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Biology and Potential Use of Chicken Bone Marrow-derived Cells

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Abstract

Developmental aspects of chicken embryos showed dramatic difference compared with those of mammals and consequently, such difference in various developmental events leads to different feasibility in both clinical and industrial application. We have concentrated on the studies for using of chicken bone marrow cells and currently we found number of unique cellular properties. Through this article, we reviewed characteristics and cell signaling of osteogenic cells during endochondral ossification in chicken long bone.

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Difference in Embryogenesis between Aves and Mammals

The aves such as chickens, quails and pheasants have unique characteristics, which is based on its evolutional position between mammals and other species. Ex vivo development of the aves is the most prominent difference from the mammalian species which undertake in vivo organogenesis and relevant or addition difference are found during embyogenesis and organogenesis. Differing from mammals, physiological polyspermy is usually induced at the time of fertilization and numerous sperms are visible, regardless of embryonic nuclei formation. Consequently, asymmetric cleavage yielding pre-blastodermal cells is observed throughout early embryogenesis and the intrauterine eggshell formation consisting of three stages is underwent before laying. According to developmental stage, the eggs consisting of 1 to 3,000 cells form yellowish soft eggshell membrane at the phase I. Phase II eggs consisting of 3,000 to 30,000 cells have light yellowish, flexible eggshell and phase III eggs consisting of 30,000 to 60,000 cells have milky white stiffened eggshell. Such difference from the mammals may induce unique cell fate determination in various systems, which may be prominent in mesenchyme-derivative tissues and organs.

Anatomic Feature of Chicken Skeletal System

Endochondral ossification is responsible for the formation of the long bones such as the femurs and humeruses. Unlikely to intramembranous ossification, cartilage tissue is formed prior to ossification, and subsequent ossification acquires the activities of rudimentary long bone formation, extension of its length and natural healing against various damages. Intramembranous ossification induce bone formation without cartilage development. In both process, rudimentary bone formation is completed before birth, while

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the extension of bone length continues until puberty. In mammals, endochondral ossification was completed until the birth and primitive cells for ossification usually disappeared in neonatal offsprings.

The long bones are the source of bone marrow cells, of which endochondral ossification following hypertrophic proliferation and calcium deposition occurs. There has been no report on the details of long bone development in chicken. Consequently, we collected the femurs from 4-day-old neonatal chicks and examined their histological characteristics. As shown in figure 1, no growth plate (hypophyseal line) is detected and proliferate chondrocytes are dominant on the middle of long bone. Probably, cell niche and microenvironment is unique in the long bones collected from neonatal chicks.

Bone Formation (Osteogenesis)

Two different processes, endochondral and intramembraneous ossifications, are involved in bone formation and mesenchymal stem cells differentiating into osteoblasts and chondrocytes play a pivotal role in inducing bone morphogenesis (Shapiro, 2008; Zhang et al., 2011a). A variety of signaling pathways are involved in mesenchymal stem cells differentiation and of those, BMP, Wnt, Notch signaling are important as extracellular mediators of differentiation (Lin and Hankenson, 2011).

The bone marrow cells retrieved from chicks may contain various undifferentiated progenitors and precursors cells and those cells can provide enormous information on cell differentiation when they can be manipulated under a laboratory condition (Csaki, Matis, Mobasheri, Ye, & Shakibaei, 2007; Dai et al., 2014; Friedenstein, Chailakhyan, & Gerasimov, 1987; Hudson et al., 2011; Pittenger et al., 1999). The lineaged cells of bone morphogenetic cells that derives from mesenchymal stem cells includes osteochondro progenitor cells, preosteoblasts, osteoblasts, osteocytes, chondrocytes, proliferate chondrocytes and hypertrophic chondrocytes (Aubin, 2001; Hofstetter et al., 1991). Osteochondro progenitor cells have the ability to become osteoblasts or chondroblasts. Osteochondral progenitor cells are located in the inner layer of the perichondrium and the periosteum, and in the endosteum. From these locations, they either derive new osteoblasts or chondroblasts or differentiate into osteoblastic or chondroblast lineaged cells (Friedenstein, Piatetzky, & Petrakova, 1966; Morikawa et al., 2009; Orlic et al., 2001; Toma, Pittenger, Cahill, Byrne, & Kessler, 2002). Osteochondro progenitor cells having the activity to differentiate into osteoblasts and chondrocytes and continuously find inner surface of the long bones. Isolation of the progenitor cells may be helpful to develop novel therapy for tissue regeneration. For clinical application, optimized and standardized process of cell isolation, purification and amplication for tissue regeneration is essential.

As stated, the osteochondro progenitor cells have the potential to develop into specific lineaged cells related bone morphogenesis when they respond to appropriate each lineage related signals (Garside et al., 2015; Langhans, Yu, & Tuan, 2016; Visvader & Stingl, 2014). Multiple gene and surface protein markers of osteochondro progenitor cells are used for detection. Of those, Sox9 is a transcription factor, which regulates chondrocyte differentiation and anti-Mullrian hormone (AMH) gene transcription (Garside et al., 2015). Sox9 is key regulatory signal for the osteochondro progenitor cells that undergo osteogenic and chondrogenic differentiation and it can control lots of signal pathways. Sox9 is also expressed in osteochondro progenitor cells, which may demonstrate its importance for early signal pathways of bone formation (Pan et al., 2008). At the end of each pathway, osteoblasts form a bone and chondroblasts make a cartilage. Runx2 is known as a core-binding factor subunit a-1 (CBF-a-1) and Osx (a zinc finger containing transcription factor) is necessary for lineage differentiation of osteochondro progenitor cells (S. Chen et al., 2009; Shahi, Peymani, & Sahmani, 2017). Runx2 regulates the expression of major bone matrix genes on differentiation (Chung, 2004; Fujita et al., 2004) and acts as a factor to mature hypertrophic chondrocyte. Wnt/β-catenin signaling influences Runx2 function. A β-catenin signal pathway regulates cell fate determination and WNT/β-catenin signal affects Runx2 expression (Dong, Soung do, Schwarz, O'Keefe, & Drissi, 2006; Hill, Spater, Taketo, Birchmeier, & Hartmann, 2005). In the presence of Wnt3a, an activator of the canonical WNT signaling pathway, β- catenin transport to the nucleus, where it binds TCF/LEF-1 and leads to the transcription of Runx2 (Komori, 2011; Voronkov & Krauss, 2013).

Through Sox9 as a transcriptional mediator, transforming growth factor (TGF)-β and bone morphogenetic protein (BMP) signaling are responsible for initiating expression of cartilaginous extracellular matrix such as aggrecan, collagen types II and XI, fibronectin and tenascin as shown in in vitro cultures (Hatakeyama et al., 2004; Chimal-Monroy and Diaz de Leon, 1999). TGF-β is a representative marker for cell proliferation and differentiation during the stage of bone and cartilage development (Sakaki-Yumoto, Katsuno, & Derynck, 2013). TGF-β signal relates with Sox9 and Runx2 signalling pathways and regulates cell lineages during endochondral ossification (G. Chen, Deng, & Li, 2012; Yang et al., 2001). TGF-β signal stimulates differentiation potential into chondrocytes and osteoblasts with multiple signals like FGF, Msx1, and Ctgf signalling pathways (Guo & Wang, 2009; Parada, Li, Iwata, Suzuki, & Chai, 2013). Alkaline phosphatase (ALP) is a functional gene expressed early in the process of calcification and cartilage formation (Amano et al., 2009). Regulatory signaling pathways involve osteogenic and chondrogenic differentiations, and alkaline phosphatase expression is controlled with WNT/beta-catenin, BMP, Runx2 signaling cascades. Collagen type I is abundant in extracellular matrix component and its gene is listed as a marker for the end product of bone and tendons (Liu, Yang, al-Shaikh, & Lane, 1995). Fully differentiated cells reduce expression of collagen type I and II and increase collagen type X and Aggrecan (Aigner, Dietz, Stoss, & von der Mark, 1995; Haaijman, D'Souza, Bronckers, Goei, & Burger, 1997).

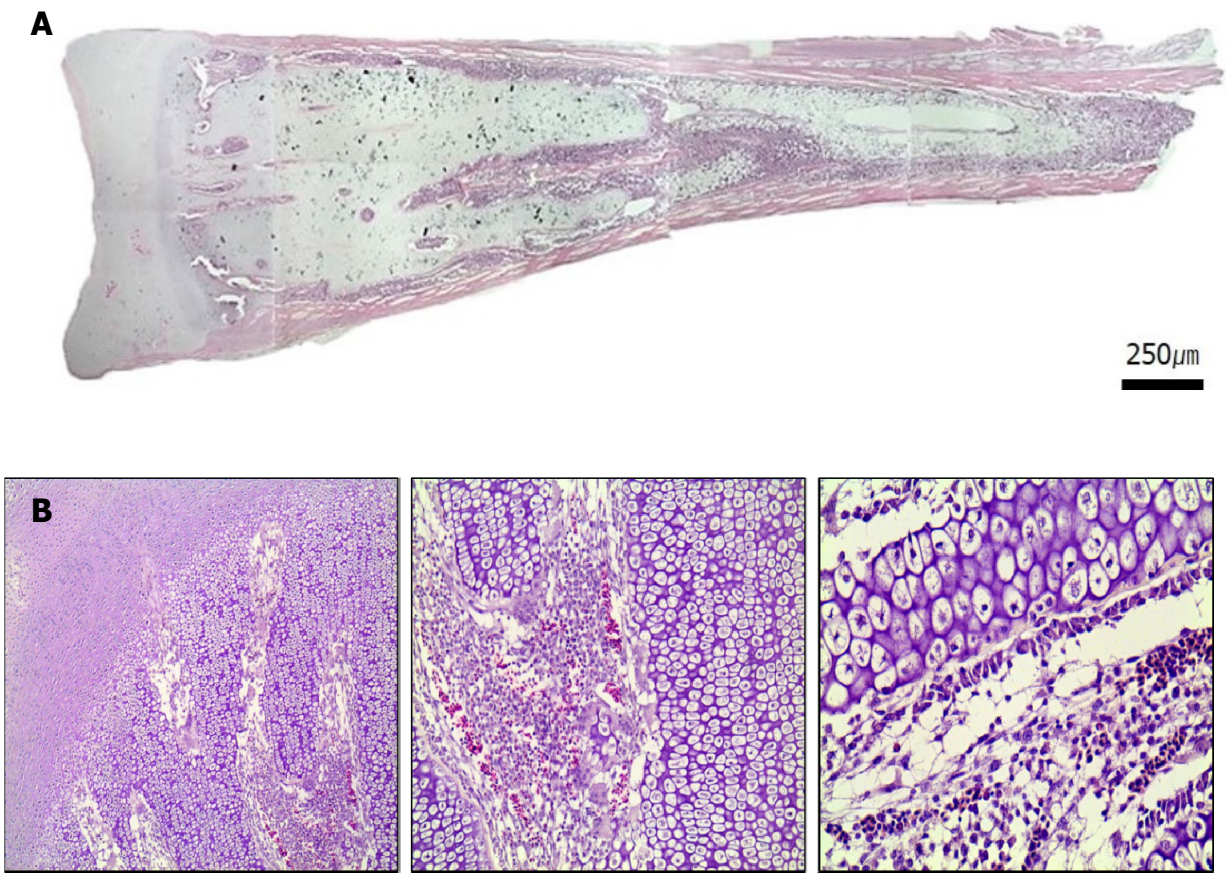

Figure 1. Morphology of the femur retrieved from 4-day-old chicks. (A) The femur has not formed epiphyseal line, which the stromal tissue of diaphysis and epiphysis are mixed. Hematoxylin and eosin staining demonstrated numerous positive cells spreaded into both the epiphysis and the diaphysis regions in the femur. (B) The picture of each site of femur.

Chicken Bone Marrow Cells

Bone marrow tissue is soft and flexible, which finds in interior hollow spaces of bones. Bone marrow consists of red and yellow marrow, which is determined by the predominance of hematopoietic tissue or fat composition (Malkiewicz & Dziedzic, 2012). It is well known that red marrow tissue contains the hematopoietic cells that produces red blood cells, white blood cells and platelets. Hematopoietic cells matured in bone marrow subsequently migrate into capillary sinusoids for entering blood stream (Lang et al., 1992). In adult tissue, red bone marrow is found on the flat bones such as the pelvic girdle and the sternum. In children, red bone marrow is found in the medullary cavity of the long bones, such as the femur (Gurevitch, Slavin, Resnick, Khitrin, & Feldman, 2009; Moore & Dawson, 1990). Yellow marrow acts as a stromal cell producing tissue that derives subcomponents of cartilage and bone (Gurevitch, Slavin, & Feldman, 2007; Zakaria & Shafrir, 1967). As the age is increased, bone marrow becomes yellow tissue and main function of bone marrow changes to store the adipocytes that serves as an energy source (Ortiz-Nieto, Johansson, Ahlstrom, & Weis, 2010; Tavassoli, Houchin, & Jacobs, 1977).

In addition to osteoblasts and osteoclasts, cellular components of bone marrow tissue contain fibroblasts and macrophages, which contribute to producing red blood cells by delivering iron for hemoglobin production. Also, fat cells and sinusoid-constituring endothelial cells are included in bone marrow tissue (Gordon, Pluddemann, & Martinez Estrada, 2014; Mansour et al., 2012; Yin & Li, 2006). Most cells derive from the endothelial stem cells that are also present in the bone marrow. The mesenchyme of embryonic connective tissue, which is derived from the mesoderm, differentiates into hematopoietic and connective tissues, whereas mesenchymal stem cells do not differentiate into hematopoietic cell (Phinney & Prockop, 2007). Mesenchymal stem cells are multipotent stem cells that have various abilities to differentiate into multi-lineage cells (Pittenger et al., 1999) such as osteoblasts (Heino & Hentunen, 2008), adipocytes, chondrocytes (Mackay et al., 1998), myocytes (Xie, Wang, Cao, & Zhang, 2006). Mesenchymal-derived, multipotent cells are valuable resources for cell-to-tissue regeneration and experimental modelling for differentiation and reprogramming (Anbari et al., 2014). Also, Mesenchymal-derived cells are crucial for developing novel stem cell biotechnologies. Various characteristics of bone marrow cells are considered as the basic material for clinical application for treating traumatic injuries and degenerative diseases (Krebsbach, Kuznetsov, Bianco, & Robey, 1999). Stable maintenance of these cells is important for optimizing manipulation protocol and cell amplication.

Bone marrow-derived mesenchymal stem cells can differentiate into multi lineages of mesodermal, ectodermal and endodermal (Woodbury, Reynolds, & Black, 2002). They can differentiate into bone, fat, chondrocyte, muscle, neuron and liver cells under a specific cellular environment (Campagnoli et al., 2001; Pittenger et al., 1999). Differentiation is controlled by the specific genetic regulation pathway that can lead to specific lineage cells. Induction chemicals and growth factors are further accelerate for differentiation and proliferation (Indrawattana et al., 2004; Kim et al., 2005; Li et al., 2007). However, osteochondro progenitors are different from stem cells. In osteogenic differentiation, differentiation medium usually contain dexamethasone, ß-glycerophosphate, ascorbic-2-phosphate was conducted to induce differentiation into osteoblast (Eslaminejad, Fani, & Shahhoseini, 2013). Alizarin red can be used for detecting differentiation with accumulating of calcium (Gregory, Gunn, Peister, & Prockop, 2004). In chonrogenic differentiation, differentiation medium usually contains insulin-transferrin-selenium, bovine serum album, linoleic, ascorbic acid-2-phosphate, TGF-β1 (Day et al., 2005; Joyce, Roberts, Sporn, & Bolander, 1990; Kolambkar, Peister, Soker, Atala, & Guldberg, 2007; Roark & Greer, 1994). As a differentiation marker, alcian blue staining to detect the formation of proteoglycan is used (H. Akiyama, Chaboissier, Martin, Schedl, & de Crombrugghe, 2002; Day et al., 2005).

Gene expression regulates lineage repression. PPAR-γ and C/EBP are involved in adipogenesis and SOX9 induce chondrogenesis (Hu, Tontonoz, & Spiegelman, 1995; Wu, Xie, Bucher, & Farmer, 1995). There is a correlation between PPAR-γ and Cbfa-1 genes. Overexpression of the PPAR-γ gene in adipogenesis represses Cbfa-1 gene expression in osteogenic cells (Heim et al., 2004; Lian et al., 2004).

Possible Application

Limitation of stem cell therapy

Stem cell therapy majorly targets autologous and allogenic cell transplantation into patients by local transplantation or systemic infusion. Regarding bone-derived, cell therapy, mesenchymal stem cells may be available for various clinical applications, which has been continuously studied in regards to stem cell transplantation and autoimmunity (Alexander et al., 2013; Daikeler & Tyndall, 2007; LoCascio, Spinelli, & Kurtz, 2011). Mesenchymal stem cells have low levels of major histocompatibility complex (MHC) class I antigens and do not express MHC class II and other stimulatory molecules (Koch, Lemke, & Lange, 2015). However, different expression pattern is detected, which is influenced by cell type and the cell characteristics and culture condition may effect on mesenchymal stem cells immunogenicity. Based on stem cells characteristic, immunosuppressive properties of transformed (differentiated) cells can be imaged as a potential risk (Flores-Figueroa, Montesinos, & Mayani, 2006; Merino-Gonzalez et al., 2016). Recent study on mesenchymal stem cell injection in the rats showed bone-like tissue differentiation and triggering into calcification of heart tissue (Lv, Liu, Wang, & Zhang, 2016). To avoid potential risk of cell therapy, good manufacturing practices (GMP), standard operating procedures (SOP) for stem cell manipulation is essential to be established.

Clinical therapeutics of Bone marrow derived cells

Mesenchymal stem cells can differentiate into osteoblasts and chondrocytes. The osteochondro-progenitor cells are developed into osteoblast and chondroblast then deposit into outer layer of bones and cartilages during intramembranous and endochondral ossification (Shapiro, 2008). In current clinical therapeutics for cartilage and joint injuries, bone marrow-derived, mesenchymal stem cell directly injects into articular space. (Jin et al., 2007; Frisbie et al., 2009). To advance cell transplantation technology, isolation of osteochondro progenitor cells and optimization of a culture technique are required. Advanced regenerative medicine field is currently using a cartilage autograft implantation system. The current trend leads to the short-term recovery of bone- and cartilage-related symptoms using progenitor cells of osteogenesis and chondrogenesis (Ankrum et al., 2010).

CONCLUSION

Bone marrow cells including bone marrow-derived, multipotent cells are crucial for the development of novel biotechnologies for cell therapy. The bone marrow cells will become a resource of cell-to-cell tissue regeneration and experimental modelling for differentiation and reprogramming (Wagers & Weissman, 2004). Also, they are the critical for the therapy of bone injuries and degenerative diseases (Lu, Shen, & Broxmeyer, 1996). For further clinical application of bone marrow-derived cells, stable maintenance of the cells is important and the optimal culture system for large-scale expansion is necessary and to obtain clinical feasibility, basic researches on identifying target cells and their controlling signals are required.

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