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Mini review

Resident mesenchymal progenitors of articular cartilage

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ABSTRACT

Articular cartilage has poor capacity of self-renewal and repair. Insufficient number and activity of resident mesenchymal (connective tissue) progenitors is likely one of the underlying reasons. Chondroprogenitors reside not only in the superficial zone of articular cartilage but also in other zones of articular cartilage and in the neighboring tissues, including perichondrium (groove of Ranvier), synovium and fat pad. These cells may respond to injury and contribute to articular cartilage healing. In addition, marrow stromal cells can migrate through subchondral bone when articular cartilage is damaged. We should develop drugs and methods that correctly stimulate resident progenitors for improvement of repair and inhibition of degenerative changes in articular cartilage.

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1. Introduction

Articular cartilage does not possess a satisfactory ability for self-renewal and repair, especially at middle and elderly ages. Once articular cartilage is damaged by injury, overload or wasting over age, the defect site does not usually regain original structure and function and may undergo degeneration, leading to chronic joint degenerative disorders such as osteoarthritis. Limited regenerative capacity of articular cartilage is likely due to low metabolic activity of articular chondrocytes and scarcity of resident mesenchymal (connective tissue) progenitors. Marrow stimulation techniques have been established to induce blood supply and recruit mesenchymal stem cells into the affected lesion from bone marrow through the subchondral bone. The procedures for marrow stimulation include transcortical Pridie drilling, abrasion arthroplasty and microfracture (Hunziker, 2002; Schindler, 2011). The autologous chondrocyte implantation (ACI) and ACI with biomaterials (MACI) are other techniques to supply autologous chondrocytes and/or chondrogenic progenitors to a focal lesion from the healthy unloading site of articular cartilage (Schindler, 2011; Oldershaw, 2012). Recently tissue engineering approaches using adult mesenchymal cells and progenitors derived from several types of tissues have been intensively studied. In many

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cases, the cells are grown under various stimuli and led to express a chondrogenic phenotype in culture prior to transplantion to the defect with biomaterials (Oldershaw, 2012; Tuan et al., 2013). These approaches provide favorable outcomes in developing an effective therapy for articular cartilage injury at young ages although long-term observation is required for reaching conclusion (Pastides et al., 2013). In addition, transplantation or injection of undifferentiated mesenchymal stem cells, bone marrow stromal cells or synovial mesenchymal cells into cartilage defects has been attempted in preclinical and clinical studies (Koga et al., 2008; Filardo et al., 2013; Kim et al., 2013) and demonstrated improvement of macro or micro-appearance in defects or better clinical outcome compared to the control without cell transplantation. Nevertheless, successful activation of resident progenitors must be an ideal solution for maintenance of homeostasis and repair of minor injury in articular cartilage. Furthermore, it would enhance repair of big defects in conjunction with transplantation of extrinsic chondrocytes or stem/progenitor cells. In this review, we summarize current information on resident progenitor cells that could contribute to development, maintenance and repair of articular cartilage, and discuss their potential and limitation.

2. Progenitor cells during articular cartilage development

2.1. Interzone cells

Articular cartilage is an essential component of synovial joints and has unique structure, biomechanical properties and function comparing to those of transient growth plate cartilage (Cohen et al., 1988; Wardale et al., 1994; Williams J.A. et al., 2010). How does articular cartilage originate and how is it organized? There are still many issues to be clarified in understanding the entire mechanisms. However, it is now recognized that articular cartilage and other synovial joint components share cell origin and are derived from a unique mesenchymal cell population, called interzone. Interzone appears at the future joint site between two cartilaginous skeletal elements and shows distinct histology and gene expression profile from those of adjacent cartilage elements (Archer et al., 2003; Pacifici et al., 2006; Khan et al., 2007). The two independent studies using a cell lineage tracing technique indicate that interzone cells constitute synovial joint components including joint capsule and articular cartilage, but not majority of the growth plate. Koyama et al. (2008) have generated the compound transgenic mice of *Gdf5*-Cre (Rountree et al., 2004) with *RosaR26R-LacZ* reporter mice and examined the fate of the resulting LacZ-positive cells. The labeled cells first constituted the entire interzone at an early stage, gave rise to the articular cartilage and synovial capsule at later stages but were mostly absent in the growth plate. Hyde et al. (2007) have used the transgenic mice that encode Cre-recombinase in the *Matrillin 1* allele in *RosaR26R-LacZ* background. The LacZ-positive chondrocytes appear in the growth plate, but not in articular cartilage in these mice. These findings indicate that interzone is a source of progenitors for articular cartilage and synovial joint components at embryonic stages, and that the interzone-derived cell population constitutes articular cartilage through life, and may supply progenitor cells to articular cartilage for its renewal.

2.2. Slow-cycling cells in synovial joints

Articular cartilage development is tightly synchronized with the development of other synovial joint structures (Hunziker et al., 2007; Las Heras et al., 2012) and the formation of the secondary ossification center (Blumer et al., 2007, 2008). The mature articular cartilage is divided into the following zones, beginning at the surface: the superficial zone, the transition or mid zone, the deep or radial zone and the calcified zone, and is lined by subchondral bone (Poole, 2003; Hunziker et al., 2007; Becerra et al., 2010; Las Heras et al., 2012). Which mechanism supports growth of articular cartilage and organization of the zonal structure? Where do articular cartilage and other synovial joint tissues obtain stem/progenitors to organize and maintain their structure and function?

The cell labeling studies with tritium thymidine or bromodeoxyuridine have been performed in the developing synovial joints in animals such as rats, opossums and rabbits, and attempt to identify localization of stem cells since the long-term labeling of cells is one of the characteristics of stem cells (Ohlsson et al., 1992; Hayes et al., 2001; Hunziker et al., 2007). Ohlsson et al. (1992) have administrated the tritiated thymidine starting in embryo or young rats and examined the distribution of labeled cells 2 to 4 weeks after isotope administration was stopped. They have found that the long-term labeled cells are present in the proximal portion of growth plate, the perichondrial ring and the surface of articular cartilage, suggesting that the surface of articular cartilage would provide stem cells to articular cartilage. Karlsson et al. (2009) have demonstrated that the groove of Ranvier contains long-term labeled cells with bromodeoxyuridine (BrdU) in the knee joint of 3-month-old rabbits. Along with the finding that this region shows immunoreactivity of the antibodies against progenitor markers Stro-1 and Jagged1, they conclude that the groove of Ranvier has the properties of a stem cell niche and that the groove of Ranvier would directly or indirectly support renewal of articular cartilage. As there is very limited information on the slow-cycling cells in developing synovial joint in mice, we performed cell labeling experiments with a new nucleoside derivative, 5-ethynyl-2′-deoxyuridine (EdU) in mice. Chemical detection of EdU makes the specificity higher and reduces the background. The mice received daily intraperitoneal injections of EdU from postnatal day 4 to day 7 or from E12 to E15. One day after the last EdU injection, a large population of cells in synovial joint cells including articular chondrocytes was labeled. Six weeks after the last injection, the number of EdU-labeled cells dramatically decreased, but a small number of them were still clearly detectable in synovium (Fig. 1A, yellow arrows), infrapatellar fat pad (Fig. 1A, orange arrows), perichondrium/periosteum (Fig. 1B) and ligament attachment sites (Fig. 1E). In articular cartilage, the labeled cells are dominantly present in the articular surface, but also detectable in the other zones (Fig. 1D). These data suggest that slow-cycling cells are present in articular cartilage surface and the adjacent tissues to articular cartilage such as synovium and infrapatella fat pad. Further investigation is required to define whether these EdU-labeled cells are the progenitors that support renewal of articular cartilage and exert a role for joint tissue repair.

2.3. Chondroprogenitors in superficial zone

Several independent research groups including our group have demonstrated that the cells isolated from the superficial zone of postnatal bovine or mouse articular cartilage have progenitor characteristics including high colony formation capacity and expression of mesenchymal stem cell markers and can acquire and express a chondrogenic phenotype after multiple passages (Dowthwaite et al., 2004; Hattori et al., 2007; Yasuhara et al., 2011). Furthermore, the presence of stem/progenitor cells in the superficial layer has been reported in human articular cartilage (Tallheden et al., 2006; Muinos-Lopez et al., 2012). These cells respond to transforming growth factor βs (TGFβs) and enhance synthesis of proteoglycan 4 proteins (also called superficial zone proteins or lubricin) and stimulate expression of cartilage matrix such as aggrecan and collagen 2B in micromass culture (Dowthwaite et al., 2004; Hattori et al., 2007). Using a mouse system, we have demonstrated that treatment of Wnt3a maintains expression of *Prg4* and *Erg* in the superficial layer of cells in culture, while ablation of *β-catenin* strongly impairs proliferation and expression of these genes in the cultured cells and stimulates chondrogenesis in the transplants (Yasuhara et al., 2011). High Mobility Group Proteins 2 (HMGB2) has been shown to be restrictedly expressed in superficial zone and involved in chondrocyte survival and functional maintenance in articular cartilage through the Wnt/β-catenin signaling pathway (Taniguchi et al., 2009a,b). These findings indicate that TGFβ and Wnt/β-catenin signaling are key regulators of proliferation and