The Role of Progenitor Cells in Osteoarthritis

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Abstract: It remains a great challenge to enhance the regeneration potential of hyaline cartilage tissue. Tissue degeneration activities initiated after major injury or due to age-related processes override the generally limited self-renewal capacity of this tissue. Numerous catalytic enzymes lead to chondrocyte apoptosis and extracellular matrix deterioration. During early embryonic development, some of the embryonic stem cells of the inner cell mass of the blastocyst will turn into the mesoderm. This will be the founder of the mesenchymal cells in connective tissues of adult life, such as bone, tendon, muscle, and cartilage. Some of these embryonic mesenchymal cells are believed not to differentiate, but to reside in each of the tissues. These are now collectively described as adult mesenchymal stem cells, which are thought to be capable of repairing injured tissue. To date, various populations of bone marrow stroma cells, one of the various populations of adult stem cells, have been described and have been experimentally differentiated into cartilage tissue *in vivo* and *in vitro*. In this review, we will briefly summarize the current knowledge about stem cell related cells in cartilage tissue that are potentially involved in regeneration processes in osteoarthritis. Our unpublished results indicate that a cell population already present in the diseased cartilage tissue might be a starting point for a regenerative therapy for osteoarthritis.

FROM EMBRYONIC STEM CELLS TO MESENCHYMAL CELLS

The original stem cells reside in the inner cell mass of the embryo proper at the blastocyst stage. Before this developmental stage, one could argue, that every cell of the morula is a stem cell. Obviously, cells of the morula are pluripotent and capable of developing into each of the three germ layers that will later, during the course of embryogenesis, develop into the specific tissues to form the organs [1]. These cells, embryonic stem cells, have also been isolated and differentiated *in vitro* [2], and are believed to be a promising starting point for regenerative therapies. However, not only are there ethical problems with the harvesting of embryonic stem cells, they are also known to form teratomas, and, as such, enhanced tumor development after embryonic stem cell treatment is a major problem [3].

During early embryonic development, some of the embryonic stem cells of the inner cell mass of the blastocyst will turn into ectoderm. Derivatives of this germ layer will, for example, develop into skin and brain - tissues devoted to connecting organisms to the exterior world. Some will turn into endoderm. This germ layer will form internal organs - mainly of the gut and liver. The third germ layer, the mesoderm, will develop from the ectoderm, known as the first ectoderm-mesenchyme transition. This mesoderm will be the founder of the mesenchymal cells later found in the connective tissues. In adult life, these tissues, like bone, tendon, muscle, and cartilage, are the building blocks of the skeletal system. Chisa Hidaka and Mary B. Goldring brilliantly discuss the underlying mechanisms of chondrogenesis in this issue. How the commitment of embryonic stem cells towards the mesoderm and from there on to the mesenchyme is regulated is largely unknown [4]. However, Franz Jacob *et al*. reviewed the known molecular mechanisms that regulate mesenchymal stem cell fate in this issue.

To complicate matters, recent reports have highlighted the possibility that isolated, adult differentiated cells like chondrocytes, which were kept in culture on plastic dishes for prolonged periods, dedifferentiate to an extent that allows for the reoccurrence of characteristics of stem cells [5-7]. Also adult skin fibroblasts have been turned into stem cell-like cells with the help of the transfection of the transcription factors oct3/4, sox2, c-myc and klf4 [8]. These results indicate that the nature of the stem cell still remains to be clarified. Moreover, there are several groups of heterogeneous populations called mesenchymal stem cells present in the body. We would prefer to call them mesenchymal progenitor cells, as all those cells described *in vivo* and *in vitro* are migratory cells that have left their original stem cell niche and, therefore, belong to the transient amplifying pool of cells. *Per definitionem*, the stem cell resides in its niche composed of adjacent, more differentiated, cells and extracellular matrix molecules [9, 10]. This concept is beginning to be unraveled for hematopoietic stem cells [11], but yet properly defined for adult mesenchymal stem cells. Recently, decorin and biglycan, two small proteoglycans responsible in collagen fiber generation, have been shown to be extracellular matrix proteins related to a postulated niche of stem cells in the human tendon [12].

Cells of Mesenchymal Origin Can Be Differentiated into Cartilage-Like Tissue *In Vitro*

Friedenstein [13] was the first to describe fibroblast colonies derived from guinea-pig bone marrow and this paved the way to the further characterization of bone marrow stroma cells. To date, various populations of bone marrow stroma cells have been described as stem cells and differentiated into cartilage tissue *in vivo* and *in vitro* [14-21]. Due to space limitation, we can only highlight a few of these studies here. Johnstone *et al*. described rabbit mesenchymal cells differentiated *in vitro* into a tissue staining positive for colla-

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gen type II protein [14]. Mackay *et al*. also described the chondrogenic differentiation of mesenchymal stem cells derived from bone marrow to produce a chondrocyte-like extracellular matrix in pellet culture [15]. Micro-mass culture of human bone marrow stromal cells with the addition of the chondrogenic mediators BMP-6 and TGFß$_3$ resulted in the formation of cartilage-like tissue *in vitro*. Toluidine blue staining to detect the glycosaminoglycan side chains of proteoglycans is often regarded to be sufficient to define the cartilage nature of the resulting tissue [16]. Gronthos *et al*. have also applied bone marrow derived mesenchymal stem cells partially characterized by their expression of STRO-1 [17]. However, this marker is not only associated with cells exhibiting stem cell characteristics, but is also found on fibroblast subpopulations. The other marker related to stem cells that this group applied is CD106 or VCAM-1, which is an adhesion molecule also found on endothelial cells. Chondrogenesis of these STRO-1$^+$/CD106$^+$ cells was proven by the detection of mRNA for collagen type II, type X and aggrecan.

Stem cells from tissue sources other than bone marrow have also been described. Synovia-derived stem cells and cells isolated from the synovial fluid can be differentiated into cartilage-like tissue [22, 23]. The Hoffa fat pad in the knee joint has been described as the origin of stem cells driven into the chondrocyte lineage *in vitro* [24]. Culturing these cells under hypoxic conditions has been shown to enhance their differentiation into cartilage-like tissue [25].

Scaffolds have been introduced to enhance the practicability of transplanting stem cells into a cartilage defect. Of these, nanofibers seem to be most promising [26]. Also, bioreactors should help to generate cartilage tissue *ex vivo*. Here, mechanical compression is essential for the expression of cartilage-like extracellular matrix [27]. The transplantation of chondrocytes into the osteoarthritic defect is not possible because these cells can not be effective in the overall catabolic milieu with its wealth of matrix degrading enzymes present in osteoarthritis [28]. Therefore, chondrocyte transplantation in its present form as described by Klinger and Baums in this issue, is not applicable for osteoarthritis treatment. Up until now, only one study in a goat animal model has described the use of mesenchymal stem cells to treat an osteoarthritic defect. In this case, intra-articular injections of mesenchymal stem cells resulted in minor improvement of the disease process of osteoarthritis. However, the cells migrated to all of the tissues of the knee, except the cartilage tissue itself [29].

Are Mesenchymal Progenitor Cells Involved in Cartilage Repair?

Especially in connective tissues, adult stem cell-like cells have been long known to be responsible for tissue repair after injury. In muscle, stellate cells are found [30] and broken bone heals *via* activation of mesenchymal cells derived from the inner layers of the periost, the connective tissue surrounding each bone [31]. There is evidence that mesenchymal cells characterized by their surface antigens are found in osteoarthritic cartilage tissue [32-34]. Alsalameh *et al*. isolated CD105$^+$ and CD166$^+$ cells from osteoarthritic cartilage tissue by enzymatic digestion and drove them into cartilage-like tissue with the help of micro-mass culture *in vitro*. In addition Fickert *et al*. isolated cells, this time positive for CD9, CD90 and CD166 and were able to demonstrate their differentiation into such a tissue [34]. Moreover, microfracture and Pridie drilling to open the bone marrow underneath the cartilage defect are still used as a therapeutic option and result in a fibrocartilaginous repair tissue. This repair tissue is thought to originate from migrating mesenchymal cells [35-38]. However this regeneration tissue exhibits less resistance to mechanical stress and is composed mainly of collagen type I, which is not typically present in healthy articular cartilage tissue [39, 40]. Finally, postnatal stem cells have been identified in the superficial zone of healthy bovine cartilage believed to be responsible for the appositional growth of the joint surface [41].

PROGENITOR CELLS IN REPAIR TISSUE OF LATE-STAGE OSTEOARTHRITIS

Physiological repair mechanisms of diseased hyaline cartilage tissues are sparse and overridden by matrix destruction resulting in less functional fibrocartilaginous, collagen type I-rich scar tissue [39, 40]. Despite the evidence that stem cells might be involved in regeneration activities seen in osteoarthritis, no studies to date have identified an already committed chondrogenic progenitor cell population in late-stage osteoarthritis. We found evidence that migratory cells derived from repair tissue of late-stage osteoarthritis posses a high chondrogenic potential and progenitor cell characteristics (unpublished results). These chondrogenic progenitor cells seem to be a suitable starting point for the development of a regenerative therapy for osteoarthritis. We isolated a cell population from human osteoarthritic tissue and showed that they posses a multipotent differentiation capacity, especially towards the chondrogenic lineage, as well as a migratory potential. Because these cells show heterogeneity in these properties and because of their migratory potential, we prefer to call them chondrogenic progenitor cells. Furthermore, with the help of RNA knock-down, we have shown that sox-9 and runt-related transcription factor 2 (runx-2) play a central role in the chondrogenic differentiation process of these cells that are also influenced by mediators from the extracellular matrix.

However, there are key limitations inherent to any cell biological therapy of osteoarthritic defects that have to be overcome before a regenerative therapy with progenitor cells will be applicable. First, it has to be shown that these cells can be manipulated to enhance their chondrogenic potential and that it remains present over a long time. The cells present in osteoarthritic tissue are not able to alter the disease process. Their physiological repair capacity is not sufficient. Therefore, it remains to be shown that these cells will produce an extracellular matrix that results in a repair tissue with a higher physical resistance to mechanical stress than the fibrocartilaginous tissue developed during the course of the disease. Finding the optimal conditions to manipulate such cells will be crucial for the development of a cell biological therapy for the treatment of osteoarthritis. Our unpublished results indicate that such a cell population is already present in the diseased cartilage tissue and it might be a good starting point for a regenerative therapy of osteoarthritis.

FUTURE PERSPECTIVES

Resident cells that are already used as a physiological response to the cell biological stimuli of the cartilage tissue, the tissue they are supposed to repair, may be more sufficient as a therapeutic starting point than cells derived from a totally different source such as adipose tissue stem cells. Furthermore, stem cells have been shown to gradually lose their differentiation potential with age. Mesenchymal stem cells from patients with osteoarthritis exhibited a reduced potential for differentiation towards a cartilage-like tissue [42]. Thus, a new concept sees mesenchymal stem cells as a therapeutic means to positively influence the microenvironment of the stem cells already present in the diseased tissue and to direct those cells in their regeneration activities [18]. This is derived from results demonstrating a positive immunomodulatory effect of mesenchymal stem cells, even in allogenic transplantations [43-45]. However, there is also the possibility that stem cells found in osteoarthritic tissue are responsible for the disease process. Before a cell biological therapy of osteoarthritis becomes a clinical reality, numerous scientific questions remain to be addressed *in vitro*. Christopher B. Little and Margaret M. Smith discussed the need to transfer new therapeutic strategies to a proper animal model to carefully evaluate this issue. The manipulation of cells with a regeneration capacity already present in the diseased tissue will be an ideal starting point towards this goal.

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