ISSN : 0974 - 7427 Volume 6 Issue 2

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Minireview

BCAIJ, 6(2), 2012 [45-48]

Signaling pathways in endochondral ossification: Implication for cartilage tissue engineering

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Received: 4th January, 2012 ; Accepted: 30th January, 2012

ABSTRACT

Mesenchymal stem cells (MSC) can differentiate into different kind of cell types such as osteocytes, chondrocytes and adipocytes. Therefore, MSC is very promising for tissue engineering, such as cartilage tissue engineering. However, the signaling pathways for cartilage development are also involved in bone formation, either directly or via a cartilage template. Therefore, for cartilage engineering, the choice of factors that promote cartilage development that devoid of bone formation is of great importance, and this mini review addrressed the stages of endochondral bone formation, the signaling pathway that are involved in endochondral bone formation, and its implication in cartilage tissue engineering.

KEYWORDS

Cartilage;
Endochondral;
Ossification;
Tissue engineering;
Chondrcytes.

INTRODUCTION

Mesenchymal stem cells (MSC), which originated from the mesenchyme, are getting more attention in tissue engineering due to their ability to undergo multilineage differentiation. Given the right stimulating condition, mesenchymal stem cells can differentiate into different kind of cell types such as osteocytes, chondrocytes and adipocytes[1]. Cartilage is an avascular tissue and therefore has a poor ability to regenerate itself. Therefore, this chondrogenic potential has a great importance in cartilage tissue engineering.

MSC for autologous use can be isolated from various adult tissues, among others, from bone marrow and adipose tissue. Afizah et al (2007) has published a comparison of chondrogenic potential between MSC isolated from adipose tissue and from bone marrow taken from the same donor. It was reported that bone-marrow derived mesenchymal stem cells (BMSCs) were superior to MSC from adipose tissue in term of chondrogenic potential; and concluded bone marrow as the better MSC source[2].

However, in endochondral ossification, bone formation is initiated through cartilage development[3], and there is no guarantee that cartilage tissue engineering will not proceed to endochondral ossification. Therefore, understanding of the signaling pathway in endochondral ossification is of great importance, as interfering

the pathway may prevent the cartilage from proceeding into ossification. Therefore, this mini review addresses the stages of endochondral bone formation, the signaling pathway that are involved in endochondral bone formation, and its implication in cartilage tissue engineering.

STAGES OF ENDOCHONDRAL BONE FORMATION

Endochondral bone formation consists of five different stages i.e.: MSCs commitment, chondrogenesis, proliferation of chondrocytes, development of hypertrophic chondrocytes and bone formation[4].

MSCs commitment

MSC's committed into chondrocyte differentiation migrate to chondrogenesis site, proliferate, and therefore become condensed. These processes are regulated by mesenchymal-epithelial interaction[5].

Chondrogenesis

Chondrogenesis is a process of cartilage development from mesenchymal stem cells, in which the committed MSCs differentiate into chondroblasts[5].

During early chondrogenesis, committed MSCs start to form extracellular matrix typical for hyaline cartilage such as type II collagen, N-cadherin and tenascin C. After MSCs differentiated into chondrocytes, aggrecan deposition takes place[6]. At the distal end of the developing cartilage, the chondrocytes remain round in shape and form the resting zone of bone formation, which finally will become the articular cartilage.

Proliferation of chondrocytes

Further, chondrocytes proliferate, and undergo early step of maturation. Proliferating chondrocytes become flattened and stacked in a row to form a column parallel to the longitudinal axis of the cartilage[6]. The proliferating chondrocytes form the proliferation zone of endochonfral bone formation.

Development of hypertrophic chondrocytes

In this stage, chondrocytes undergo maturation and hypertrophy and become the maturation zone. As chondrocytes become larger, they secrete alkaline phosphatase and type X collagen into the extracellular matrix[6]. Further, chondrocytes deposit calcium salts in the matrix, and when the matrix became calcified, the region become the calcification zone. Calcified matrix causes impairment in diffusion of nutrition and chondrocytes die by apoptosis, leaving spaces in the calcified matrix, and the region become degeneration zone[4].

Bone formation

The empty spaces left by the dead chondrocytes are invaded by blood vessels and MSCs that differentiate into osteoblast in oxygen-rich condition. The osteoblast lay bone matrix that later becomes calcified, which turn the cartilage into bone and the region become the ossification zone[4].

SIGNALING PATHWAY THAT ARE INVOLVED IN ENDOCHONDRAL BONE FORMATION

The commitment of MSCs to differentiate into chondrocytes is regulated by transcription factors such as Sox and Runx, in addition to signaling molecules such as fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), Indian Hedgehog (Ihh)[7], and transforming growth factor β (TGF-β)[8].

TGF-β signaling pathway stimulates chondrogenesis through activation of Smad2/3 protein, and BMP signaling pathway stimulates chondrogenesis by activating Smad1/5/8 protein. Both signaling pathways interact to each other during different phases of chondrogenesis. Their interaction regulate Wnt signaling through β-catenin in non-canonical and/or canonical pathways, and play an important role in the progress of chondrogenesis into endochondral ossification[8, 9], which is negatively regulated by the Indian hedgehog (Ihh) and parathyroid hormone-related protein (PTHrP)[10]. Further, Wnt/β-catenin pathway is supposed to set the balance of FGF and BMP pathway in mesenchymal stem cells that opt to favor chondrocyte instead of osteocyte fate[11].

In resting zone, the chondrocytes express specific hyaline cartilage extracellular matrix proteins, as well as sex-determining region Y-box 9 (*Sox9)* that is an important transcription factor in early chondrogenesis, and low level of fibroblast growth factor (FGF) receptor 3

(FGFR3)[6].

In proliferation zone, the chondrocytes express low levels of Runx2 and Osterix (Osx)[6], as well as Ihh[12], and high levels of FGFR3, Nkx3.2 and Ptch1[6, 8]. Runx2 and Osx are transcription factors that play a role on chondrocyte differentiation and cartilage mineralization[6], and differentiation into osteoblast[13], directly from mesenchymal stem cells[14]. Ihh stimulates chondrocyte proliferation and induces PTHrP, and together they cooperate to inhibit terminal chondrocyte differentiation, thus chondrocyte hypertrophy. A few PTHrP is secreted by proliferating chondrocytes and it is upregulated when the cells stop proliferating[12]. Nkx3 is a PTHrP signaling dependent transcription repressor, which is expressed by proliferating chondrocytes. It inhibits Runx expression and is supposed to mediate PTHrP function, which prevent chondrocyte hypertrophy and progress to ossification[12]. Ptch1 is a receptor that mediates Ihh signaling, and is upregulated in hypertrophic chondrocytes, thus involved in the progress into ossification[8, 15].

In maturation zone that usually overlapped with calcification and degeneration zone, the chondrocytes initially express Ihh and PTHrP receptor, as well as abundant alkaline phosphatase, Runx2, Osx, matrix metalloprotein13 (MMP13), Vegfa and various growth factors. MMP13 is a matrix degrading enzyme that aids in clearing the remnants of cartilage matrix in the degeneration zone, which creates the bone marrow spaces. Vegfa plays a role in vascular invasion into the spaces that is left by the dead chondrocytes and degraded cartilage matrix[6]. Vascularization follows to form bone marrow and to accommodate surrounding osteoblasts[5]. The growth factors are crucial in the signaling pathway to induce the cells in perichondrium to differentiate into osteoblasts, which secrete the bone matrix in the ossification zone[6].

Further, during chondrocyte maturation and hypertrophy, three important factors play a role in the maturation process, i.e. thyroid hormone, retinoids and BMP[10]. Thyroid hormone is known as skeletal growth influencing hormone during childhood development. Even though cartilage has no vascularization, the thyroxine (T4) and tri-iodothyronine (T3) molecules are able to diffuse in extracellular matrix to bind to their receptors, and in doing so, T3 and T4 molecules work to counteract upregulated PTHrP and stimulate chondrocyte to express type X collagen and alkaline phosphatase, which are molecular markers of hypertrophy[12], and to induce matrix mineralization[10].

Retinoids act in similar manner as thyroid hormones by stimulating cells to express type X collagen. However, retinoids enhance chondrocytes maturation in dose-dependent manner, high concentration of retinoids may lead into inhibition of type X collagen deposition[10]. At the end of the endochondral ossification process, another osteogenic marker, Ihh is upregulated and extracellular matrix mineralization will take place[5].

IMPLICATION OF SIGNALING PATHWAY IN CARTILAGE TISSUE ENGINEERING

In future clinical setting, close connection between chondrogenesis and endochondral ossification process could generate some problems, for instance, during MSC therapy for cartilage repair, on which transplanted cells are expected to remain as chondrocytes, they may undergo further process and initiate bone formation on the cartilage template. Another possibility is the MSCs differentiate into osteoblasts and form bone instead of cartilage. Therefore, for cartilage engineering, the choice of factors that promote cartilage development that devoid of bone formation is of great importance.

Some factors that are supposed to inhibit chondrocyte maturation and thus endochondral bone formation are noggin, chordin, Ihh, PTHrP, Smad6/7[16], Runx2[7], NKx3[12], parathyroid hormone-like hormone (PTHLH), Sox9, stanniocalcin-2 (Stc2), and S100 calcium binding protein A1 and B (S100A1, and S100B)[17]. Ihh, Nkx3, PTHLH and PTHrP cooperate to inhibit chondrocyte hypertrophy, while noggin, chordin[16, 17] and Smad 6/7 are known to have inhibiting effect on both TGF-β and BMP pathway of chondrogenesis[8].

However, using TGF-β and BMP pathway inhibiting factors that prevent the progress into ossification may prevent chondrogenesis as well, when given at the beginning of the process. In addition, Wnt[8, 9, 11], Runx2[6, 7, 13, 14] and Ihh[8, 12, 15] may have dual action depending on the stage of endochondral ossification and with other molecules they interact[6-9, 11-15].

A recent study showed that compared to osteo-

phytic chondrocytes, articular chondrocytes expressed increase in transcript levels of Gremlin-1 (GREM1), FRZP, and Wnt inducible signaling pathway protein-3 (WISP3), which are BMP- and Wnt-signaling pathway inhibitors. The most prominently increased was GREM1 that was confirmed by imunohistochemistry of tissue sections, which may be useful in cartilage engineering as it may stabilize the chondrocyte phenotype permanently[17].

In conclusion, for future cartilage engineering, more researches are necessary to prove whether the key factors that are important in the stimulation of MSCs to undergo chondrogenesis and prevent the progress to endochondral ossification really yield new cartilage devoid of ossification.

ABBREVIATIONS

MSC	=	mesenchymal stem cells
BMSCs	=	bone-marrow derived mesenchymal stem cells
FGFs	=	fibroblast growth factors
BMPs	=	bone morphogenetic proteins
Ihh	=	Indian Hedgehog
TGF-β	=	transforming growth factor β
PTHrP	=	parathyroid hormone-related protein
Sox9	=	sex-determining region Y-box 9
FGFR3	=	fibroblast growth factor (FGF) receptor 3
Osx	=	Osterix
MMP13	=	matrix metalloprotein13
PTHLH	=	parathyroid hormone-like hormone
T4	=	thyroxine
T3	=	tri-iodothyronine
Stc2	=	stanniocalcin-2
S100A1	=	S100 calcium binding protein A1
S100B	=	S100 calcium binding protein B
GREM1	=	Gremlin-1
WISP3	=	Wnt inducible signaling pathway protein-3

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