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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.

Keywords

Progenitors; chondroprogenitors; articular cartilage; injury; repair

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

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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 (Schindler, 2011;Hunziker, 2002). 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 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 are 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 (Williams J.A. et al., 2010;Wardale et al., 1994;Cohen et al., 1988). 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 (Khan et al., 2007;Pacifici et al., 2006;Archer et al., 2003). 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., 2008;Blumer et al., 2007). The mature articular cartilage is divided into the following zones, beginning at surface: the superficial zone, the transition or mid zone, the deep or radial zone and the calcified zone, and is lined by subchondral bone (Hunziker et al., 2007;Las Heras et al., 2012;Becerra et al., 2010;Poole, 2003). 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 (Hunziker et al., 2007;Ohlsson et al., 1992;Hayes et al., 2001). 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 surface of articular cartilage would provide stem cells to articular cartilage. Karlsson et al (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, presence of stem/progenitor cells in the superficial layer has been reported in human articular cartilage (Muinos-Lopez et al., 2012;Tallheden et al., 2006). 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., 2009;Taniguchi et al., 2009). These findings indicate that TGFβ and Wnt/β-catenin signaling are key regulators of proliferation and differentiation of the superficial cells and may be important for articular cartilage long-term function.

3. Contribution of resident progenitors to articular cartilage repair

3.1 Superficial zone

In osteoarthritic joints, degenerative changes start from cellular disorganization, gradual stiffening and irregular surface of superficial zone followed by loss of matrix, appearance of proliferative chondrocyte clones of fibrillation zones, clefts and osteophyte formation in articular cartilage (Pritzker et al., 2006;Glasson et al., 2010). The superficial zone exhibits reorganization of cell arrangement and cell proliferation at an early osteoarthritis stage (Rolauffs et al., 2011;Rolauffs et al., 2010). Incidence of apoptosis induced by blunt impact, experimental wound or reactive oxygen species (H_2O_2 treatment) was higher in superficial zone than that in deeper zone in immature or mature articular cartilage in explant culture of bovine articular cartilage (Tew et al., 2000;Gilbert et al., 2009;Khan et al., 2008). When such cell death was inhibited by Necrostatin-1 or a pan-caspase inhibitor, tissue deformation and matrix loss were greatly reduced (Gilbert et al., 2009). These findings strongly indicate that superficial zone is an onset of osteoarthritis and thereby a critical target for prevention of osteoarthritis. As we described above, superficial zone of articular cartilage contains chondroprogenitor cells. Interestingly, Seol et al. (2012) have shown that chondrogenic progenitors have superior migration abilities compared to chondrocytes and migrate to

injured sites in response to blunt impact. Thus, chondroprogenitors in superficial zone could play a role in articular cartilage repair. Once articular cartilage is damaged, chondroprogenitors present in the superficial zone may respond to biomechanical and chemical stress and undergo cell death, resulting in poor healing and degeneration of articular cartilage. Further studies are required to clarify how these cells respond to injury, mechanical or chemical stress or inflammation and alter their proliferative and differentiation ability and activity.

3.2 Articular cartilage

Articular cartilage by itself may contain more chondroprogenitor cells than we expected. Several membrane-associated proteins including Notch-1, CD44, CD151, CD105 and CD166 have been selected as biomarkers of mesenchymal progenitor cells in cartilage (Dowthwaite et al., 2004;Grogan et al., 2007;Alsalameh et al., 2004). Interestingly both normal and osteoarthritic articular cartilage contain high number of CD105+/CD166+ cells in the radial (deep) zone, and the cell population containing CD166-positive cells has strong chondrogenic potential (Pretzel et al., 2011). Furthermore, the cells positive to Notch-1, Stro-1, and VCAM-1 -markers of mesenchymal progenitors-have been broadly found throughout normal human articular cartilage from the superficial zone to deep zone and that the number of these cells increased at the middle zone in the non-fibrillated OA (Grogan et al., 2009). The authors emphasize that widely accepted markers may not be applicable for detection of progenitors in articular cartilage. Interestingly the articular cartilage progenitors have a phenotype distinct from bone marrow stromal cells. The former can organize chondrogenic induction maintaining chondrogenicity without calcification or expression of hypertrophic traits in 3D-pellet culture system (Williams R. et al., 2010;McCarthy et al., 2012). The autologous chondrocyte implantation (ACI) (Schindler, 2011;Mastbergen et al., 2013) could be dependent on chondrogenic differentiation of such cell populations as well as proliferation of articular chondrocytes.

3.3 Synovium and infrapatellar fat pad

Recent studies have demonstrated that synovium and infrapatellar fat pad contain mesenchymal stem cells in humans (De Bari et al., 2001;Dragoo et al., 2003;Wickham et al., 2003) as well as in animals (Futami et al., 2012;Koga et al., 2008;Yoshimura et al., 2007;Jones et al., 2008), and that these cells can be utilized for cell-based tissue engineering and treatment for cartilage regeneration (Tuan et al., 2013;Filardo et al., 2013). Interestingly, these cells have superior chondrogenic ability compared to the mesenchymal stem cells derived from other tissues such as bone marrow and muscles (Futami et al., 2012;Yoshimura et al., 2007;Segawa et al., 2009). Mesenchymal stem cells have also been found in synovial fluid in the normal knee joint and their number increase in the affected knee joints (Jones et al., 2008;Morito et al., 2008). The source of these mesenchymal stem cells may be synovium or infrapattelar fat pad because their nature resembles that of the mesenchymal cells isolated from these tissues (Futami et al., 2012;Yoshimura et al., 2007;Segawa et al., 2009). Kurth et al. (Kurth et al., 2011) have demonstrated that slow-cycle cells in the synovium rapidly and strongly respond to the joint injury in an articular cartilage injury model in rabbits. These findings suggest contribution of endogenous progenitors in synovium and infrapaterllar pad to renew and repair articular cartilage.

Recent studies have shown that the LacZ-labeled cells are detected in synovial fibroblasts in *Col2a1-Cre;ROSA26RLacZ* mice and these cells are expanded in the antigen-induced arthritis model of inflammatory arthritis without up-regulation of Cre expression (Fosang et al., 2013). We performed articular injury surgery in the femoral condyle of the knee joint of the *GDF5Cre;ROSA26RLacZ* mice and chased the LacZ-labeled cells. In the control mouse that had received a skin incision only, the LacZ-labeled cells were found in articular cartilage and synovium (Fig. 2A, AC and Synovium, respectively), suggesting these cells are interzone-derived cells (Koyama et al., 2008). One week after surgery, the synovium was thickened and contained a large number of the LacZ-positive cells (Fig. 2B) while the injured site in articular cartilage had less number of the LacZ-positive cells (Fig. 2B, blue box). Three weeks after surgery, the number of positive cells was decreased in synovium (Fig. 2C, synovium). These results suggest that interzone-derived cells rapidly respond to inflammation and injury in synovium. It is important to clarify whether these interzone-derived cells contain progenitor population and how they participate in articular cartilage repair.

3.4 Bone marrow

Bone marrow is rich in adult hematopoietic stem cells and mesenchymal stem cells (Li et al., 2005). The surgical procedures for articular cartilage repair such as transcortical Pridie drilling, abrasion arthroplasty and microfracture (Schindler, 2011) largely rely on mesenchymal stem cells and growth factors coming from bone marrow. It has been demonstrated that chondrogenic progenitors migrate to damaged articular cartilage from the bone marrow cavity underneath the subchondral bone and have stem cell characteristics (Koelling et al., 2009). It is not clear whether these cells contribute to intrinsic repair of damaged articular cartilage. On the other hand, mesenchymal stem cells in bone marrow may impair homeostasis of articular cartilage receiving TGF-β signaling. Zhen et al. (2013) have shown that TGFβ signaling activates nestin-positive mesenchymal stem cells to make clusters and that inactivation of this signaling pathway attenuates osteoarthritic changes in articular cartilage. It is suggested that bone marrow stromal cells can be utilized for osteoarthritis and articular cartilage regeneration, but inadvertent stimulation of these cells could worsen degeneration of articular cartilage.

4. Concluding remarks

Chondroprogenitors are present in articular cartilage, especially in the superficial zone and its neighboring tissues including synovium, fad pad and perichondrium (Ranvier groove). At this moment, we do not have direct evidence that these progenitors support self-renewal and healing of articular cartilage. They may do so in some cases, but not always. Obviously resident chondroprogenitors are attractive therapeutic targets to develop drugs and cell-based tissue engineering for articular cartilage repair. How can we stimulate their potential and increase their life span? The candidate signaling pathways that regulate resident mesenchymal (connective tissue) progenitors include TGFβ signaling (Hattori et al., 2007;Embree et al., 2010) and Wnt/β-catenin signaling (Yasuhara et al., 2011;Taniguchi et al., 2009;Taniguchi et al., 2009;Yuasa et al., 2009). Recently an axis of CBFβ-Runx1 is highlighted for articular cartilage repair (Johnson et al., 2012). In addition, EGFR signaling

might be involved in regulation of proliferation of progenitors in articular cartilage because deficiency of Mig-6, a negative regulator of EGFR signaling increases the number of cells that express putative progenitor cell markers in articular cartilage (Shepard et al., 2013). However, we should keep in mind that these signaling pathways also do induce inhibition of chondrogenic differentiation and/or catabolic actions of cartilage matrix (Zhen et al., 2013;Yuasa et al., 2008;Enomoto-Iwamoto et al., 2002;Zhang et al., 2013;Zhang et al., 2011). Thus stimulation of resident chondroprogenitors requires special cautions. If chondroprogenitors proceed toward differentiation of transient cartilage or fail or stop to express chondrogenic phenotype, it would induce ectopic bone formation or fibrous tissue formation, leading to further degeneration of articular cartilage and deformity of joints. We should continue investigating the mechanisms that support survival and maintenance of these cells and carefully consider how to modulate their proliferation, migration and differentiation regarding level, location, time and/or duration.

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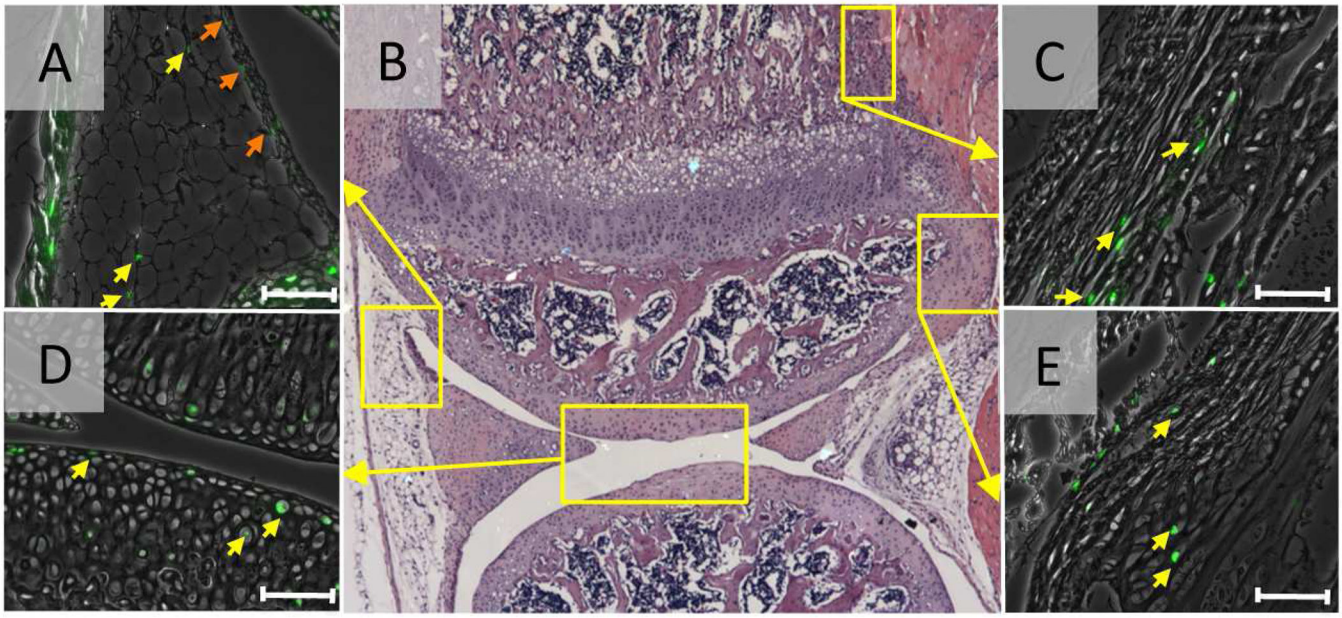

Figure 1.

Slow-cycling cells in synovial joints. The C57BL6/j mice received 4 daily intraperitoneal injections of EdU (5-ethynyl-2'-deoxyuridine, 5 μg/10 μl/ mouse) from postnatal day 4. Scale bars represent 50 μm. Six weeks after the last EdU injection at P7, the knee joints were harvested and subjected to EdU staining. The EdU fluorescence images (green) were superimposed to the phase contrast images. B, Low magnification hematoxyline-eosin staining image of the knee joint. Small number of EdU-labeled cells were detected in the synovium (A, orange arrows) and fat pad (A, yellow arrows), perichondrium/periosteum (C), articular cartilage (D) and ligament attachment site (E). Scale bars represent 50 μm.

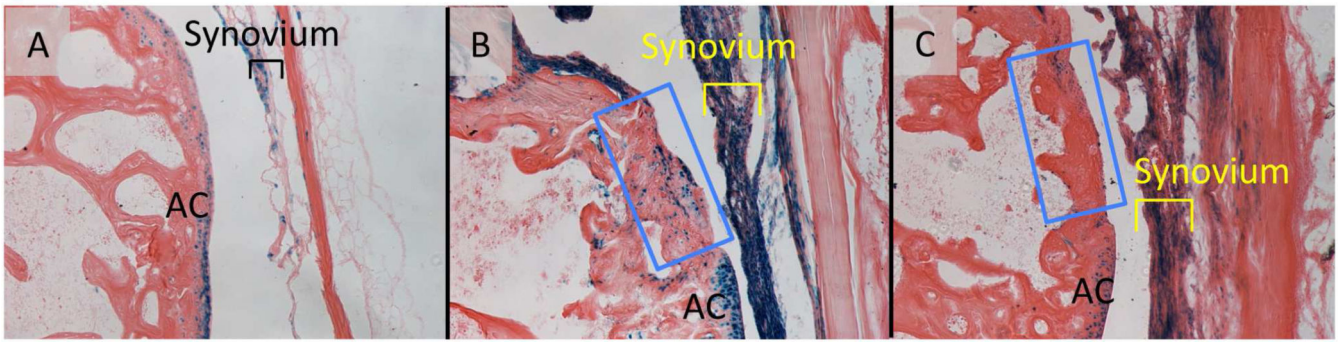

Figure 2.

The LacZ-labeled cells in synovium in the *GDF5Cre; ROSA26RLacZ* mice after articular cartilage injury. Joint surface injury was made in distal femoral articular groove in *GDF5Cre; ROSA26RLacZ* mice. The mice were sacrificed 1-week (B) or 3-weeks (C) after the surgery. The injured (B and C) or sham-operated articular cartilage (A) sections were subjected to β-galactosidase. Injured areas were indicated by blue boxes. AC, articular cartilage.