been linked to a number of factors, including nutrition, lifestyle choices, and exposure to chemical agents. For instance, a comprehensive investigation conducted by a PCa prevention trial group (Seattle, USA) discovered strong associations between consumption of polyunsaturated fat and the emergence of aggressive

PCa. This study's findings are supported by a significant association between obesity and invasive PCa development among African and Caucasian men (above 50%). For example, PCa is more frequently associated with higher socioeconomic levels in Brazil. Sedentarism, an increase in animal fat consumption, and a decrease in fiber consumption have all been linked to greater risks of PCa progression along with other cancer types. Consuming fat appears to be a significant risk factor for PCa, thus. Since the late 1990s, there has been intense discussion over the connection between pesticide exposure and hormone-related malignancies, such as PCa. On the other hand, numerous studies have found an inverse relationship between a mild amount of sun exposure and a higher risk of mortality or PCa incidence. The precise causes of a prospective PCa induction, meanwhile, are still not well understood.

It can take up to 4 to 10 years for a prostatic tumor in a man to develop to a size of 0.4 inches. The semen-secreting prostate gland cells transform into tumor cells and begin to proliferate at greater mitotic levels, which is how PCa develops. The prostate gland's periphery initially experiences tumor formation as a result of the prostate cells' initial proliferative phase. The seminal vesicles, rectum, bladder, and urethra are only a few of the adjacent organs that these cancer cells eventually multiply to further penetrate. Malignant cells from the main tumor separate from their original site and travel through blood and lymphatic arteries in the early stages of metastatic disease. Cancer cells eventually expand to further-reaching organs in the latter stages, including the bones, liver, and lung.

Due to the close organ localization, surgery and/or radiotherapy have been the main modes of PCa treatment. An favorable prognosis with a minimal incidence of PCa-related death following surgery can be expected with a prostatectomy. However, aberrant androgen signaling pathway mutations, angiogenesis, local migration, invasion, intravasation, circulation, and extravasation of the tumor, as well as deregulated production and secretion of growth factors by cells that reside within the pica microenvironment, may result in systemic recurrence of the cancer, including the development of a focal tumor in an advanced stage. The optimum course of action in this situation is androgen-deprivation therapy (ADT), which typically includes luteinizing hormone-releasing hormone. Despite its transient effectiveness (often lasting between 18 and 24 months), ADT continues to be the most effective therapy in the early stages of advanced PCa.

Numerous cell lines that replicate both androgen-dependent and androgen-independent carcinogenic kinds have been widely used to study These cell lines have allowed researchers to assess the genomic underpinnings as well as to further elucidate the biological characteristics involved in cancer development, as well as to directly test a number of antitumor drug candidates, such as tumor apoptosis inducers or enhancers of antitumor immune response. Several animal models have been created in addition to in vitro studies in order to validate in vitro findings using a more therapeutically applicable methodology. Mouse models for PCa can be created using xenografts, doxycycline-based inducible systems to overexpress certain target genes, such as AKT, which in turn triggers tumorigenesis or systemic induction of gene mutations.

Pica induction may be caused by a range of genetic changes, whereas mutations in genes involved in the expression of proteins involved in a number of cell signaling systems might influence the choice between cell death and survival. In this overview, we'll talk about some

potential preventative measures for as well as the function that key cellular signaling pathways play in the disease's evolution. The proteins that transmit signals into a cell through cell surface receptors are the first step in the signaling pathways, a collection of signal transduction pathways. A combination of the terms Wingless and Int-1 led to the creation of the name signaling pathways either rely on same-cell or adjacent cell-cell contact (paracrine or autocrine). Since they have undergone significant evolutionary conservation in animals, they are shared by all animal species, including humans and fruit flies.

The canonical route, the noncanonical planar polarity of cells pathway, or the noncanonical calcium pathway are the three signaling pathways that have been defined. When a protein ligand binds to a Frizzled family receptor, the signal is transmitted to the disheveled protein inside the cell, activating all three pathways. The gene is hypothesized to have a role in the negative regulation of the canonical Want pathway, which controls the transcription of genes. The non-canonical planar cell polarity pathway controls the cytoskeleton, which gives cells their shape. The non-canonical Want/calcium pathway controls the cell's calcium levels.

signaling was originally discovered to play a part in the development of cancer, and then of the embryo. The body axis patterning, cell fate specification, cell proliferation, and cell migration are among the embryonic activities it regulates. These mechanisms are essential for the correct development of critical tissues like bone, the heart, and the muscle. When genetic abnormalities in pathway proteins resulted in malformed fruit fly embryos, its function in embryonic development was discovered. Later studies discovered that the genes in charge of these anomalies also affected the mouse model of breast cancer. Adult bone marrow, skin, and gut tissue regeneration are likewise under the influence of Want signaling. The mutations that cause a number of diseases, such as type II diabetes, glioma, breast and prostate cancer, and others, have shown the clinical significance of this pathway. Recently, scientists reported the first successful application of went pathway inhibitors in disease-modeling mice.

DISCUSSION

Route Description

The androgen receptor (AR) signaling pathway encourages the differentiation of epidermal cells into male urogenital tissues and encodes proteins required for spermatogenesis as well as the appropriate operation of the prostate. Like many other steroid-hormone receptors, AR is a nuclear receptor that functions as a transcription factor. It is composed of four unique functional domains. The first region consists of a constitutively active N-terminal domain with a transcriptional activation function which is carried out by two transcriptional activating units The second section is a heavily conserved binding domain which is in charge of DNA binding specificity as well as making it easier for the complex to dimerize and stabilize. Another receptor region, the -terminal ligand-binding domain (LBD), is moderately conserved and crucial for mediating the binding to steroids, which is the fundamental function of the AR signaling pathway.

The chaperone complex (Hsp90), which maintains the receptor in an inactive state yet in a spatial conformation that permits affinity for androgens, is directly bound to the AR at this position as well. After this complex bind to androgens, Hsp separates it and releases AR, which continues to dimerize and then translocate to the nucleus. The hinge region, a brief amino-acid sequence that divides LBD from DBD and includes a nuclear localization signal

(NLS), is found in a fourth AR region. Through its association with the cytoskeletal protein Filamin- whose cytoplasmic distribution is connected with metastatic or hormone-refractory phenotype, this area is also crucial for the AR translocation to the nucleus [1]–[3].

PCa-Related Pathway Disruptions and Therapeutic Targets

AR overexpression, which can be linked to gene amplification, transcriptional and/or translational upregulation, and reduced degradation, is one of the main causes of CRPCa. The most frequent genetic mutation in Croc patients is AR gene amplification, which is seen in about 80% of these cases. But gene amplification alone cannot fully account for AR overexpression, and various other mechanisms that support this improvement have been studied. Through the binding of the AR-ligand complex to DNA, specifically to the androgen receptor binding sites (ARBSs) or testosterone-responsive elements (AREs), AR regulates a large number of genes. These binding sites could be distant enhancers or located proximal to the target genes. Numerous androgen-regulated genes, such as UBE2C, CND1, p21, and p27, are up-regulated as PCa progresses. Prostate cells have greater sensitivity to lower ligand concentrations in the majority of CRPCa situations where AR overexpression is present.

In the early stages of PCa, AR mutations are uncommon, but they are quite common in CRPCa. These alterations may increase the range of molecules that the AR is selective for or they may eliminate the requirement for a ligand for appropriate transcriptional activity. The promiscuous behavior of the receptor is activated by adrenal androgens and other steroid hormones, including as a hormone known as (DHEA), progesterone, estrogen, and cortisol, according to a large number of AR mutations that have been characterized. The prostatic epithelial cells can develop in an androgen-refractory manner as a result of this occurrence For this, there are three distinct AR sites where mutations seem to confer special features. The resistance to adrenal androgens, glucorticoids, and progesterone is enabled by the first area, which is located between residues Mutations such as blame for this effect. mutation has been identified as the most prevalent in Copa in the second region, which is between residues This modification broadens the range of ligands that can bind AR by altering the stereochemistry within the binding pocket, which appears to have an impact on the AR ligand specificity. As a result, AR can be activated by other hormones like DHEA, estrogen, progesterone, cortisone, and cortisol. The transcription response of AR to hormones such adrenal androgens or antiandrogens is similarly increased by another mutation (H874Y). Between residues 670 and 678, at the intersection of the hinge and LBD domains, is the third mutational site that improves the transactivation activity of AR in response to testosterone (DHT). Although less frequently, there are additional variations in the amino terminus [4]–[6].

Transcription factors are essential for the production of AR and can either favorably or adversely affect the regulation of genes. For instance, it has been noted that cAMP response element-binding proteins (CREB) considerably rise as PCa progresses, which ultimately boosts AR transcriptional levels. It is widely known that the proto-oncogene Myc contributes to the development of cancer and that it also takes part in AR transcription, serving as a predictor of biochemical recurrence following radical prostatectomy The activator protein subunit c-Jun is known to inhibit the production of the AR, but it also coactivates this receptor. FOXO3a, a transcription factor that binds to the Foo-response element in the AR promoter region, is another transcription factor that positively controls AR transcription. Due

to the activation of LEF1 by Wnt1 and the subsequent increase in AR transcription, LEF1 is a nuclear transducer that suggests a connection between Wnt signaling and PCa. A significant role in the development of the CRPCa state may be played by other transcription factors, such as NF-B and Twist-1, as shown by their favorable connection with AR expression.

Route Description

Inflammation, autoimmune diseases, and cancer are just a few of the physio pathological illnesses that the nuclear factor kappa B (NF-B) signaling pathway is connected to. Five proteins make up the NF-B family in humans: p65 Rel, and Reb After activation, NF-B proteins assemble into homo- or heterodimeric complexes that bind to DNA's B enhancer sites to operate as transcriptional factors. The inhibitory I-B proteins are phosphorylated by the I-B kinase complex (IKK), which is made up of the catalytic subunits IKK and IKK and the regulatory scaffolding protein NEMO. This causes I-B to be ubiquitinated and further degraded by the proteasome, allowing the NF-B dimers to move to the nucleus and activate target genes that respond to I-B. A non-canonical NF-B pathway, in contrast, is found to process p100 in an IKK-dependent manner as opposed to the usual I-B degradation, and it is more cell-specifically detectable in lymphoid tissue and immune-related cells. Lymphotoxin- (LT) and B cell-activating factor (BAFF) are examples of specific stimuli that activate the non-canonical pathway. In contrast, the canonical pathway is activated by a broader range of stimuli, such as tumor necrosis factor (TNF) and interleukin and is frequently linked to tumorigenesis, such as leukemias, lymphomas, and certain solid tumors. Certain tumor cells may originate, advance, and become resistant as a result of the crucial antiproliferative and apoptotic activities played by some NF-B target genes.

PCa-Related Pathway Disruptions and Therapeutic Targets

Prostate cancer has been found to be suppressed by molecular tactics that target NF-B, both in terms of prevention and after therapy. For instance, it has been established how particular IKK inhibitors affect the development and survival of androgen-dependent and independent PCa cell lines. According to the findings, cell proliferation is noticeably impacted regardless of AR status and androgen dependence. Therefore, finding NF-B responsive genes associated with PA development is crucial for improving our knowledge of and ability to treat this condition. The differentiating mRNA expression between tumor tissues and normal tissues has helped scientists discover several genetic abnormalities. For instance, both mRNA and protein levels of NF-B are increased during androgen-independent carcinogenesis in the prostate. These data suggest that the NF-B pathway can be constitutively active in PCa since interleukin expression was consistently raised in androgen-independent PCa lineages constitutive NF-B activation is the main cause of the dysregulation of IL-6 production in prostate cancer cells, and this activation is brought about through signal transduction involving the upstream effectors NF-B inducing kinase (NIK) and IKK. Consequently, NF-B additionally targets a transcription regulatory component of PSA, an essential marker for the emergence and evolution of PCa.

PCa exhibits high amounts of proinflammatory cytokine TNF-, a prototypical NF-B inducer and downstream target gene, as well as tumor epithelial expression of TNF receptors TNFR1 and TNFR2 in comparison to healthy prostate epithelium The pathological information and prognosis of PCa patients are related to the serum levels expression has been linked to altered chemotherapeutic drug responses, angiogenesis, metastasis, and enhanced pica cell survival

and proliferation [66]. Studies with the TNF-inhibitor pastoralizing on the PC-3 and DU145 cell lines suggest that this cytokine may be a potential therapeutic target. Through p65 and other upstream molecules, such as the survival protein families IAPs (inhibitor of apoptosis proteins), psoralen's reduction of TNF- suppresses NF-B. The mitochondrial (intrinsic) and death receptor (extrinsic) pathways, which ordinarily start the activation of the cysteine protease caspases, are both inhibited by the IAP proteins. Since IAPs regulate apoptotic processes and TNF impacts cell survival and proliferation via NF-B, the combined suppression of IAPs and TNF may be appealing for PCa therapy [7]–[9].

Route Description

A vital signal transduction network, the phosphoinositide 3-kinase/AKT (PI3K/AKT) pathway connects various kinds of membrane receptors to numerous vital physiological processes, including G-protein coupled receptors, and oncogenic proteins, such as small G protein RAS, to transduce their signals, are divided into three major classes, while class II and III molecules, which have a single catalytic subunit and can bind to several receptors, such as RTKs or cytokine receptors these molecules can recruit and activate the serine/threonine-specific protein kinase AKT (also known as protein kinase B, PKB) after activating PI3K. Through the plectron homology domain a conserved protein module found in numerous proteins involved in cell signaling or as components of the cytoskeleton, PIP3 can recruit AKT. Multiple additional proteins, including mTOR, glycogen synthase kinase 3, and FOXO members (the forked box family of transcription factors), can be phosphorylated and activated as a result of activated AKT. AKT's action ultimately triggers and controls a wide range of cellular functions. It makes sense to associate PI3K/AKT with the emergence of cancer given that PI3K/AKT signaling is connected to cell survival and proliferation.

PCa-Related Pathway Disruptions and Therapeutic Targets

In the majority of solid tumors, the pathway is dysregulated. According to estimates signaling is increased in 30% to 50% of pace patients, frequently as a result function loss which results in AKT hyperactivation. The dephosphorylation is carried out by PTEN (phosphatase and tensing homolog), negatively regulates the activity of PI3K/AKT signaling. It's interesting to note that it's unclear whether or how direct mutations can cause As observed in artificially produced mouse models its genetic dosage is linked to development, where absolute loss of function can be correlated with more advanced Complete PTEN inactivation in the prostate results in a noninvasive phenotype in mouse models, which raises the possibility that additional alterations may be responsible for the emergence of more invasive tumors. In fact, more aggressive in vivo has been associated with mutations in p53 or the cyclin-dependent kinase inhibitor other pathways appear to be involved in the loss function in addition to PTEN gene deletion. With the identification of miR-22 and as PTEN-targeting, the effect of microRNAs (miRNAs), small, single-stranded RNA sequences that act as posttranscriptional regulators of gene expression, on inactivation has recently been described. Additionally, it is well-known that nuclear exclusion plays a crucial role in the growth of malignancies, including In fact, it has been noted that nuclear PTEN interacts via the anaphase-promoting complex and stimulates its association with CDH1 (the APC/C activator protein), increasing the complex's ability to suppress cell division, indicating a function for nuclear PTEN in PCa suppression.

High levels of proliferation and resistance to apoptosis are brought on by AKT hyperactivation, with resistance being one example of the latter. Tumor necrosis factor superfamily member selectively encourages apoptosis in cancer cells. In fact, cells treated with the PI3K inhibitor become more susceptible to TRAIL-induced apoptosis. Certain PI3K subunits, including p110, which are not typically expressed in non-hematopoietic cells, are present together with the increased PI3K/AKT activation seen in PMCA cells. Increased PTEN activity inhibition and more AKT activation are associated with increased p110 expression. In addition to p110, transgenic mice that express p110 continuously show that this molecule can also be connected to neoplasia formation [10]–[12].

CONCLUSION

The signaling processes that generate and sustain have been the subject of extensive research over the past few decades. The development of specific drugs that might encourage the blockage and/or induction of particular molecules that could result in the control of tumor progression depends critically on our growing understanding of the connections between various signaling cascades that ultimately promote the advance. In reality, a number of medications are now undergoing clinical trials or animal testing, the majority of which work as precise inhibitors of aberrant signaling pathways like those discussed in this article. However, there is still a need for a more thorough and interactive assessment of the extrinsic elements that can cause the deregulation seen in the PCa microenvironment. In order to create completely functional countermeasures against PCa, it is necessary to acquire a deeper understanding about the cascade-dependent cues that underlie PCa induction. This will also boost our understanding of PCa propensity tests, which will undoubtedly result in improved prevention plans and early treatments for this disease.

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CHAPTER 11

CHONDROCYTE AND HYPERTROPHIC DIFFERENTIATION ASSOCIATED SIGNALLING PATHWAYS

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ABSTRACT:
Instead of direct cell-to-cell contact, diffusible signals are the primary means of communication between chondrocytes. Mesenchymal stem cells' (MSCs') chondrogenic development is tightly controlled by the interplay of several growth factors, cytokines that and signaling molecules. The differentiation of mesenchymal progenitor cells into hypertrophic chondrocytes has been regulated by a number of key signaling molecules, including bone morphogenetic proteins related high-mobility group-box genes 9 (Sox9), parathyroid hormone hormone-related peptides Indian hedgehog, fibroblast growth factor receptor 3 (FGFR3), and -catenin. Adenosine, O2 tension, and reactive oxygen species (ROS), in addition to these chemicals, are important contributors to the development of chondrocytes and cartilage. In this article, we described the intricate transcriptional network and its major players' roles in determining and controlling the inherited program of chondrogenesis or chondrocyte differentiation.

KEYWORDS:
Communication, Diffusible, Mesenchymal, Transcriptional.

INTRODUCTION

Key processes in bone development include chondrocyte differentiation and hypertrophy. In order to generate bones, two processes must take place. The first is intramembranous ossification, in which mesenchymal embryonic stem cells (MSCs) transform into osteoblasts. This is how irregular bones, including as the skull bone, clavicle, and a portion of the jaw, develop. On the other hand, endochondral ossification, another bone-forming process, creates the long bones and vertebrate skeleton. Endochondral ossification, to put it simply, is the process by which MSCs condense to create chondrocytes, which subsequently differentiate to produce a cartilage template. Minerals from the bone gradually take the place of the cartilage template. Sox9, a crucial regulator of chondrogenesis, will be expressed by MSCs during condensation. In addition, these cells divide into two subpopulations of chondrocytes: rounded, low-proliferating chondrocytes near the distal ends of the condensation that remain to express Sox9 and high-proliferating chondrocytes arranged in columns toward the centre that eventually go through maturation. The core part of cartilage anlagen has chondrocytes that withdraw from the cell cycle throughout the maturation process and differentiate into PR hypertrophic and hypertrophic chondrocytes, which have a 20-fold increase in volume. Extracellular matrix that has been mineralized by hypertrophic chondrocytes serves as a blueprint for bone to replace it later.

The perichondrium, which encloses the cartilage component, is made up of a layer of fibroblast-like cells. The heavily vascularized periosteum is created by the differentiation of the perichondrium's cells into osteoblasts. Then the calcified matrix created by hypertrophic

chondrocytes is invaded by blood vessels from the periosteum, osteoblasts, and osteoclasts, leading to the replacement of mineralized cartilage by bone to create the primary ossification centre. A hematopoietic habitat will be created by further remodeling the matrix to create the cortical bone and the bone marrow cavity. At the distal extremities of long bones, secondary ossification centres typically form during the postnatal growth period.

The different stages of endochondral ossification have varied effects on gene expression. The transcription variables structural proteins collagen type II, aggrecan, are expressed by immature chondrocytes. These are all indicators of the differentiation of chondrocytes. The next step is chondrocyte PR hypertrophy, which is characterized by the expression of the Indian hedgehog gene and the parathyroid hormone 1 receptor (Pth1r). Early hypertrophic chondrocytes that express collagen type X, subtype 1 (Col10a1) then enter the stage. And as a result, Sox5, Sox6, Sox9, and Col2a1 expression reduced. When osteopenia, matrix metalloproteinase 13, and vascular endothelial growth factor A (VEGFA) were expressed, chondrocytes advanced towards a late hypertrophic condition. These gene expressions signal the invasion of the matrix by osteoblasts, osteoclasts, and endothelia cells, which will result in the replacement of the cartilage templates with bones. According to studies, the equilibrium between chondrocyte differentiation and proliferation is necessary for bone growth. The length and stability of bones will change once the balance is upset.

Numerous human chondrodysplasias and transgenic mice with skeletal abnormalities during the past few years have provided evidence for the critical role that chondrocyte differentiation plays in the development of cartilage and bone. These studies have also provided insight into the fundamental biology of cartilage and bone. Numerous skeletal diseases will result from the chondrocyte maturation process' fine-tuning. Therefore, a detailed investigation of the mechanisms underlying chondrocyte differentiation and hypertrophic differentiation is required. The molecular events of how the signals are translated into gene expression remain largely unknown, and our data about the mechanisms that regulate the initial steps of chondrogenesis are limited. Despite the fact that a number of critical signaling and transcription factors have been identified as playing an important role in regulating cartilage formation as well as chondrocyte differentiation from initial MSCs into mature terminal enlarged chondrocytes by amount of work both in vivo and in vitro, the molecular events of how in this study, we compiled the most recent information on the transcription factors and pertinent signaling networks that control chondrocyte development.

Undifferentiated stem cells from the mesoderm can differentiate into a wide range of generative cells, including osteochondrogenic (also known as osteogenic, chondrogenic, osteoprogenitor, etc.) cells. The initially undifferentiated mesenchymal stem cells lose their pluripotency, multiply, and cluster together in a dense aggregate of chondrogenic cells (cartilage) near the site of chondrification (bone, or in this case cartilage). These chondrogenic cells differentiate into chondroblasts, which produce the extracellular matrix (ECM) of cartilage, which is composed of fibers and a ground material (proteoglycans and glycosaminoglycan for low osmotic potential). The chondroblast is now a fully developed chondrocyte, capable of secreting and degrading the matrix even though it is normally dormant.

Excess cell culture studies Vitamin A prevents chondrocytes from producing chondroitin sulfate and prevents chondrogenesis in the developing embryo, which can lead to limb

deformities. When chondrocytes become hypertrophic during endochondral ossification, they go through terminal differentiation. The cell undergoes significant phenotypic alterations throughout this final stage. In the cartilage matrix, the chondrocyte has a rounded or polygonal shape. The articular surfaces of joints, for instance, where chondrocytes may be flattened or discoid, are an exception to this rule. A synthetically active cell has characteristics that are internal to the cell. Between the ages of 20 and 30, the cell density of full-thickness, mature human femoral condyle condor is maintained at Although chondrocyte senescence develops with age, typical adult articular cartilage does not exhibit mitotic figures. Depending on where it is located, a mature chondrocyte has a different structure, density, and synthetic activity. In the superficial zone, which has the maximum cell density, flattened cells and collagen fibers are positioned parallel to the surface. The chondrocytes are larger, more rounded, and distributed more randomly in the intermediate zone, where the collagen fibers are likewise distributed more sporadically. Along with the collagen fibers, chondrocytes form columns in the deeper zones that are perpendicular to the cartilage surface.

Chondrocytes may behave differently depending on where they are located inside the various layers. These zonal disparities in synthetic characteristics may endure in primary chondrocyte cultures. The primary cilia, which are sensory organelles in chondrocytes, are important for the spatial orientation of cells in growing growth plates. Primary cilia have mechanosensitive receptors and serve as hubs for wingless type (Wnt) and hedgehog signaling. Numerous different genes and proteins can affect how many chondrocyte cells are produced as well as how they mature. Bone morphogenetic protein 4 (BMP-4) and fibroblast growth factor 2 (FGF2) are two proteins that have been found to affect how much cartilage is differentiated into chondrocytes. Both proteins have been linked to the differentiation of embryonic stem cells into mesodermal cells via signaling with BMP-4 and the stimulatory effects of FGF2. Cells will continue to develop downward into numerous different types of cells from the mesodermal germ layer. BMP-4 and FGF2 therapy has been demonstrated to increase the number of cells that differentiate down into chondrogenic and osteogenic cells when cultivated in chondrogenic and osteogenic media, respectively, in addition to promoting the mesodermal germ layer. The treatment boosted the expression of the transcription factor Sox9 in chondrogenic cells, which is important for the process of chondrogenesis, which results in the production of cartilage from condensed mesenchymal tissues, which then develop into chondrocytes.

DISCUSSION

Signaling Sox9

A crucial transcriptional factor in the growth and maturation of cartilage is Sox9, which is a member of the family of "high-mobility group-box" transcription factors. One of the earliest indicators of condensing chondrocytes, it is expressed starting at the multipotent skeletal progenitor stage and is kept constant in permanent cartilage cells of healthy articular cartilage throughout life. But in hypertrophic chondrocytes, Sox9 expression will be suppressed. Multiple signaling pathways control the expression and activity of Sox9 during chondrogenesis, according to studies. These studies also showed that Sox9 plays a significant part in the chondrogenic differentiation program. Sox9 heterozygous mutations have been linked to the severe skeletal deformity condition, and Sox9 deficiency can entirely prevent

chondrogenesis. Furthermore, Sox9 controls the transcription of numerous genes required for the development and upkeep of healthy cartilage, which is what underlies chondrocyte differentiation. And numerous additional genes also play a part in controlling chondrocyte differentiation, most likely by working with Sox9 or influencing Sox9 expression. Through coextrusion with Sox9, Sox5 and Sox6, two SOX family members, can stimulate chondrocyte differentiation. Although Sox9 overexpression can aid in MSC chondrogenic differentiation, it can also inhibit chondrocyte hypertrophy by altering the expression of a few important genes. According to Bi et al., the absence of Sox9 caused immature chondrocytes to develop into hypertrophic cells. According to all of these investigations, Sox9 is necessary for determining the chondrogenic lineage [1]–[3].

Sox9 inhibits Won't signaling, which has been shown to promote chondrocyte hypertrophy, primarily through the following mechanisms It can block the activation of the transcription factor Runx2, which is essential for reducing chondrocyte development Topol et al. reported that Sox9 interacted with -catenin to inhibit Won't signaling may directly repress expression of the genes expressed in hypertrophic.

Signaling via Bone Morphogenetic Protein

BMPs have been found to positively regulate endochondral ossification and ectopic chondrogenesis According to a paper, blocking BMP signaling will prevent cartilage from forming. Their receptors, BMPR1 (BMPR1a and BMPR1b) and BMPR2, facilitate BMP signaling. It has been shown that these receptors regulate the expression of target genes in early xenopus embryos by phosphorylating SMAD transcription factors such SMAD1, SMAD5, and SMAD8. In contrast, animals with a deletion of each of these receptors show reduced levels of Sox5, Sox6, and Sox9 in PR cartilaginous condensations, which prevents the production of new chondrocytes and leads to abnormal chondrocyte maturation [4]–[6].

Signaling by Won't

Many developmental processes, such as the skeletogenous process, depend on wnt signaling. The two main skeletal system cell types that differ from typical mesenchymal progenitors are chondrocytes and osteoblasts. It has been demonstrated that Wnt signaling can control how the ecchondroses progenitor cells differentiate into chondrocytes and osteoblasts. While suppressing chondrocyte differentiation in MSCs, Wnt signaling activation increases osteoblast differentiation. Wnt may be classified into two classes: Wnt-1 class, which activates the canonical Wnt pathway and Wnt-5a class, which activates the noncanonical Wnt pathway Reports suggested that genetic inactivation of -catenin increased Sox9 expression and induced chondrocyte differentiation at the expense of osteoblast differentiation both in the process of intramembranous and in endochondral ossification. Canonical Wnt signaling acts through -catenin to promote chondrocyte hypertrophy. Furthermore, it has been shown that chondrocytes evolve into osteoblast precursors that lack -catenin. Wnt3a greatly suppressed chondrogenesis and chondrocyte gene expression, as demonstrated by Reinhold et al. There are further research about non-canonical Wnt signaling as well. According to Liu et al.'s hypothesis overexpression improved MSC chondrogenic development by stimulating the gene expression of chondrogenic regulators and working in concert with TGF-. By controlling the expression of the chondrocyte-specific gene Col2a1, Yang et al. reported that Wnt5a and Wnt5b appear to work together on chondrocyte proliferation and differentiation. Together, our results imply that noncanonical Won't signals have distinct effects on

chondrocyte production and maturation than canonical Wnt signals and that they tend to impede chondrocyte differentiation while promoting chondrocyte growth.

Won't signaling is crucial for maintaining the correct alignment of chondrocyte columns in the development plate. According to reports, Wnt5a and Wnt5b appear to control the area where proliferating chondrocytes, which are found at the distal ends of the condensation, are located. In Wnt5a-deficient animals, the rate and area of proliferating chondrocytes were reduced. Bradley and Drissi reported that Wnt5b activated the Wnt planar cell polarity pathway to regulate mesenchymal cell aggregation and chondrocyte development. When the Wnt planar cell polarity pathway is disrupted in living tissue, columnar growth plate architecture is lost. Conversely, when this route is activated in chondrocyte cell pellets, typically randomly oriented cells exhibit columnar organization. In the chondrocyte pellet culture paradigm, Randall et al. demonstrated that activation of the Won't planar polarity of cells pathway in the presence of either Wnt5a or Wnt5b accelerated the beginning of columnar morphogenesis.

Signaling of Fibroblast Growth Factor

Fibroblast growth factors (FGFs) are signaling proteins that belong to a vast family. It has been established that FGF signaling plays crucial functions in the development of both endochondral and intramembranous bone. It has been shown to be crucial in controlling chondrocyte proliferation and the start of chondrocyte hypertrophy. Condensing mesenchyme that will develop into cartilage expresses both fibroblast growth factor simultaneously. Since hypertrophic chondrocytes express temporary increase in the hypertrophic zone is caused by deficiency. Condensing mesenchyme exhibits significant levels of early FGFR2 expression, which thereafter appears to be downregulated in proliferating chondrocytes. Additionally, the absence of FGFR2 causes postnatal dwarfism and a thinner hypertrophic zone. In order to control cell proliferation and differentiation, FGFR3 is expressed in proliferating chondrocytes and is downregulated in the hypertrophic zone. Activating mutations in FGFR3 speed up late hypertrophic differentiation while inhibiting chondrocyte proliferation and the start of chondrocyte hypertrophy.

Only FGF9 and FGF18 have been demonstrated to be related to chondrogenesis, despite the fact that the FGF ligands involved in skeletal development have been thoroughly described. In the early phases of endochondral ossification, FGF9 both directly and indirectly encourages chondrocyte proliferation and hypertrophy, while in the later stages it governs vascularization In the early stages of FGF18 signaling, it encourages chondrocyte proliferation and the beginning of chondrocyte hypertrophy, while in older embryos, it inhibits chondrocyte proliferation or delays chondrocyte hypertrophy [7]–[9].

Signaling of the parathyroid hormone-related peptide by Indian Hedgehog

Ich, which is produced and secreted by PR hypertrophic and early hypertrophic cells, is a crucial regulator of endochondral ossification. In proliferating chondrocytes, Ihh signaling directly stimulates proliferation, and mice lacking Hihi exhibit significantly reduced chondrocyte proliferation, premature chondrocyte hypertrophy, and an inability to properly form osteoblasts in endochondral bones. Additionally, regulates the expression of When Phra is overexpressed, chondrocyte differentiation is delayed, and when is deleted, chondrocyte proliferation is reduced, chondrocytes mature in the wrong place, and bone formation is

hastened. It has been demonstrated that deletion of can reverse the suppression of chondrocyte hypertrophy caused by elevated Ihh. Studies show that Ihh signaling activation upregulates null explants and inhibits chondrocyte hypertrophy, indicating that Ihh regulates chondrocyte proliferation and maturation through a PTHrP-dependent route Additionally, independent mechanism that positively controls chondrocyte proliferation also exists, mostly through transcription factors of the GLI family.

Factors in the Runs Family Transcription

Members among the Runx family transcription factors, Runx2 and Runx3, are crucial for fostering chondrocyte hypertrophy. Numerous studies show that the expression of hypertrophic markers including Col10a1, MMP13, and VEGF is induced by the ectopic expression of Runx2 in juvenile chondrocytes. It has been discovered that Runx2 can interact with BMP-regulated Smads proteins to activate the gene expression of hypertrophic chondrocytes and directly bind to the Ihh gene's promoter region to powerfully induce the reporter gene that is driven by the Ihh promoter When Runx2 is knocked out in mice, chondrocyte hypertrophy is delayed and significantly diminished, but chondrocyte hypertrophy and vascular invasion into cartilage are only somewhat delayed in Runx3-deficient mice [50, 52]. Furthermore, mice with Runx2 and Runx3 knockouts displayed a complete lack of chondrocyte development [50]. All of these findings suggest that Runx2 and Runx3 are necessary for the development of chondrocytes.

Signaling by Adenosine

Important cellular metabolites known as purines are engaged in a wide range of biological functions. Adenine nucleotides can be broken down by enzymes both inside and outside of cells to produce adenosine, a nucleoside. Increased intracellular adenosine concentration is brought on by transporters when extracellular adenosine levels rise. Adenosine is known to participate in numerous distinct metabolic pathways by activating cell surface G protein-coupled receptors, and it plays a significant physiological signaling role in the peripheral and central nervous systems. Adenosine levels outside of normal range, however, may be harmful to health. Adenosine deaminase (ADA) is a degradative process that transforms adenosine to inosine. The skeletal, central neurological, endocrine, and gastrointestinal systems will all be impacted by an adenosine deaminase Adenosine is converted to AMP by adenosine kinase (AK) in the second pathway, which is then further processed to produce adenosine triphosphate Effective DNA repair depends on maintaining the right equilibrium between ATP and dATP. Vertebrates have four adenosine receptors called, which are further classified into two subclasses: those that stimulate (A2A and A2B) or those that are negatively connected to (A1 and A3) adenylate cyclase. A2A has a strong affinity, in contrast to A2B's low affinity. Additionally, these receptors can influence other signaling pathways including serine-threonine-specific kinases and mitogen-activated protein kinase (MAPKs) to affect various systems.

Recent research has shown that adenosine is essential for the growth of bone and cartilage. Patients with ADA deficiency frequently exhibit myeloid dysplasia symptoms and hypo cellular bone marrow. Manson et al. shown that patients with ADA deficiency could exhibit atypical ecchondroses characteristics, which would disappear after 6–12 months of ADA enzyme replacement medication once adenosine levels were restored to normal. By boosting Ca2+ oscillations in monolayer-cultured chondrocytes, it was discovered that ATP, a Ca2+

modulator, may maintain proteoglycan levels close to those of normal tissue and increase collagen synthesis and functional attributes of synthetic cartilage. Increased adenosine concentrations within chondrocytes and preventing adenosine's breakdown by an ADA inhibitor adenine (EHNA)) result in cell death, according to research in MC615 chondrocytes. Adenosine can be released by chondrocytes in response to many different physiological events. Adenosine levels, both extracellular and endogenous, play a critical role in controlling cartilage degradation during inflammatory processes. Nitric oxide (NO), matrix metalloproteinases and glycosaminoglycan (GAG) will all express more when levels decline, favoring chondrogenic development. But if the chondrocytes are incubated with an ADA inhibitor, the increased adenosine concentration results in a decrease in the release of prostaglandin which damages the cartilage but lessens inflammation. In addition, stimulation of the adenosine receptor A2AR decreased the generation of MMPs and proinflammatory cytokines in mouse articular chondrocytes that had previously been activated with interleukin-1 by suppressing NF-kB activation. Adenosine may therefore play a dual role in cartilage destruction, as well as a role in preventing bone and cartilage degradation in rheumatoid arthritis via activating A2AR.

Oxygen Tension's Effect on Chondrocyte Development

Articular cartilage is a type of avascular tissue that receives some of its nutrients from subchondral bone and synovial fluid According to reports, the tissue contains between 1% and 6% oxygen Chondrocytes must therefore adjust to a low O2 tension environment. However, glucose uptake, lactate generation, and cell ribonucleic acid (RNA) synthesis are all inhibited by O2 tension below 1%. In other words, chondrocytes require at least some O2 for their basic metabolism. Both anoxia and hyperoxia, according to studies, impair chondrocytes by affecting glycolysis and matrix synthesis. How does O2 alter the actions of chondrocytes? It is clear that a cell coming from tissue with low oxygen tension survives better at 5% than at 20% O2. In an environment with high O2 tension, the morphology of chondrocytes will resemble a spindle-like phenotype Collagen type II and glycosaminoglycan are two extracellular matrix components that are synthesized in greater amounts and in higher-quality conditions under hypoxic.

Hypoxia has also been shown to induce MSC differentiation into chondrocytes The stability and transactivation of HIFs are crucial for the effect of hypoxia on the chondrogenic division of MSCs, which is mediated by changes in oxygen tension. Hypoxia inducible factors (HIFs) are a group of vital transcription factors that mediate cellular responses to variations in oxygen tension. HIF-1 is often instantaneously present in the cytoplasm under nonmonic circumstances and is transitorily broken down by the ubiquitin-proteasome pathway. Under hypoxia, HIF-1 will translocate to the nucleus where it will bind its DNA binding site and stimulate the production of genes relevant to hypoxia. According to studies, hypoxia chondrogenic induction increased the expression of HIF-2 compared to hormonic chondrogenic induction pathways, which prevent apoptosis, may also contribute to increased chondrogenic differentiation under hypoxia, in addition to HIFs. Low oxygen tension in the work by Poltroon et al. inhibits hypertrophic differentiation of human adipose MSC and murine cells by changing the transcriptional activity. This inhibits the early chondrogenic differentiation of these cells. Furthermore, a gradient in oxygen tension concentration can guide human MSC to differentiate into either permanent tissue or hypertrophic cartilage that

will be replaced by bone Human MSC chondrogenic differentiation was aided by hypoxia (2.5% O2), while hypertrophic differentiation was boosted by Nordonia [10]– [12].

These results show that chondrocyte phenotypic is sensitive to O2 tension even though chondrocytes are well acclimated to hypoxia. Low O2 tension encourages the development of the cartilage-specific matrix and the expression of the chondrogenic phenotype, indicating that low O2 tension plays a significant role in chondrogenesis and cartilage breakdown and may be crucial for cartilage tissue engineering and stem cell therapy.

CONCLUSION

The final fate of hypertrophic chondrocytes is still in question, despite the fact that numerous signaling factors have been shown to be controllers of both chondrocyte proliferation and hypertrophy. The established view is that late hypertrophic chondrocytes undergo cell death during the creation of endochondral bone, and that osteoblasts and chondrocytes are separate lineages descended from a single osteochondroprogenitor. However, Yang et al. recently investigated the fate of murine seriously differentiated hypertrophic chondrocytes using a cell-specific tamoxifen-inducible genetic recombination approach. They discovered that while some hypertrophic chondrocytes die, a sizable portion of these cells continued to differentiate into osteoblasts and osteocytes during the cartilage-to-bone transition. In other words, more research should be done on chondrocyte-based and/or stem cell-based techniques of cartilage regeneration because the long-term fate of hypertrophic chondrocytes is still not fully understood. Numerous signal transduction pathways and a sophisticated transcriptional network work together to control chondrocyte development. Significant progress has been made over the past few decades in our understanding of the intricate transcriptional network that controls chondrocyte development. The development of healthy cartilage and chondrocyte differentiation depend on the balance of these signaling pathways. A better comprehension of these signaling pathways will enable us to better understand the regulation of bone and cartilage repair as well as the process of bone formation and vertebrate development.

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CHAPTER 12

KEY GENES & SIGNALING PATHWAYS IN THE TERM ENDOGENOUS ARDS

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ABSTRACT:
To examine the pathogenesis and early diagnostic molecular markers utilizing entire transcriptome data, as well as to assess the differentially expressed genes in rats with endogenous acute respiratory distress syndrome lung injury. Methods. Twelve male Sprague Dawley 8-week-old rats were chosen and randomly and evenly divided into two groups: one for lung injury and the other for normal control. Both groups' left lung tissues had their RNA extracted, and the Illumina Hisses sequencing platform's paired-end sequencing mode was used to sequence the extracted software was used to screen the DEGs of miRNA, was used to build the regulatory network used in the Gene Ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes enrichment analyses. The 15 essential genes were found using and polymerase chain reaction Results. The ARDS lung injury group displayed and 1 downregulated gene) based on various screening conditions control group. According to GO, the DEGs of mRNA were primarily engaged in biological processes such cytokine-mediated signaling, leukocyte and neutrophil chemotaxis, and defensive responses to lipopolysaccharide and other pathogens. The DEGs mostly carried out their biological functions through taking part in chemokine signaling pathways, according to KEGG enrichment analysis. A total of 281 node proteins and 634 interaction edges were identified by the PPI analysis. were the top 15 important genes that were examined. The miR-21-3p, Camk2g, and Stx2 genes were the three major gene nodes in the network analysis, which revealed 69 nodes and 73 association relationships. Conclusions. In the occurrence and progression of endogenous acute lung injury during ARDS, the chemotaxis, migration, and degranulation of inflammatory cells, cytokine immune response, autophagy, and apoptosis play important biological roles. Therefore, the signaling pathways may offer fresh perspectives and opportunities for further research into the mechanism of lung injury and therapeutic approaches.

KEYWORDS:
Apoptosis, Chemokine, Degranulation, Identified**.**

INTRODUCTION

In the realm of critical care medicine, acute respiratory distress syndrome (ARDS) is a typical acute and critical sickness According to a global epidemiological study, roughly 10% of patients in intensive care unit’s severe acute respiratory syndrome) in flu in and novel coronavirus pneumonia corona virus disease the latter is still spreading worldwide are just a few examples of recent severe acute respiratory infections that pose a threat to human health. The majority of severely ill patients develop ARDS, which has a mortality rate of more than The pathophysiology of ARDS has undergone great improvement in the past 20 years, although the fundamental mechanism of acute lung injury in endogenous ARDS is still not

entirely understood. Additionally, there is a dearth of defined biomarkers and efficient therapeutic targets [6]. Rapid analysis and great resolution are two benefits of the recently created high-throughput sequencing technology known as RNA-sequencing (RNA-Seq). The important genes and pathways were screened in the current study using RNA-Seq technology to examine the expression profiles of lung damage genes in the endogenous subtype of lipopolysaccharide (LPS)-induced ARDS. The goal of this study was to investigate treatment targets and particular biomarkers while also offering new perspectives on the mechanism of endogenous ARDS lung injury. A chemical or physical signal is transferred through a cell as a succession of molecular events through a process known as signal transduction. Protein kinases are most frequently responsible for the catalysis of protein phosphorylation, which ultimately causes a physiological response. Although in some instances the term sensor is used, generally speaking, proteins that detect stimuli are referred to as receptors. A signaling route, which is a series of biochemical events known as a biochemical cascade, is initiated by the changes brought about by ligand binding (or signal detecting) at a receptor.

Signaling pathways connect with one another to build networks that enable the coordination of cellular responses, frequently through combinatorial signaling events. At the molecular level, these responses can affect how genes are translated or transcribed, as well as how proteins are post-translationally altered and how they conform. The fundamental mechanisms governing cell growth, proliferation, metabolism, and many other functions are these molecular occurrences. Signal transduction pathways control cell communication in multicellular organisms in a wide range of ways. Each part (or node) of a signaling pathway is categorized based on the function it performs in relation to the initial stimulus. First messengers are ligands, and signal transducers are receptors, which in turn activate primary effectors. These effectors, which are primarily proteins, are frequently connected to second messengers, which in turn can activate further effectors. Signal gain is the idea that a signal can be amplified so that one signaling molecule can cause a reaction involving hundreds to millions of molecules depending on how effective the nodes are. Similar to other signal transduction, biological signal transduction is characterized by delay, noise, signal feedback and feedforward, and interference, which can be minimal or severe.

Analysis of signaling pathways and networks has become crucial to understanding biological processes and illness, including the signaling rewiring mechanisms driving acquired drug resistance. This is thanks to the development of computational biology. Most signal transduction pathways entail the attachment of signaling molecules, or ligands, to receptors that set off cellular activities. Receptor activation, which results from a change in receptor conformation brought on by the binding of a signaling molecule to a receptor, is a biological process. The majority of ligands that bind to cell surface receptors are soluble molecules from the extracellular media. These consist of neurotransmitters, cytokines, and growth factors. Fibronectin and hyaluronan, both of which are found in the extracellular matrix, can bind to these receptors (integrins and CD44, respectively). Additionally, some chemicals, like steroid hormones, are lipid-soluble and can pass through the plasma membrane to reach receptors in the cytoplasm or the nucleus. When steroid hormone receptors are stimulated, they bind to the promoter region of genes that respond to steroid hormones.

The chemical makeup of each class member is not taken into account in all classifications of signaling molecules. For instance, neurotransmitters, which range in size from tiny molecules like dopamine to neuropeptides like endorphins, as well as odorants, belong to a variety of

molecular groups. In addition, some molecules may fall under more than one category. For instance, epinephrine is both a neurotransmitter and a hormone depending on where it is released from in the body. When overexpressed or altered, certain receptors, including HER2, can activate without the need for a ligand. As a result, the route becomes constitutively activated, which compensation mechanisms may or may not be able to reverse. Constitutive activation causes cancer and hyperproliferation in the instance of HER2, which functions as another EGFR dimerization partner. Most receptors are made up of extracellular receptors, which are essential transmembrane proteins. They cross the cell's plasma membrane, with one receptor segment on the outside and the other on the inside. When a ligand binds to the receptor's outer area (i.e., does not pass through the membrane, signal transduction takes place. It is sometimes referred to as "receptor activation" when the shape of the receptors inside changes as a result of ligand-receptor contact. This causes the signal to eventually spread into the cytoplasm by either activating the receptor's enzyme domain or exposing a binding site for additional intracellular signaling proteins.

Tyrosine kinase and phosphatases are two examples of intracellular proteins that have an enzymatic activity in eukaryotic cells when they are triggered by a ligand/receptor interaction. Such enzymes frequently have a covalent bond with the receptor. Some of them produce second messengers such cyclic AMP and IP3, the latter of which regulates the release of calcium reserves from intracellular reservoirs into cytoplasm. In order to coordinate the signaling complexes and facilitate contacts between signaling proteins, other activated proteins engage in interactions with adaptor proteins. Both adaptor proteins and enzymes respond to different second messenger molecules. Activated adaptor proteins and enzymes frequently have specialized protein domains that bind to particular secondary messenger molecules. For instance, the EF hand domains of calmodulin bind calcium ions, enabling it to bind and activate calmodulin-dependent kinase. Proteins with receptors, including nuclear and cytoplasmic receptors, are soluble proteins that are confined to their specific regions. Non-polar hormones like the steroid hormone’s testosterone and progesterone and derivatives of the vitamins A and are the typical ligands for nuclear receptors. The ligand must passively diffuse through the plasma membrane in order to start signal transduction. The ligands enter the nucleus through the nuclear membrane after interacting with the receptor, changing how genes are expressed.

The promoter region of the genes that the hormone-receptor complex activates contains receptor-specific hormone-responsive element (HRE) sequences where activated nuclear receptors can bind to the DNA. They are also known as inductors of gene expression because they promote gene transcription. Due to the relatively slow turnover of the majority of enzymes and proteins that would normally either deactivate or terminate ligand binding onto the receptor, all hormones that act by regulating gene expression have two consequences in their mechanism of action: their effects are produced after a characteristically long period of time and their effects persist for another long period of time, even after their concentration has been reduced to zero.

The zinc fingers in the DNA-binding domains of nucleic receptors, which also feature a ligand-binding domain, help to stabilize DNA binding by retaining the phosphate backbone. Hexametric repetitions of any kind are often the DNA sequences that match the receptor; while the sequences are similar, their orientation and proximity set them apart. Additionally, the ligand-binding domain is in charge of dimerizing nucleic receptors before binding and

supplying structures for transactivation that are utilized to interact with the translational apparatus.

DISCUSSION

Groups and Animals Used in Experiments

The Medical Ethical Committee of Lianyungang Clinical School of Nanjing Medical University accepted this work, and all animal studies were carried out strictly in accordance with the standards of animal ethics Twelve male Sprague Dawley rats in good health were chosen. Rats weighed between 230 and 250 grams and were 6 to 8 weeks old. Six rats from each group were randomly assigned to the blank control group (N group) and the experimental group (LPS group). LPS (Sigma-Aldrich, St. Louis, MO, USA; 10 mg/kg) was injected into the rats' airways to create the endogenous ARDS lung damage models (LPS group) [1], [2].

Lung Tissue Pathological Observation

Rats were given intraperitoneal injections of xylazine (8 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) and ketamine (80 mg/kg; Zhengqui, China) to put them to sleep after 36 hours. The rats were sacrificed by heart puncture and bloodletting after a successful anesthesia, and the sample specimens were gathered for analysis. The degree of pulmonary inflammation and edema was calculated using the middle lobes of the right lung's wet/dry weight ratio. The right upper lobe lung tissues of the rats were collected, dried with gradient alcohol, inserted into paraffin, and sectioned. Using an Olympus microscope, damage to the lung tissue was seen at low, medium, and high magnification.

Extraction of Total RNA from Lung Tissues

Using RNA extraction kits from Thermal Fischer Science, Waltham, Massachusetts, USA, the total RNA was isolated from the lung tissues. In order to meet the requirements of subsequent sequencing quality, the purity and RNA integrity factor of the extracted RNA samples were determined using Themo Nanodrop one ultra-micro spectrophotometer and Agilent2100 bioanalyzer, respectively (both from Santa Clara, CA, USA).

Sequence pre-processing and annotation for classification

The high-throughput sequencing of several samples was done in this study using the Illumina sequencing platform's paired-end mode. The adaptor sequence and low-quality fragments were dynamically removed from the three ends of the sequencing reads using the Skewer software The quality control study of the pre-processing data was carried out using Festuca software The pre-processed sequence reads for each sample were aligned with the reference genomes of the sequenced species using the STAR program and the statistical comparison analysis was performed using. In order to predict new miRNAs, annotate known miRNAs in alignment, and analyse miRNAs quantitatively, the sequence data was aligned, filtered, and then submitted to the Roam database, Rebase sequence database, and miRBase database, respectively. When transcripts were constructed using Springtime, the transcripts were categorized by comparing results with the locations of known genes in the reference genome.

For additional screening, the transcripts with the letters O, I, X, J, and U as well as those with lengths larger than 200 bp and a low number of exons were kept. Using software system

platforms like PLEK and Pfau, the incomplete transcripts that could code for proteins were eliminated. Using the software platform, the long non-coding sequences discovered from sample sequencing were compared with the known lncRNA sequences. Then, a quantitative analysis of known lncRNA sequences was performed. The front and rear positions of circular RNAs were predicted for the STAR alignment findings using the CIRCexplorer2 software (2.2.6). Based on the information about where the chromosomes are located, the annotation & gene structure evaluation of derived genes were carried out [3]– [5].

Analysis of Gene Expression Levels and Function

The number of fragments per kilo base in length from a protein-coding gene per million fragments, or FPKM technique, was used to calculate the levels of mRNA expression. Using the number of transcripts per million and the number of fragments per kilo base length per million fragments from a transcript, quantitative estimations of miRNA and lncRNA were done, respectively. The number of transcripts per million spliced was used to calculate circa quantitatively. Dees software was used to identify the differentially expressed genes (DEGs), was used for statistical analysis and visualization. Volcano plots and heat maps were used to show the differences in gene expression levels between the two groups. For the purpose of functional identification and classification of the DEGs, analyses of KEGG pathway enrichment and Gene ontology (GO) function enrichment were carried out. GSE32707, a dataset for the mRNA expression profile, was retrieved from GEO This dataset contains information on the mRNA expression of 34 control samples (free of sepsis or ARDS) and 33 ARDS samples. The entire blood samples were processed to remove the total RNA, and microarrays were created under screening parameters of and to identify the DEGs. The co-expressed DEGs in the two datasets (human DEGs and rat DEGs) were identified using a Venn diagram. In order to analyze protein-protein interactions (PPI), co-expressed DEGs were imported into the online STRING database. Statistical significance was assigned to proteins in the PPI network graph with a total score of >0.7. The top 15 most important genes were determined using the cytoHubba plug-in for Cytoscape [6]– [8].

Network construction and analysis for mRNA, miRNA, lncRNA, and circRNA

The base sequences were utilized to estimate the miRNA-target relationship pairings from the whole transcriptome sequencing findings, which also included information on lncRNA, circinate, and miRNA sequences. The target recognition sites of miRNA in the genomic sequence were predicted using Miranda. The threshold parameters for this process are and, where and stand for the free energy of duplex formation and the single residue-pair match scores, respectively. The Pearson's correlation coefficients for the miRNA-projected target correlation pairings were computed based on the expression levels of miRNAs and their predicted targets, including lncRNA, circa, and mRNA. We filtered out any pairs that had a substantial negative correlation with controlling the expression of the miRNA-targets. The correlational possibility was ruled out when the expression's correlation coefficient was greater than 0.05 and the absolute value of Pearson's correlation coefficient was less than 0.7. For a later competing prediction investigation, these base sequences and expression predictions were compiled. The miRNA-target, miRNA-circa, and mRNA-lncRNA (only DEGs) were found and integrated based on the screening results. A was used to present the identified Cernan connection pairings.

lung tissue RNA extraction and quality control

The two groups' combined RNA extraction yielded >0.2 g of total RNA with a mass-volume concentration of >20.0 ng/L. High purity and good integrity of the extracted RNA met the requirements for building a library. The samples' quality, distribution, and homogeneity were sufficient, and they complied with the requirements for library building. Principal component analysis (PCA), a method of comparing samples by lowering the dimension, was used to assess the repeatability of the data within the group. Rats in the control group are represented by blue circles while rats in the LPS group are represented by red circles. Rats in the LPS group are represented by dots. The trend of gene expression in the samples is more closely correlated with sample proximity. The combined findings demonstrated the two groups' RNA full transcriptome data to have good repeatability.

Gene Set Enrichment Analysis (GSEA) and GO/KEGG Enrichment Analysis

The 836 DEGs' enriched KEGG pathways and GO function were examined. There were 577 biological processes (BPs), 32 cellular components (CCs), and 52 molecular functions (MFs) under the conditions of and. Leukocyte chemotaxis, neutrophil chemotaxis, defensive responses to other species, and cytokine-mediated signaling pathways were the main BPs of DEGs. Ion channel complexes, membrane transporter structures, transport complexes, and extracellular matrix were the CCs where DEGs were most highly enriched. The cytokine activity, chemokine activity, receptor modulation activity, and cytokine receptor binding of MFs were considerably enriched in the A total of 33 signaling pathways were highly enriched in the LPS group, according to KEGG pathway enrichment analysis. Interleukin-17 tumor necrosis factor (TNF), nuclear factor kappa-B (NF-B), and chemokine signaling pathways were all significantly influenced by DEGs. The possible underlying mechanism of the ARDS-related lung damage process was determined using GSEA. The findings demonstrated a substantial positive connection between the collection of genes engaged in autophagias and apoptotic activities and the ARDS lung injury process [9]–[11].

PPI Analysis and Construction of the carnal Network

A dataset for measuring mRNA expression, was retrieved from GEO 449 human DEGs were chosen under the screening criteria and. The screening parameters produced 2524 DEGs in the rat mRNA expression datasets. The co-expressed DEGs in the two datasets were determined using a Venn diagram for PPI network analysis, the 130 co-expressed DEGs were imported into the online STRING database The PPI network analysis identified a total of 130 node proteins with 419 interacting edges (compared to the 140 predicted interactions), a node degree average of 6.45, a local clustering coefficient average of 0.432, and an enrichment value of 1.0e-16. This means that the proteins interacted more than would be predicted by a random group of proteins with a similar size and degree distribution. This enrichment suggested that the differential proteome may be biologically connected or may participate in a common biological activity during ARDS lung injury. The top 15 key proteins include hematopoietic cell kinase (HCK), fetal growth restriction, chromium b-245 beta chain (CYBB), salmonella pathogenicity island 1 chemokine C-X-C ligand 10 (CXCL Additionally, ceRNA analysis demonstrated that miRNA target genes were accurately identified utilizing the two-part computational prediction stages, which included comparing the sequences of the and assessing the results of the energy stability calculation.

As a result, a total of were produced. The differential miRNA was utilized to forecast the lncRNAs controlled by the rat miRNA miR-21-3p using which demonstrated that there was no lncRNA regulation by this miRNA. To determine the distinct mRNAs regulated by rno-miR-21-3p, Miranda was used to predict the targets of differentially expressed miRNAs. To acquire the targets, integrated miRNA-target, the target prediction was adjusted to 100 kb upstream and downstream of the lncRNA in accordance with the distance between the and known protein-coding gene. As shown in Figure 5(d), we then built a regulatory with 69 nodes and 73 relational pairs. Green circles represent downregulated mRNAs, red triangles represent upregulated miRNAs, blue diamond's represent downregulated known lncRNAs, gray diamonds represent downregulated predicted lncRNAs, and orange hexagons represent Shiga toxin 2 (Stx2), calcium ion/calmodulin-dependent protein kinase II gamma.

Current research in the area of acute and critical clinical care is focused on defining the homogenous subgroups in critical sickness syndromes, such as ARDS. The clinical characteristics (phenotype), molecular mechanism-related therapeutic responses (endogenous type), and/or prognostic risk (prognosis) of critically unwell subgroups have also been gradually revealed to differ significantly from one another. According to clinical studies, patients with the pulmonary intrinsic ARDS phenotype are sicker than those with exogenous pulmonary ARDS. This is evidenced by the fact that they recover from lung injury more slowly, have a lower exhumation success rate, have higher short-term mortality rates, and may have worse long-term prognoses. Nevertheless, despite the emphasis of numerous studies on this, the intricate and varied mechanisms underlying endogenous lung injury remain little understood, and neither are the reasonably efficient therapeutic approaches nor the early diagnostic biomarkers. Based on the current circumstances, the current study used the complete transcriptomic data to explore the key genes and signaling pathways involved in the pathogenesis of endogenous lung ARDS, a unique but widespread subtype of lung injury. It also explored the mechanism of lung injury and provided insights into the prevention and treatment targets.

The oxygenation index, respiratory distress symptoms, and pulmonary pathological alterations in rats were used to diagnose ARDS in this investigation. A rat model of the ARDS lung injury was created by injecting LPS into their airways. Rat ARDS models accurately reflected the typical clinical symptoms and histological alterations seen in individuals with endogenous ARDS lung damage. The LPS (ARDS) group's lung tissue displayed substantial pathological damage, which primarily appeared as dispersed alveolar damage, including atelectasis, edema in the alveoli and interstitial, and the production of hyaline membranes. Additionally, there was a considerable infiltration of inflammatory cells along the pulmonary arterioles, alveoli, and bronchi. This analysis integrates the profiles in endogenous ARDS, to the best of our knowledge. In addition, the endogenous ARDS injured lung contained, and miRNAs in total.

The DEGs were found to be enriched in many BPs, including defensive responses to lipopolysaccharide-induced, the leukocyte chemotaxis, neutrophil chemotaxis, and cytokine-mediated signaling pathways, according to the functional enrichment analysis of whole transcriptomic data from endogenous ARDS lung tissue. By binding to chemokine receptors and exhibiting chemokine activity through transcriptional proteins, DEGs assisted in mediating the inflammatory cells' invasion and infiltration of alveoli, peri bronchioles, pulmonary arteries, and pulmonary capillaries. This results in the release of inflammatory

factors by inflammatory cells through autocrine, paracrine, and degranulation processes. These inflammatory cells also exert receptor regulatory activity and receptor-ligand activity to mediate inflammatory cascade reactions. In addition to generating pulmonary edema, atelectasis, refractory hypoxemia, and respiratory distress signs, these responses harm alveolar epithelial cells and alter pulmonary capillary permeability. The pulmonary arterioles are infiltrated by a large number of inflammatory cells, which causes vascular smooth muscle cell edema, inflammatory proliferation of fibrous connective tissue, and severe hypoxia, all of which play a role in the pathophysiological process of ARDS and induce acute pulmonary hypertension. 33 signaling pathways were highly enriched in the LPS (ARDS lung damage) group, according to KEGG pathways enrichment analysis, and the DEGs were significantly implicated.

CONCLUSION

This research has several restrictions. First, we did not validate any differentially expressed or miRNAs; instead, we only validated a few DEGs of mRNA targets. Second, we only used high-throughput techniques to collect complete transcriptome data for the rat ARDS model at 36 hours without dynamic trend monitoring; next research will need to consider more time points. Thirdly from our study demonstrated that the expression amounts in the ARDs group and the uncontrolled group had different outcomes. We speculate that it might be caused by variations in gene expression between various animals or tissues. Additionally, there could be variations in cytokine expression at various stages of the disease, such as the pro-inflammatory stage or the anti-inflammatory stage, as well as variations in gene expression levels. Finally, future research should explore the process underlying these findings. In the occurrence and progression of endogenous acute lung injury during ARDS, chemotaxis, migration and breakdown of inflammatory cells, cytokine immune response, autophagy, or apoptosis play essential biological roles. The might offer fresh perspectives and opportunities for further research into the mechanism of lung injury and therapeutic treatment.

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