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Regeneration of Articular Cartilage by Adipose Tissue Derived Mesenchymal Stem Cells: Perspectives From Stem Cell Biology and Molecular Medicine

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Adipose-derived stem cells (ASCs) have been discovered for more than a decade. Due to the large numbers of cells that can be harvested with relatively little donor morbidity, they are considered to be an attractive alternative to bone marrow derived mesenchymal stem cells. Consequently, isolation and differentiation of ASCs draw great attention in the research of tissue engineering and regenerative medicine. Cartilage defects cause big therapeutic problems because of their low self-repair capacity. Application of ASCs in cartilage regeneration gives hope to treat cartilage defects with autologous stem cells. In recent years, a lot of studies have been performed to test the possibility of using ASCs to re-construct damaged cartilage tissue. In this article, we have reviewed the most up-to-date articles utilizing ASCs for cartilage regeneration in basic and translational research. Our topic covers differentiation of adipose tissue derived mesenchymal stem cells into chondrocytes, increased cartilage formation by co-culture of ASCs with chondrocytes and enhancing chondrogenic differentiation of ASCs by gene manipulation.

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Cartilage defects due to trauma, tumor ablation, or age-related abrasion, lead to constant pain and functional limitations of joints and cause serious medical and social problems. It is believed that even small lesions can severely affect the structure and function of articular cartilage and may predispose to the development of osteoarthritis (Alford and Cole, 2005a). The reason for this is quite obvious: no vascularization is present in articular cartilage tissues. Therefore, normal events in tissue repair like inflammation and fibrin clot formation do not happen in cartilage defects. Only chondrocyte and synoviocytes which reside in the local environment can fill up the defects by slow proliferation and matrix deposition (Mankin, 1982; Vincent et al., 2002). In cartilage defects deep into the subchondral bone, bone marrow cells as well as blood cells can migrate to the articular surface by bleeding to fill the gaps with rapid proliferation and matrix synthesis (Furukawa et al., 1980). However, the newly synthesized matrix is usually fibrous. And fibrous cartilage is inferior to hyaline cartilage in mechanical properties (Nehrer et al., 1999). Troubled by the poor self-regeneration of cartilage tissue, clinicians and basic scientists have been working for years on new techniques to find the perfect treatment for cartilage defects.

The most popular treatments for cartilage defects nowadays, are micro-drilling and autologous chondrocytes implantation (ACI). In the micro-drilling technique also known as microfracturing, tiny fractures are induced into the subchondral bone plate by drilling small holes which allow blood and bone marrow to seep out in the defect. This creates a blood clot with incorporated pluripotent mesenchymal stem cells (MSCs). These MSCs eventually heal the defect with scar tissue consisting of a mixture of fibrous tissue, fibrocartilage, and hyaline-like cartilage (Gilbert, 1998). Regarding the clinical outcome, improvements in joint function and pain relief have been reported in 75% of young patients, with even higher success rates in young athletes (Sledge, 2001). However, the quality of the newly formed cartilage is generally out of control, since it may depend on various factors including the gender and age of the patients, the size and location of the defects, the surgical protocols used, and the post-surgery rehabilitation (Alford and Cole, 2005b). In addition, the mechanical properties of scar tissue are inferior compared to native cartilage which may predispose the defected joint to early onset osteoarthritis in the medium to long run. Another treatment called ACI was first introduced by Brittberg et al. (1994). The rational behind ACI is to fill the cartilage defects with autologous chondrocytes which are expanded in vitro. The classical procedure includes arthroscopic excision of biopsies from low-weight bearing areas of healthy cartilage, isolation and expansion of chondrocytes in the laboratory, and implantation of chondrocyte suspension into the defects which is then covered by a periosteal flap sutured to the surrounding healthy tissues.

Nowadays, new technique called matrix-induced autologous chondrocyte implantation (MACI) is becoming more popular. Instead of injection into defects as cell suspension, chondrocytes were seeded on a bilayer of porcine-derived type I/type III collagen, after in vitro expansion. The MACI membrane is then secured directly to the defect by fibrin glue without a cover (Bartlett et al., 2005). Clinical studies with a

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follow-up period of 2–10 years indicated that 90% of treated patients developed well-integrated tissue in the defect sites (Peterson et al., 2003). Despite the success of ACI in clinical practice, there are some drawbacks of this therapeutic method that limit its broader application. One major issue is that the success rate of the procedure severely drops with age limiting the application of ACI to patients under the age of 50 years. Other drawbacks include expensive surgical procedures, donor site morbidity, and dedifferentiation of chondrocytes during in vitro expansion. In vitro expansion is required since relatively large quantities of healthy chondrocytes from the patient are required to fill up the defect site. Replacement of chondrocytes with other cell sources like stem cells gives hope to tackle this problem.

Differentiation of Adipose Tissue Derived Mesenchymal Stem Cells Into Chondrocytes

Adipose tissue, like bone marrow, is derived from the embryonic mesenchyme and contains a stroma that can be easily isolated. It was first reported in 2001, that a group of multipotent cells can be isolated from the stromal vascular fraction (SVF) of collagenase digested human adipose tissue (Zuk et al., 2001). These cells called adipose tissue-derived stromal cells or adipose stem cells (ASCs) can differentiate into adipocytes, osteoblasts, chondrocytes, and myocytes under specific culture conditions in vitro (Zuk et al., 2002). From that point on, many documents have emerged to describe the chondrogenic potential of ASCs isolated from diverse animal models including mouse (Lin et al., 2005b), rat (Lopez and Spencer, 2011), rabbit (Han et al., 2009), dog (Reich et al., 2012), and pig (Wang et al., 2008).

Chondrogenic potential of ASCs

When cultured in medium containing proper growth factors (TGFβ-1, TGFβ-2, TGFβ-3, BMP-2, BMP-6, or BMP-7), ASCs differentiate into chondrocytes in vitro (Lin et al., 2005b; Knippenberg et al., 2006; Mehlhorn et al., 2007). With a few days pre-conditioning in chondrogenic medium, ASCs could form cartilage tissue in vivo (Lin et al., 2005a). Unlike bone marrow stromal cells (BMSCs), ASCs can be isolated in large quantities with minimal morbidity and discomfort clinically (Parker and Katz, 2006). In view of these practical advantages, ASCs are an alternative for chondrocytes or BMSCs in cell based cartilage regeneration strategies.

Regarding the application of ASCs in cartilage repair, infrapatellar fat pad (IFP) could be a more attractive clinical source of ASCs. IFP can give rise to cells that fulfill all the criteria of MSCs, including most importantly significant chondrogenic potential (Dragoo et al., 2003; Khan et al., 2008; Buckley et al., 2010). It was even reported that ASCs derived from osteoarthritic (OA) IFP showed higher chondrogenic capacity than that of bone marrow MSCs and subcutaneous fat-derived ASCs (Sakaguchi et al., 2005; Mochizuki et al., 2006). Moreover, it was reported that chondrogenic potential of IFP derived ASCs was better preserved during in vitro expansion process compared to OA-cartilage derived chondrocytes which rapidly lose their phenotype (English et al., 2007).

Micro-environment needed for cartilage matrix deposition of ASCs

The differentiation medium required to induce chondrogenic differentiation of ASCs usually contains a cocktail of growth factors. Transforming growth factor-β (TGF-β) is considered as the most important component. There are three TGF-β isoforms: TGF-β1, -β2, and -β3. Their distinct roles in embryonic development have been studied intensively in mouse and human (Gatherer et al., 1990; Millan et al., 1991; Schmid et al., 1991). However, their differential functions on extracellular matrix (ECM) formation were just discovered recently. Studies showed that TGF-β3 and TGF-β2 led to significantly higher collagen type II expression and glycosaminoglycans deposition of BMSC than TGF-β1 (Barry et al., 2001). Cals et al. (2012) reported that no significant differences in total collagen and glycosaminoglycans (GAGs) formation could be observed among BMSCs cultured in medium containing the three TGF-β isoforms respectively. However cells induced by TGF-β3 had significantly higher mineralization level than cells cultured in TGF-β1 containing medium. Although we did not find any study in which the differences of TGF-β isoforms on chondrogenic differentiation of ASCs were tested, these data suggest that differences between isoforms of TGF-β may affect ASCs differentiation and ECM deposition as well.

BMP-6 is another important growth factor commonly used in the differentiation medium. It was reported that BMP-6 when combined with TGF-β significantly increased chondrogenesis of ASCs by up-regulating the expression of aggrecan and collagen II with minimal side-effects such as increased collagen type X expression or other characteristics of a hypertrophic phenotype (Estes et al., 2006). The mechanism of the synergistic effects of BMP-6 and TGF-β is that BMP-6 could induce the expression of TGF-β receptor I which is usually not expressed by ASCs (Hennig et al., 2007).

BMP-2 was used as a stimulator for osteogenic differentiation of ASCs (Lin et al., 2008b). However, BMP-2 was also applied to promote the chondrogenic differentiation of MSCs (Kurth et al., 2007; Noth et al., 2007). The cross talk between TGF and BMP signaling suggests an important role of BMP-2 in cartilage matrix deposition (Luyten et al., 1992; Keller et al., 2011). Notably, BMP-2 induced chondrogenic differentiation of MSCs would eventually lead to hypertrophy and endochondral-ossification (Carlberg et al., 2001; Steinert et al., 2009).

BMP-4 is traditionally considered as a trigger of adipogenic differentiation of embryonic stem cells (Taha et al., 2006). A recent article presented BMP-4 as a promising growth factor for ASCs' in vitro expansion since a low dose of BMP-4 increased their viability and maintained their multipotency (Vicente et al., 2011). Addition of BMP-4 in the differentiation medium significantly enhanced the chondrogenic phenotype of ASCs compared to TGF-β1 alone (Kim et al., 2010).

The role of BMP-7 in ASCs differentiation is not as clearly defined as other BMPs. On one hand, BMP-7 has been shown to be an important regulator of brown fat adipogenesis and energy expenditure (Tseng et al., 2008); on the other hand, it is also commonly used in bone tissue engineering to promote healing of critical size bone defects (Yang et al., 2005; Koh et al., 2008; Zhu et al., 2010). To make it even more complex, there are reports claiming that BMP-7 could initiate a more chondrogenic phenotype in ASCs than BMP-2 (Knippenberg et al., 2006). It looks like BMP-7 is involved in all the three mesenchymal lineages and might play multiple roles in the differentiation of ASCs.

In many studies, serum free medium was used for chondrogenic differentiation. It was reported that serum free medium maintained the expression of Sox 9 in chondrocytes during in vitro expansion and sustained their phenotype, while serum caused the de-differentiation of chondrocytes (Malpeli et al., 2004). Another report claimed that fetal bovine serum (FBS) in the differentiation medium inhibited the production of glycosaminoglycans (GAGs) and type II collagens in synovial cells (Bilgen et al., 2007). However, the negative effects of serum on chondrogenic differentiation of ASCs appears to be weak, since differentiation of ASCs towards chondrocytes was observe with the presence of serum (Ogawa et al., 2004; Lin et al., 2005b).

Conventionally, chondrocytes or MSCs must be placed in a three dimensional culture environment such as a micro-mass or a pellet culture before they start depositing cartilage matrix (Koch and Gorti, 2002). One misconception is that 3D (3 dimensional) culture is required for chondrogenic differentiation of ASCs. Actually, chondrogenic differentiation of ASCs involves two biological events: commitment into chondrogenic lineage and deposition of cartilage matrix. There is ample evidence showing that 3D culture environment is not essential for chondrogenic commitment of ASCs. In vitro induction of ASCs in 2D culture was sufficient to make these cells express chondrogenic genes and form cartilage tissue in nude mice (Lin et al., 2005a; Merceron et al., 2011).

Molecular cascades in ASCs during chondrogenic differentiation

We previously identified a group of osteo-adipo progenitors (OAPs) in SVF from adipose tissue (Lin et al., 2008c). This group of cells possess bidirectional differentiation potential which are derived from the Scal-1 negative cell population. They simultaneously express adipogenic and osteogenic genes (RUNX2 and PPAR-γ). Interestingly, PPAR-γ moved from cytoplasm to the nucleus when OAPs differentiated into adipocytes, while RUNX2 stayed in the cytoplasm. In contrast, RUNX2 moved from cytoplasm to the nucleus when OAPs differentiated into osteoblast, while PPAR-γ remained in the cytoplasm (Lin et al., 2008c). This article together with other studies (Enomoto et al., 2004; Heim et al., 2004; Backesjo et al., 2006) demonstrated an interesting reciprocal relationship between osteogenesis and adipogenesis: osteogenic induction enhanced expression of osteogenic genes and inhibited expression of adipogenic genes, while adipogenic induction enhanced expression of adipogenic genes and inhibited expression of osteogenic genes.

When ASCs lose their potential to differentiate into the adipogenic lineage, they seem to be able to differentiate into both chondrocytes and osteoblasts. From a developmental point of view, osteoblasts and chondrocytes share the same progenitor (Zou et al., 2006). During endochondral ossification, mesenchymal progenitors first differentiate into an intermediate bipotential progenitor cell that can give rise to both the chondrocytes which give rise to primary growth plate and the osteoblasts in the bone collar. After a period of proliferation, growth plate chondrocytes become hypertrophic, die and are replaced by osteoblasts depositing bone on the cartilaginous matrix (Mackie et al., 2008). Osteochondral progenitors are not only observed during development, but are also found in vitro. A number of bipotential cell lines have been described to differentiate into both the osteogenic and chondrogenic lineages simultaneously (Grigoriadis et al., 1990; Tominaga et al., 2009). Reciprocal relationship between osteogenesis and chondrogenesis was also found in osteochondral progenitors. Hypertrophic differentiation of chondrocytes, is tightly controlled by the balance of Sox9 and Runx2: Sox9 preserves the chondrogenic phenotype, while Runx2 accelerates hypertrophic differentiation. RunX2 also acts as the master transcription regulator of osteoblastic differentiation (Ding et al., 2011; Dy et al., 2012).

Once ASCs are committed to the chondrogenic lineage, molecular events become clear and simple. Cells stably express Sox9, and then Sox9 triggers the expressions of cartilage matrix proteins, including collagen type II (COL II), collagen type IX (COLIX), aggrecan (ACAN), and cartilage oligomeric matrix protein (COMP) (Lin et al., 2005a). Then a group of cytokines is secreted by mature chondroyctes to maintain the expression of Sox9 and other chondrogenic marker genes such as COL II and ACAN (Polacek et al., 2011). The molecular events regulating the step-wise differentiation from tri-potential ASCs into bi-potential osteochondral progenitors and then into committed chondrocytes are summarized in Figure 1.

Increased Cartilage Formation by Co-Culture of ASCs With Chondrocytes

Cartilage is a unique tissue in which only one cell population resides. Cellular interactions between chondrocytes and other cell types are rare occasions that can only occur at the superficial zone of cartilage and at the interphase between cartilage and the subchondral bone. When co-culture was first introduced into the cartilage field as a research tool (Goldring et al., 1984), it was mainly used to study the pathophysiology of rheumatoid-arthritis and osteoarthritis by investigating the cross-talk between chondrocytes on one hand and synoviocytes on the other (Lubke et al., 2005), or between chondrocytes and osteoblasts (Sanchez et al., 2005). Only recently, it has become clear that co-culture has great potential in cartilage regeneration (Hendriks et al., 2007).

Synergistic effects in co-culture of ASCs and chondrocytes

To reduce the cell number need for ACI, chondrocytes may be partially replaced by other more easily obtained cell types. Tsuchiya et al. (2004) first reported that co-culture of BMSCs and articular chondrocytes enhanced matrix production. The synergistic effects of co-culture were confirmed by other researchers in similar co-culture models (Mo et al., 2009; Hendriks et al., 2010). Meanwhile, increased cartilage matrix formation was also reported in co-culture of chondrocytes with ASCs (Hildner et al., 2009).

To explain the mechanism of increased cartilage formation in co-cultures, two hypotheses have been proposed: (1) increased cartilage formation is due to chondrogenic differentiation of MSCs triggered by signals from chondrocytes; (2) increased cartilage matrix is a result of enhanced activity of chondrocytes stimulated by MSCs. Two hypotheses are illustrated in Figure 2.

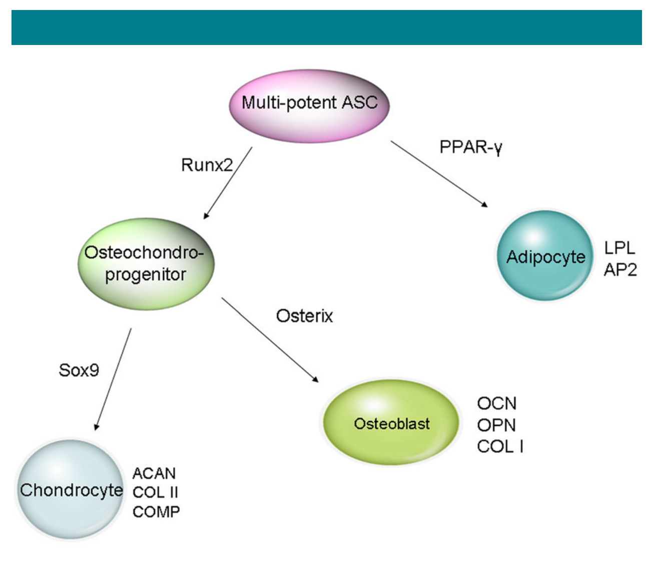

Fig. 1. Schematic representation of molecular events during step-wise differentiation of ASCs from tri-potential stem cells into bi-potential osteochondral progenitors and eventually into committed chondrocytes. LPL, lipoprotein lipase; AP2, adipocyte fatty acid-binding protein 2; OCN, osteocalcin; OPN, osteopotin; COL I, collagen type I, ACAN, aggrecan; COL II, collagen type II; COMP, cartilage oligomeric matrix protein.

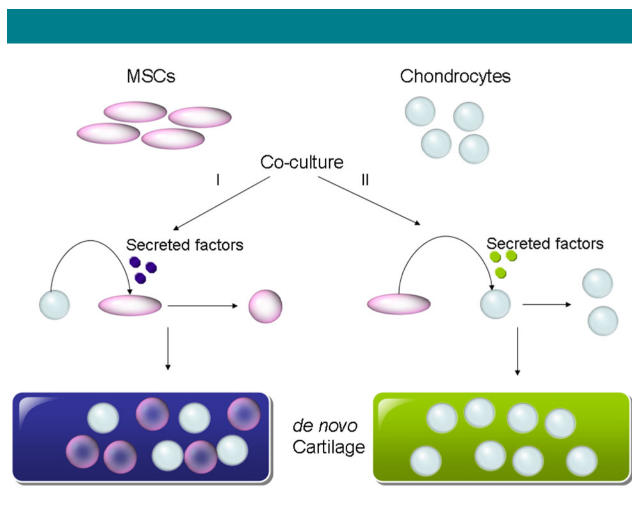

Fig. 2. Two hypotheses explaining the mechanism of increased cartilage formation in co-culture of MSCs and chondrocytes. In hypothesis I (left), signals from chondrocytes induce the chondrogenic differentiation of MSCs, while in hypothesis II (right), MSCs secreted soluble factors to increase the proliferation and matrix deposition of chondrocytes.

Chondrocytes promote differentiation of ASCs

It was suggested that beneficial effects of co-culturing chondrocytes with MSCs are largely due to the differentiation of MSCs into chondrocytes. Soluble factors released from chondrocytes have been shown to support chondrogenesis in an indirect co-culture model of human embryonic stem cells (hESCs) and primary chondrocytes by significantly enhancing the expression of proteoglycans, collagen I and II (Vats et al., 2006). Conditioned medium of chondrocytes could induce osteo-chondrogenic differentiation of BMSCs (Hwang et al., 2007). It was also reported that co-culture of BMSCs and chondrocytes in a 3D environment induced chondrogenic gene expression in BMSCs (Vadala et al., 2008). In a trans-well co-culture system, chondrogenic differentiation of BMSCs is increased by chondrocytes (Chen et al., 2009). More specifically, several studies revealed that ASC could respond to soluble factors released by nuclear pulposus cells by up-regulating cartilage-specific gene expression such as of COL II and aggrecan (Li et al., 2005a; Lu et al., 2007, 2008). A conflicting study reported that direct cell–cell contact was required for the differentiation of BMScs when co-cultured with nucleus pulposus cells (Yamamoto et al., 2004; Richardson et al., 2006b). Nevertheless, many studies so far indicate secreted soluble factors may be responsible for the differentiation of BMSCs in co-culture with chondrocytes.

Trophic effects of MSCs

In a recently published work, we tracked the two cell populations by using a xenogenic co-culture model of human MSCs and bovine chondrocytes (Wu et al., 2011). Their contributions to cartilage matrix formation were therefore separately studied. Our data showed a significant decrease of MSCs in co-culture pellets, resulting in an almost homogeneous cartilage tissue. Thus the beneficial effect of co-culture is largely due to increased chondrocyte proliferation and matrix formation. Chondrogenic differentiation MSCs was shown to be a minor contribution to cartilage formation. Furthermore, these observations are not specific to certain species (combination) or donors. It is the first time a trophic role of MSCs has been demonstrated in stimulating chondrocyte proliferation and matrix production.

Arnold Caplan first proposed MSCs as a trophic mediator for tissue repair (Caplan and Dennis, 2006). Term TROPHIC traditionally refers to the non-neurotransmitters bioactive molecules produced by nerve terminals in neurology (Singer, 1974). When first being introduced in the field of MSCs, the term "trophic effect" referred to the effects that MSCs secrete factors that stimulate releasing of functional bioactive factors from surrounding cells (Caplan and Dennis, 2006). Its definition then expanded to the MSC produced factors that promote cell viability, proliferation, and matrix production in the surrounding environment. The picture has been changed about the roles MSCs played in tissue repair since the introduction of trophic effects into MSCs research. Based on the first pioneer studies, people tend to believe that MSCs repair damaged tissues by differentiating into specific cell types and replacing lost cells (Bruder et al., 1994). But now, more and more researchers considered the trophic roles of the MSC as more important feature of MSCs in tissue repair (Kassis et al., 2011). Evidences supporting the trophic role of MSCs in tissue repair include MSCs improved gain of coordinated functions into brain stroked rats without differentiating into any neuronal related cell type (Li et al., 2005b) and MSCs stimulated cardiomyocyte proliferation (Sassoli et al., 2011) and vascular regeneration (Tang et al., 2005).

As illustrated by recent co-culture studies (Wu et al., 2011; Acharya et al., 2012), the trophic effects of MSCs in cartilage regeneration can be dissected into several layers: (1) MSCs promoted ECM formation of chondrocytes; (2) MSCs increase proliferation of chondrocytes; (3) MSCs died overtime in the co-culture with chondrocytes. Furthermore, our follow-up study demonstrated that the trophic effects MSCs in co-culture pellets stimulating cartilage formation are independent of the culture conditions or MSCs origins (Wu et al., 2012). Co-culture pellets grow in medium stimulating chondrogenic differentiation gave similar results as pellets cultured in proliferation medium. The origins of the MSCs are also proved to be unimportant for their trophic effects since co-culturing chondrocytes with MSCs isolated from bone marrow, adipose tissue and synovial membrane all showed similar results. This implies that it is a very general observation that the MSCs play as trophic mediators in co-cultures with chondrocytes.

Enhanced Chondrogenic Differentiation of ASCs by Gene Manipulation

Besides co-culture ASCs with chondrocytes, over-expression of regulatory genes in ASCs is another strategy to enhance chondrogenic differentiation (Gafni et al., 2004). Our previous studies have shown that ASCs are good cell source for genetic modification (Wu et al., 2007, 2008; Lin et al., 2008b). Genes related to muscle-skeleton development have been introduced into ASCs to improve the differentiation of ASCs (Gimble et al., 2011; Peng et al., 2011). On the list of genes involved in cartilage development, there are generally two groups of genes which are potentially useful for genetic manipulation to boost cartilage regeneration (Trippel et al., 2004). These are genes encoding anabolic growth factors, such as TGF-β, BMPs and insulin-like growth factor (IGF), and transcription factors like Sox-5, -6, and -9 that control chondrogenesis.

Growth factors: TGF-β

TGF-β1 has been regarded as the most powerful chondrogenic growth factor, which induces significant chondrogenic phenotype of ASCs both in vitro and in vivo (Lin et al., 2005a; Lin et al., 2005b). Guo et al. (2006a) reported that a plasmid DNA encoding TGF-β1 could be entrapped into a chitosan-gelatin based biomaterial to enhance ECM deposition of chondrocytes which were incorporated in the same materials. In a similar

study, Guo et al. (2006b) used a slightly different strategy in which a plasmid encoding TGF-β1 was transfected into BMSCs, then transfected cells were applied to repair full-thickness articular cartilage defects in a rabbit model. There are no reports on expressing TGF-β1 or TGF-β3 in ASCs. In contrast, TGF-β2 transduced ASCs have been used. In these studies PLGA (poly-lactic-*co*-glycolic acid)/alginate compound materials have been used to potentiate the differentiation of the genetically manipulated ASCs (Jin et al., 2007, 2008). It has also been demonstrated that TGF-β2 transfected ASCs could repair articular cartilage defects in rabbits (Yang and Tian, 2008).

Growth factors: BMPs and others

Exogenic expression of BMPs in ASCs normally leads to osteogenic differentiation. For example, BMP-2 transfected ASCs developed an osteoblastic phenotype and after loading in an alginate gel were used to repair critical size cranial defects in rat models (Lin et al., 2008b). BMP-7 was also transduced into ASCs to promote bone formation both in vitro and in vivo (Kang et al., 2007). However, there are some BMPs found to induce cartilage matrix formation when over-expressed in pluoripotent stem cells or de-differentiated chondrocytes. These BMPs might be useful to boost cartilage formation when overexpressed in ASCs. Kuroda et al. (2006) reported that BMP-4 transduced muscle derived stem cells (MDSCs) acquired chondrocyte-like characteristics in vitro and formed better cartilage in knee repair models in rats. The repairing results could even be better if BMP-4 was co-tranduced with sFit-1 (Matsumoto et al., 2009). Lin et al. (2008a) demonstrated that BMP-4 could induce re-differentiation of chondrocytes which lost their typical phenotype. The only BMP that has been ectopically expressed in ASCs is BMP-6, due to the special effects of BMP-6 that induces the expression of TGF-β receptor 1 on ASCs (Hennig et al., 2007). Diekman et al. (2010) reported a model of alginate beads to culture ASCs transfected with a pcDNA3-BMP-6 construct and confirmed the induction of chondrogenic differentiation of ASCs.

Other growth factors that were considered for over-expression in ASCs for cartilage tissue engineering purposes are IFG-1, fibroblast growth factors (FGF), and epidermal growth factors (EGF). Results from a previous study suggest that dynamic compression combined with IGF-1 over-expression could benefit cartilage tissue formation of ASCs seeded in chitosan/gelatin scaffolds (Li et al., 2012). Although FGF and EGF are believed to benefit the proliferation of ASCs while keeping their chondrogenic potential (Kilroy et al., 2007; Lee et al., 2009), no transgenic studies have ever been conducted in ASCs with these two groups of factors so far.

Transcription factors: Sox 9 and its family members

Sox 9 is considered as the "master regulator" of chondrogenic differentiation (de Crombrugghe et al., 2000), since it directly controls the synthesis of collagen type II and other ECM matrix in cartilage tissue (Lefebvre et al., 1997; Zhao et al., 1997). A few researchers used adenovirus to deliver exogenic Sox 9 gene in chondrocytes and disc cells to increase the deposition of cartilage specific ECM (Paul et al., 2003). With respect to tissue engineering, Sox 9 was over-expressed in BMSCs by adenoviral transduction (Tsuchiya et al., 2003; Richardson et al., 2006a). Infected BMSCs express higher level of Collagen II than cells without transduction. Recently researchers started expressing exogenous Sox 9 in ASCs in an attempt to boost cartilage matrix formation. Yang et al. (2011b) infected ASCs with a retrovirus expressing Sox 9. In this study, they found that collagen II and proteoglycan production was increased in Sox 9 engineered ASCs. Furthermore, co-culture of Sox-9 transduced ASCs and nuclear pulposus cells in alginate beads resulted in an increase of collagen II and GAGs production. A new trend in these studies is to co-transfect ASCs with SOX Trio (Sox 5, 6, and 9 genes), since Sox 5 and 6 are believed to cooperate with Sox 9 in cartilage development (Han and Lefebvre, 2008; Dy et al., 2010). Studies showed that transfection of SOX Trio initiated the differentiation of ASCs into chondrocyte-like cells both in vitro and in vivo (Yang et al., 2011a). It was even reported that SOX Trio retroviral-transduced ASCs seeded in fibrin gel promoted the healing of osteochondral defects and prevented the progression of experimental osteoarthritis in a rat model (Lee and Im, 2012). Besides plasmid transfection and viral transduction, the delivery method could also be seeding ASCs on PLGA hydrogel incorporated with the pcDNA vector expressing SOX Trio. This method has been successfully used to treat osteochondral defects on the patellar groove of a rabbit model (Im et al., 2011).

Conclusion

Many efforts have been made to improve cartilage regeneration during the last few decades. Advances have been achieved to efficiently differentiate ASCs into chondrocyte-like cells. These findings can be potentially translated into stem cell-based therapies for treating large size cartilage defects. Achievements in this field have shown a wide range of prospects and promise to support cartilage regeneration in the future.

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