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Advances in the Regulation of Osteogenic Differentiation of Periodontal Stem Cells by Signalling Pathways

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Abstract: *Periodontal ligament stem cells (PDLSCs) are mesenchymal stem cells derived from periodontal tissues with self-renewal and multidirectional differentiation potential, and are an important cell population for periodontal tissue repair. At present, studies have shown that various signaling pathways play a crucial role in the osteogenic differentiation of periodontal stem cells, but the mechanisms involved in the regulation of osteogenic differentiation of periodontal stem cells are still in the early stage of research. Therefore, it is necessary to investigate the interrelationship and regulation of these pathways in the osteogenic differentiation of periodontal stem cells, cell signaling and new bone formation, in order to further reveal their molecular biological mechanisms. In this paper, we review the research on the regulation of osteogenic differentiation of periodontal stem cells by Notch signaling pathway, Wnt signaling pathway, NF-κB (nuclear factor-kappa B) signaling pathway and MARK signaling pathway, and provide a theoretical basis for the clinical treatment of periodontal bone defects by targeting the signaling pathways.*

Keywords: *signaling pathway, osteogenic differentiation, periodontal stem cells*

1. Introduction

Periodontal stem cells (PDLSCs) are mesenchymal stem cells derived from periodontal tissues, which have the potential for self-renewal and multidirectional differentiation and are an important cell population for periodontal tissue repair [1]. They can differentiate into osteoblasts, lipoblasts and chondrocytes, and can also form periodontal membrane-like structures, thus PDLSCs can reconstruct periodontal tissues in the oral cavity that have been damaged by periodontal disease and are expected to promote the regenerative repair of periapical bone defects through their osteogenic potential. Signalling pathways are a series of enzymatic pathways that transmit molecular signals from outside the cell into the cell via the cell membrane to exert their effects. From the time a cellular receptor receives an external signal to the final integrated response, it is not only a process of signal transduction, but more importantly, a process of progressive amplification of the external signal. The transition of PDLSCs from a state of self-renewal to osteogenic differentiation is closely related to the differential expression of multiple signalling pathways, and it is therefore important to investigate the mechanisms affecting the signalling pathways in the osteogenic differentiation of periodontal stem cells.

Osteogenic differentiation of periodontal stem cells is a key step in the repair of periodontal bone defects, i.e. periodontal stem cells undergo a complex process of osteogenic progenitor cells, osteogenic precursor cells, osteoblasts and finally differentiate into bone cells, which involves multiple types of inter- and intracellular signaling, such as signaling pathways, transcription factors, growth factors, microRNAs, etc., forming a complete regulatory bone metabolism negative feedback loops [2,3]. In this paper, we review the key points on the progress of research on signalling pathways related to osteogenic differentiation of periodontal stem cells.

2. The Notch signalling pathway

The Notch mutation was discovered in Drosophila by Dexter in 1914 and its allele was recorded and established in Drosophila by Morgan three years later.Notch was named after the gene that causes a defect in the edge of the Drosophila plumage [4,5]. Notch signalling pathways are highly conserved specific

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signalling pathways [6] that play an important role in determining cell metabolism and fate, cell proliferation, growth and development and cell biological behaviour.The Notch signalling pathway is regulated by Notch1, Notch2, Notch3, Notch4 and Jagged1, Jagged2, Dll1, Dll3, Dll4 ligands and receptor activation occurs with adjacent cell lysis and Notch protein translocation into the cell [7-9].

Studies have shown that Notch is quite important for the maintenance of the biological properties of PDLSCs, as well as for terminal osteogenic differentiation and wound healing [10,11]. Previous literature has shown [12-14] that although the Notch signalling pathway is involved in the differentiation process of bone formation in PDLSCs, the mechanism of action of the Notch pathway in the osteogenic differentiation of PDLSCs is unclear. Studies have shown that periodontal stem cells express Notch1 [15,16] and that the behaviour of stem cells can be regulated by cellular and molecular cues from their microenvironment [16-18].

When the Notch receptor binds to the ligand, the internal part of the receptor is truncated and translocated to the nucleus, releasing the ICD (intracellular region) bound to the RBP-Jkappa transcriptional effector complex [19]. Activation of Notch1 suppressed p53 gene expression. Ectopic overexpression of p53 overcame the anti-apoptotic effect of Notch1 signalling, providing further evidence that Notch1 regulates p53 at the transcriptional level. These data suggest that in PDLSCs, ICN may also be localised in the nucleus and may act against apoptosis. The nucleus promotes anti-apoptotic effects by regulating the expression of target genes as well as target genes [20]. Furthermore, Notch receptors are very sensitive to extracellular forces and a small amount of force can identify the site of protease s2 breakdown, which induces its gene transcription and release in the ICD region. PDLSCs modulate Notch1 signalling under chemical and dynamic tension [21], and the expression of the first osteogenic markers ALP and BMP2 under dynamic stress conditions prior to activation was in a time-dependent manner significantly higher than controls. However, after activation of the Notch1 pathway, the expression of osteogenic markers was significantly lower than in the inhibitor and control groups [22].

3. Wnt/β-Catenin signalling pathway

Wnts are a group of secreted glycoproteins that bind to the frizzled protein receptor (FZD). The Wnt family secretes factors that are involved in a number of cellular physiological processes including cell polarization, differentiation, migration, proliferation and biological function.Wnt proteins can be broadly classified into two groups: those that activate the classical Wnt signaling pathway, the Wnt/β-Catenin signaling pathway, and those that are activated by Wnt5a, a non-classical Wnt pathway that is not dependent on β-Catenin and LRP5/6. Periodontal stem cells can regulate classical Wnt proteins and the classical Wnt/β-catenin pathway, and can regulate the transactivation of non-classical Wnt proteins and non-classical signalling pathways [23].

Zheng et al [24] showed that erythropoietin (EPO) (concentration 20 U/mL) could promote proliferation and osteogenic differentiation of PDLSCs by activating the Wnt/β-catenin signalling pathway, presumably by a mechanism related to osteogenesis, by activating β-catenin and increasing the expression of its target gene CyclinD1. Wnt5a may play a plays a role in the development of periodontal disease and alveolar bone loss, regulating the development of periodontal disease, the proliferation and differentiation of periodontal membrane stem cells and the maintenance of periodontal tissue homeostasis, which may, to some extent, provide a theoretical basis for periodontal tissue regeneration [25]. Hasegawa[26] found that rat PDLSCs expressed Wnt5a and human periodontal stem cells (hPDLSCs) expressed Wnt5a and its receptors (Ror2, Fzd2, Fzd4); it was also found that the expression of Wnt5a was positively correlated with the magnitude of occlusal force, and that mechanical stretching tension increased the expression of Wnt5a and its receptors[26] . The proliferation and migration of hPDLSCs can be regulated by direct activation of signalling pathways such as MAPK, Wnt/PCP and PI3K/Akt by Wnt5a[27] . It has also been found in the literature[28] that Wnt5a induces upregulation of periodontal collagen expression, upregulates TGF-β1 expression on periosteal proteins, promotes maturation of periodontal fibres and acts as a positive regulator of collagen fibres to repair periodontal tissue regeneration. After osteogenesis induction of periodontal stem cells, Wnt5a down-regulates the number and size of mineralized nodule formation and, with the expression of osteogenic marker genes, such as OPN, BSP and Osterix, has been shown to inhibit osteogenic differentiation of periodontal stem cells[29,30]. Knockdown of Wnt5a resulted in a significant increase in ALP activity after osteogenic induction of human periodontal stem cells, further showing that Wnt5a can inhibit osteogenic differentiation through regulation of the classical Wnt signalling pathway, while specific inhibition of osteogenic differentiation of hPDLSCs was also observed [31].

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4. NF-κB signalling pathway

NF-κB is expressed in almost all mammalian cells and plays an important role in the normal physiological regulatory network of the human immune and inflammatory response and in systemic diseases (e.g. cardiovascular disease, chronic inflammation, diabetes, cancer). The family includes five members: NF-κBp50 (p105/NF-κB1), NF-κBp52 (p100/NF-κB2), p65 (RelA), RelB and C-Rel. The NF-κB signalling pathway includes both classical and non-classical pathways. The classical pathway is activated by several inflammatory factors, such as tumor necrosis factor α (TNF-α), interleukin 6 (IL-6,) and interleukin 1β (IL-1β), which to some extent inhibit the osteogenic differentiation of periodontal stem cells [32-34]. The transcriptional response to innate and acquired immunity depends on several transcription factors, among which the nuclear transcription factor NF-κB activates multiple signalling pathways and controls several genes. Having multiple functions and unique regulatory mechanisms is particularly important.

The NF-κB signalling pathway can be activated in response to external stimuli, and its studies have shown that inhibition of p65 in the classical NF-κB pathway enhances the differentiation of mesenchymal osteoblasts from a mouse myogenic cell line (C2C12) in vitro [35,36]. Compared to controls, young transgenic mice transfected with specific NF-κB inhibitor genes showed a significant inhibition of osteogenesis, inhibiting NF-κB signalling along with TNF-α on TGF-β-regulated Smad signalling, but still promoting to some extent osteo-differentiation and mineralisation of nude mouse MC3T3 osteoblast progenitor cell lines and bone marrow mesenchymal stromal cells. nF-κB inhibited the osteogenic differentiation of the Saos2 osteosarcoma cell line. NF-κB signalling plays an important role in the differentiation of osteoblasts, osteocytes and chondrocytes.

5. MAPK signalling pathway

The conventional mitogen-activated protein kinases (MAPKs) comprise three subfamily members: ERK1/2, ERK5; JNK1/2/3; and p38. The MAPK pathway is primarily involved in transducing extracellular stimuli (environmental stress, growth factors, cytokines, etc.) that causes cell growth, differentiation and apoptosis. Once cells are exposed to a stimulus, MAPKK kinase (MAP3K) is activated and phosphorylates MAPK kinase (MAP2K), which in turn phosphorylates activated MAPKs[37]. MAPKs are the main kinases of mechanotransduction, and since most mechanosignals to the nucleus are mediated by the activation of these kinases, and the MAPK pathway has been implicated in CTS-induced osteogenic differentiation of hPDLSCs [38,39]. In addition, many cytokines play an important role in periodontal tissue regeneration by promoting the differentiation of osteogenic PDLSCs via the MAPK pathway [40-42].

6. Conclusion

Notch, Wnt, NF-κB and MAPK signalling pathways are all involved in the osteogenic differentiation of PDLSCs in periodontal stem cells, and act directly or indirectly on osteogenic transcription factors such as Runx2, Osterix or PPARγ to regulate the osteogenic differentiation of PDLSCs by activating or repressing transcription factor expression. However, the mechanisms regulating the osteogenic differentiation of PDLSCs into osteoblasts are complex, and the individual signalling pathways are not independent but interrelated. Therefore, there is still a need to explore the interactions between the signalling pathways and their mechanisms in more depth and comprehensively based on the existing studies for the purpose of providing new ideas for the treatment of periodontal bone defect repair.

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