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G protein-mediated pathways and stem cell proliferation and differentiation

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Abstract:

The current popular targets of GPCR activate intracellular signal transduction by binding to G proteins. The drugs developed by it can inhibit the proliferation and migration of cancer cells and promote their programmed cell death. It can be linked to the proliferation and differentiation of stem cells, providing new ideas for regenerative medicine. Research progress has shown that through different Gα subunit activation pathways, these pathways can integrate multiple extracellular signals (growth factors, chemokines, hormones, mechanical forces), and determine the behavior of stem cells by regulating the core transcription factor network. However, there is a lack of detailed depiction and mechanism analysis of the dynamicity (time, space, cellular heterogeneity) and integrative (multi-dimensional signal input, especially mechanical force input in three-dimensional physical environment) of G protein signaling networks in specific stem cell environments. This article analyzed the signals and their correlations mediated by some GPCRs, and recognized their importance in regulating stem cell activities. Regulating the activities of stem cells through GPCRs not only promotes the in vitro cultivation of tissues and organs, but also provides new drug development ideas for human self-healing. However, to understand the complexity of the signals (which may function differently under different time and environmental conditions), further research and analysis are still needed.

Keywords:-G protein-coupled receptors; Stem cell regulation; Signal transduction dynamics

I. Introduction

Stem cell research, as a revolutionary breakthrough in the 21st century, has provided new models and ideas for the medical system, and has also demonstrated huge potential in fields such as regenerative medicine, disease modeling, and drug development. The advent of induced pluripotent stem cells (iPSCs) has made the regeneration of tissues and organs and patient-specific cell therapy possible. With the maturation of iPSCs technology, stem cell research has moved from basic theoretical exploration to a new

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stage of clinical transformation.

G protein-coupled receptors (GPCRs), as a hot target in recent years, have been confirmed to be highly expressed in various types of stem cells (such as neural stem cells and osteoblasts). GPCR can be activated by various stimuli and dissociate from subunits to activate the corresponding enzymatic activity, thereby promoting the production of second messengers (G-protein subunits regulate the release of second messengers and various downstream intracellular signaling pathways, including adenylate cyclase AC, phospholipase A2, C and D, calcium mobilization, mitogen-activated protein (MAP) kinases, phosphoinositide 3-kinase (PI3K), and small GTPases), and regulating downstream signaling pathways [1]. For instance, the cAMP-PKA pathway, where GPCRs activate the activity of AC, thereby regulating the synthesis levels of its downstream factors (such as Yap1 protein which maintains muscle stem cells [2]). The self-renewal and directed differentiation of stem cells are the core mechanisms of tissue repair and regeneration, and GPCRs, by sensing external microenvironmental signals, regulate the dynamic balance of stem cells, providing an important theoretical basis for regenerative medicine.

GPCR as the largest family of cell membrane receptors, can transmit signals that regulate the proliferation and differentiation of stem cells. It can provide solutions to the problems faced by regenerative medicine, and at the same time offer new ideas for drug targets, enabling patients to repair themselves through self-growth. However, the complexity of the GPCR signaling network, receptor dimerization, and microenvironmental interaction mechanisms simultaneously pose challenges in clinical application, such as off-target differentiation, heterogeneous responses of stem cells, and low delivery efficiency [3]. This review comprehends and discusses the role and mechanism of GPCR-mediated pathways in the proliferation and differentiation of stem cells, and explains how they can be targeted in the future to regulate and treat diseases, providing new ideas for regenerative medicine.

II. GPCRs

A. Structure and Classification:

GPCRs are receptors that interact closely with a member of the G protein family. G proteins are defined as guanine nucleotide-binding proteins. As the largest membrane protein superfamily encoded by the human genome, GPCRs encompass more than 800 members. At present, there are multiple classification approaches for GPCRs. The most prevalent classification method categorizes them into Class A (rhodopsin - like receptors), Class B (the secretin family), which further includes Subclass B1 (secretin - like receptors) and Subclass B2 (adhesion receptors), Class C (glutamate receptors), and Class F (frizzled/taste family). Moreover, its key structural characteristics include a highly conserved seven-transmembrane α - helical domain and three major subunits, namely Gα, Gβ, and Gγ.

B. Mechanisms of mediation

Three basic elements determine that signal transduction is carried out through GPCRs: 1) There is a receptor in the plasma membrane with seven transmembrane helical segments. 2) There is a G protein that shuttles between active and inactive forms. 3) There is an effector or ion channel in the plasma membrane that is regulated by the activated G protein. The main mediating form is that after GPCR is stimulated, the G protein detaches from GPCR and binds to the corresponding effector enzyme, promoting the production of second messengers and acting on downstream targets.

III. The Physiological Functions of Stem Cells

Stem cells, as the core functional units for maintaining tissue homeostasis and repairing damage in the body, mainly exhibit their physiological functions in the dynamic balance between multi-directional differentiation potential and self-renewal ability. When tissue damage occurs, microenvironmental signals will activate the regeneration program of stem cells to repair the tissue damage. Mesenchymal stem cells (MSCs) can secrete growth factors, immunomodulatory factors, and exosomes to promote cell repair and regulate immune responses. In the presence of an inflammatory environment (with high levels of TNF-a and IFN-g), MSCs are activated and secrete soluble factors (such as IDO, PGE2, NO, etc.) to suppress immune responses and prevent excessive tissue damage [3]. As one of the key theories in regenerative medicine, stem cells can dynamically respond to microenvironmental signals, coordinate tissue renewal, immune regulation, and pathological repair, providing new ideas for medical treatment.

IV. Pathways Regulating Stem Cells

A. cAMP-PKA Pathway

The activation of the cAMP-PKA pathway is mainly determined by the activity of adenylate cyclase(AC), which decides the strength of the signal. After GPCR is stimulated, Gαs will detach from GPCR to activate the activity of AC, promoting the conversion of ATP to cAMP,

changing the intracellular cAMP concentration gradient, and then stimulating PKA to regulate the expression of transcription factors. It also plays an important role in regulating osteoblasts, osteocytes, and chondrocytes. GPER-1 has been proven to be expressed in the above cells, and GPER-1 can mediate the proliferation of BMSC through the cAMP-PKA pathway. The experiment utilized the specific antagonists and agonists of GPER-1 to act on and culture the tibial cells of neonatal rats and mice. The results indicated that GPER-1 mediated a positive feedback regulation of BMSC proliferation through signal transduction [4,5].

The interaction between CALCR and the cAMP-PKA pathway plays a significant role in the regulation of muscle stem cells. By conditionally knocking out PKA-tg and CalcR (calcitonin receptor) to create mutant mice, it was demonstrated after two weeks of Tamoxifen treatment that the activation of PKA would act downstream of the CALCR signal to prevent the activation of MUSC (muscle stem cells) under the condition of PKA activation [6]. Similarly, by modulating the CALCR receptor signal, we can adjust muscle stem cells. This may make it possible to cure certain muscle injury diseases, especially for some aging-related diseases, such as Parkinson's.

B. Wnt/β-catenin Pathway

The Wnt/β-catenin signaling pathway serves as one of the core molecular mechanisms regulating the fate of stem cells. It can play a significant role in tissue development, maintaining cellular homeostasis and the regeneration process, and the activity of this pathway can directly affect the self-renewal and differentiation direction of stem cells to a certain extent. The Wnt/β-catenin signaling pathway can be activated by the binding of LGR5 (a type of GPCRs) and R-spondins (RSPOs), thereby promoting cell proliferation and the maintenance of stem cells. At the same time, after the binding of LGR5 and RSPOs activates the Wnt/β-catenin signaling pathway, it will regulate the expression of transcription factors such as Gli1 and Sox9. In addition, Wnt ligands can bind to the Frizzled receptors on the cell membrane surface to be activated. After the receptors are activated, they combine with LRP5/6 to promote the phosphorylation of Disheveled (DVL), which in turn inhibits the degradation of β-catenin, allowing it to enter the nucleus and stimulate gene expression [7]. By constructing defective mice and observing the role of Wnt/β-catenin signaling during embryonic development, the results indicated that Wnt3a could stimulate the activity of the Lef1 reporter gene and inhibit the activity of Tcf3, showing a competitive relationship between the two [8]. It demonstrated the interaction mechanism between Wnt signaling and endogenous factor signaling.

C. MAPK/ERK Pathway

The MAPK/ERK pathway (also known as the Ras-Raf-MEK-ERK pathway) is widely involved in various physiological processes such as cell proliferation, differentiation, and migration, and it is a key signal transduction system that regulates the fate of stem cells. Among them, ERK is a crucial factor for cell proliferation and survival. Meanwhile, the MAPK/ERK pathway mediated by GPCR has compartmentalized characteristics, and the activity of ERK may vary in different regions. By using live cell labeling technology and detecting the expression and transport of β2AR, the data results show that the activity of GPCR stimulation to activate ERK occurs in the cytoplasm and endosomes, rather than at the plasma membrane [9]. Unlike the above two signaling pathways, the MAPK/ERK pathway can interact with the cAMP-PKA pathway to jointly regulate the proliferation of stem cells. For instance, the transmission of MAPK and PIK3 signals can reduce the activity of AC, thereby inhibiting the transmission of PKA signals.

D. Hedgehog (Hh) Pathway

The Hh signaling pathway, as a key regulatory route for embryonic development and tissue homeostasis, is associated with SMO (a member of GPCR), and it transmits GLI signals through SMO, playing a significant regulatory role in the development of the nervous system and bones. Meanwhile, it plays a significant role in the process of hair follicle development. The study observed the growth of hair follicles by injecting Hh monoclonal antibodies at different times. The results showed that blocking the Hh signal would inhibit the growth of hair follicles, but it was not involved in the initial induction of hair follicles. Its signal is crucial for the later stage of hair shaft formation [10]. The Hh signaling pathway is associated with the Wnt/β-catenin pathway. During the development of hair follicles in mouse embryos, the activation of β-catenin in the skin development process induces the expression of Shh in the epidermis, stimulating the transmission of Hh signals. However, Hh signals can stimulate the expression of Wnt5a and GLI1, among which Wnt5a can inversely inhibit the classical Wnt/β-catenin pathway [11].

V. Molecular Mechanism of Action

The cAMP-PKA pathway: After GPCRs are stimulated and activated, the Gαs subunit detaches from the GPCR and binds to adenylate cyclase (AC) to activate its activity, promoting the conversion of ATP to cAMP. As a sec-

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ond messenger, cAMP binds to the regulatory subunit (R subunit) of PKA, causing the dissociation of the catalytic subunit (C subunit) and further phosphorylating downstream target proteins to regulate the proliferation and differentiation of stem cells. In addition, PKA has multiple subtypes of regulatory and catalytic subunits as well as multiple splicing variants of the catalytic subunit, forming a vast intracellular microdomain [12].

The Wnt/β-catenin pathway: In the absence of activation of Wnt signaling, β-catenin is degraded and thus unable to enter the nucleus to regulate gene expression. Among them, the β-catenin degradation complex is composed of adenomatous polyposis coli (APC), axin, glycogen synthase kinase 3 (GSK3), and casein kinase 1 (CK1). GSK3β and CK1α phosphorylate β-catenin, which is then ubiquitinated and degraded by the proteasome [13]. The activated Wnt signal is formed by the binding of Wnt protein to Frizzled receptor (FZD) and co-receptor LRP5/6, creating a ternary complex. This activates the downstream signal, recruiting Dvl protein (Dishevelled) to the membrane, phosphorylating LRP5/6, and disrupting the formation of the complex. Consequently, the level of β-catenin in the cytoplasm increases, entering the nucleus to regulate gene expression (such as the transcription of c-Myc, Cyclin D1, and Axin2).

The MAPK/ERK pathway: A common mechanism for GPCR to activate the MAPK/ERK pathway is that after GPCR is activated, Gβγ is released, which then recruits ras-dependent cascade reaction components (such as SHC, GRB2, SRC), leading to the activation of RAF-1 and MAP kinase 1 (specific activators of ERK1/2) [14].

The Hh pathway: The initiation of the signal occurs when PTCH1 binds to Hh, releasing the restriction on SMO (a member of the G protein-coupled receptor family), allowing SMO to enter the cilia. By disrupting the SUFU-Gli complex, Gli is released. Gli2/3 is transformed into the activated form (GliA) and enters the nucleus to initiate the transcription of target genes (such as PTCH1, Gli1, and Cyclin D1). Meanwhile, the target gene PTCH1 can be upregulated through negative feedback regulation to inhibit the activity of SMO, forming a self-regulatory mechanism.

VI. Application Potential in Stem Cell Therapy

The GPCR-mediated signaling pathway is one of the core mechanisms regulating the fate of stem cells. GPCRs dynamically regulate intracellular second messengers (such as cAMP, Ca^{2+}, IP3, etc.) through the activation of heterotrimeric G proteins (such as Gs, Gi, Gq subtypes), thereby influencing the activity of key transcription factors (such as β-catenin, YAP/TAZ), and ultimately determining the self-renewal or differentiation direction of stem cells. Regulating signal transduction through agonists or antagonists to control the process of stem cells is the main research direction of current drugs. For instance, GPR52 agonists can activate the Gs/cAMP/PKA cascade to induce pluripotent stem cells to differentiate into dopaminergic neurons, thereby treating Parkinson's disease. EP4 receptor selective agonists enhance the proliferation of limbal stem cells through the Gαs-PKA pathway to repair corneal damage. At the same time, CRISPR technology can be used to modify GPCRs on the cell membrane, such as the gene editing technology of photosensitive GPCRs (such as Opto-β2AR), to achieve precise light-controlled manipulation of stem cell differentiation, making the in vitro cultured organs and tissues more accurate.

VII. Conclusion

This review introduces the structure, classification and current research progress of GPCRs, and provides a preliminary introduction and analysis of some of the signaling pathways mediated by them. With the support of data and experiments, we can see that GPCR plays an important role in regulating the proliferation and differentiation of stem cells. After analyzing the pathways simultaneously, it is clearly found that there are complex correlations among them. Not only does this regulation have high spatiotemporal specificity and environmental dependence. This undoubtedly increases the difficulty of the research, and at the same time indicates that in the next step, we not only need to conduct in-depth analysis of the influence of a single pathway under different times and environments, but also study the mutual influence and interaction among different pathways. This article only introduces at a basic level the relationship between GPCR-mediated partial signaling pathways and stem cell proliferation and differentiation, and briefly explains the connections among different pathways. However, it does not provide a detailed and in-depth analysis of signal transduction and its various effects. Finally, GPCRs hold significant research value in regenerative medicine. With the maturation of CRISPR technology, by modifying GPCRs on the cell membrane, solutions can be provided for some diseases.

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