Adipose-Derived Stem Cells: In Musculoskeletal Disorders

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Introduction

Conceptually and from a practical standpoint, bone marrow has been the most influential source of stem cells that offers a possibility of being used in a wide range of therapeutics. Clinical situations frequently demand stem cells with dependable quality and quantity to treat disorders of cellular degeneration. Challenges to bring advances to the clinical mount have expanded rapidly, engendering new perspectives concerning the identity, origin, and full therapeutic potential of various tissue-specific stem cells. Recent progress in stem cell biology has allowed researchers to investigate distinct stem cell populations in such divergent mammalian tissues and organs. Taking stem cells adaptable for regenerative medicine applications in adequate quantities at the right time is a challenge. In this respect, an emerging body of literature suggests that redundant adipose tissue serves as an abundant, accessible, and reliable source of stem cells that can be readily harvested with minimal risk to the patients. Rapidly accumulating evidence suggests that adipose tissue-derived stem cells (ADSC), especially from white adipose tissue, possess a far wider property of self-renewal and multilineage differentiation capacity, thereby highlighting their importance and effectiveness in regenerative medicine [1–5]. Despite literature supporting the capacity and plasticity of ADSC for regenerative medicine, there are functional and heterogeneous discrepancies associated with it, thus presenting ADSC research a difficult and challenging task. Promising strides are continuously being made to unravel these challenges and realize the potential of ADSC. While much progress on adipose-derived stem cells has been made in the last few years, there remain a lot to be explored.

This chapter focuses on the overview of adipose-derived stem cells. Further insight into the current knowledge on the advances in the applications of this adipose tissue-derived stem cell in musculoskeletal disorders has also been explored.

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Adipose-Derived Stem Cells

Attention in considering adipose tissue as a reservoir of stem cells was really undertaken only after the findings of Zuk and his coworkers, in the year 2001 [5, 6]. The translation of his findings associated with the easy sampling of adipose tissue with its low risk and morbidity attracted many new investigators. Subsequently, increasing evidences are accumulating on the pivotal role of subcutaneous fat-derived stem cells owing to their proliferative capacity and multilineage differentiation ability [3, 4, 7–11]. To investigate multipotency, several researchers had demonstrated the multilineage differentiation ability of subcutaneous adipose-derived stem cells [2, 3, 6, 12, 13]. For instance, Rodriguez and his coworkers [14] created a single clone from fast-adherent ASCs and proved that two out of 12 clones were able to undergo multilineage differentiation [15]. The remaining ten clones had bipotent capacity. These findings indicate that a high percentage of ASCs have multipotential as well as pluripotential capacity in vitro to differentiate into the major mesodermal and ectodermal lineages.

Adipose tissue-derived mesenchymal stem cells naturally differentiate into mature adipocytes [2, 3, 6, 12, 13]. In adipogenic induction medium such as 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), indomethacin, insulin, and so on, ASCs were found to develop intracellular lipid vacuoles which coalesce and give rise to a single, cytoplasm-filling vacuole. Besides this definite marker of adipogenesis [14], comprising glycerol-3-phosphate dehydrogenase (GPDH), lipoprotein lipase, peroxisome proliferator-activated receptor γ (PPARγ), leptin, adipocyte fatty-acid-binding protein (aP2)11, CCAAT/enhancer binding protein (C/EBP), and glucose transporter 4 (Glut4) are found to be expressed. Similarly, ASCs differentiation towards the osteogenic cell lineage is well established for in vitro as well as for in vivo animal tissue engineering models [2, 3, 12, 13, 16, 41]. A clinical observation is in part responsible for the discovery of the osteogenic differentiation capacity of ASCs. A rare disorder named "progressive osseous heteroplasia" together with the capacity of MSCs to convert into the osteogenic lineage led to the assumption that ASCs are likewise able to differentiate into osteocytes [17]. Osteogenic induction of ASCs can be achieved by similar culture conditions as used in MSCs, including supplementation with ascorbic acid together with 1-alpha,25- dihydroxyvitamin D3 (1,25(OH)2D3), the hormonal metabolite of vitamin D, or dexamethasone [18]. Under osteogenic differentiation medium, ASCs are capable of expressing diverse genes and proteins found in the osteoblast's phenotype: type I collagen, alkaline phosphatase, osteocalcin, osteonectin, osteopontin, parathyroid hormone (PTH) receptor, bone morphogenetic protein-2 (BMP-2), BMP-4, BMP receptors I and II, bone sialoprotein, and RunX-1 [5, 18, 19].

Besides, the efficacy of retention capacity of its characteristics at prolonged culture condition of both rat and human adipose tissue-derived mesenchymal stem cells had also been demonstrated. Both murine and human stem cells were found to retain their properties evidence for the possibility of their characteristics until prolonged culturing. They were able to preserve their long-term stem cell characteristics and differentiation potential even at longer passages [10, 20, 21]. The human subcutaneous adipose tissue showed high telomerase activity that could be maintained for more than 100 population doublings. Thus, evidence is available proving that subcutaneous adipose tissues possess properties of true stem cells, which were retained even after extended in vitro culturing, thereby rewarding a prerequisite for possible successful cell-based therapies [20]. Besides, secretion of various growth factors that control and manage damaged neighboring cells has been an essential function of ADSC [22–24]. It is deduced that ADSCs may exert their beneficial effects via complex paracrine mechanisms in addition to a building-block function. This paracrine effect has been well demonstrated, thereby making ADSCs an attractive therapeutic tool.

Applications of ADSC in Musculoskeletal Disorders

The basic, experimental, and clinical research on SVF/ADSC has expanded exponentially over the past decade. Cell-based therapy using ADSCs presents a unique opportunity for their use in tissue repair and regeneration. The important experimental findings using SVF/ASC in recent years in treating wide range of diseases are increasing, thereby laying a blueprint for ADSC in cellular replacement and regenerative medicine. Adipose-derived stem cells are an abundant, easily accessible, and reproducible cell source for musculoskeletal regenerative medicine applications; however, in vivo repair processes continue to present major challenges. Musculoskeletal defects due to acute trauma, congenital malformations, degenerative diseases, and neoplasia are potential targets for cell-based regenerative therapies. But, the use of collagenase in isolation of ADSCs has made FDA to declare its use as a "Drug" and demands a laborious time-consuming process to acquire permission. This might warrant further investigations into other methods of separation.

Soft Tissue Defects

One of the most intuitive uses of ADSCs is for the replacement of adipose tissue itself. Large soft tissue defects are a common problem following trauma, burns, and oncological resections, such as mastectomy. The regeneration and augmentation of soft tissues requires long-term maintenance of aesthetic results. In order to develop more physiological alternatives for soft tissue reconstruction, several laboratories have investigated the possibility of creating tissue-engineered cell-seeded scaffolds for the generation of de novo adipose tissue. Current therapies are limited, and biomaterials, which include collagen, hyaluronic acid, silicon, and other filler materials. However, the influence of variables such as porosity, biomaterial composition, and seeding density has been under continuous investigations for the optimization of the constructs to improve adipogenesis. Besides, they have several disadvantages such as high cost, immunogenicity and allergenicity, and the risk of transmitting infectious diseases. Although, autologous fat grafts including ADSC [25] are in current clinical practice, their long-term graft retention [26] might be still controversial.

Yoshimura and coworkers used adipose-derived SVF cells for soft tissue augmentation by a novel strategy called cell-assisted lipotransfer (CAL) for treatment of facial lipoatrophy and breast augmentation. It was identified that ASC supplementation has improved its efficacy and improved facial contour, with no adverse effects, although there are no statistically significant difference identified. Furthermore, breast tissue augmentation and reconstruction trial had been reported successful [27–30]. Alternatively, Kim and his coworkers were successful in transplanting the differentiating adipocytes from ADSC for treating depressed scar with up to 75 % success rate [31]. Developing strategies to reconstruct larger tissue defects, however, remains a formidable challenge. Furthermore, although the preliminary studies proved efficient in retention and volume-restoring capabilities of transplanted fat, further attention in these techniques is of utmost importance to draw any conclusion.

Muscular Dystrophies

Muscular dystrophies are a clinically and genetically heterogeneous group of disorders characterized by progressive degeneration and loss of skeletal muscles. The continuous and gradual muscle degeneration in progressive muscular dystrophies leads to depletion of satellite cells, and consequently, the capacity to restore the skeletal muscle is lost. Knowledge of the genetic and molecular mechanisms underlying muscular dystrophies (MDs) has advanced in recent times [32]. However, congenital muscular dystrophies (CMD) are disabling and often lethal disorders. The CMDs share the same muscle pathology status similar to other traditional muscular dystrophies, of which Duchenne and

Becker muscular dystrophies are the major forms. However, the mechanisms leading to the muscle pathologies (sarcolemma instability, degeneration and regeneration of muscle cells, apoptosis and fibrosis) differ between the common CMD types and other muscular dystrophies. Stem cell-based therapy holds promise for treating genetic diseases and has been utilized in animal models and human clinical trials for different types of muscular dystrophies, in particular Duchenne muscular dystrophy [33]. Although initial efforts on myoblast transfer have proven successful, poor cell survival, immune rejection, and poor migration of transplanted cells limited its applications. Cell-based therapies for muscular disease evolved out of interest to restore dystrophin levels in patients with Duchenne muscular dystrophy (DMD). The discovery of muscle-derived stem cells has led to new investigations not only in skeletal muscle disease but also in other applications [19, 34, 35]. However, muscle-derived stem cells are limited and difficult to obtain; hence, alternative way of using adult stem cells from non-muscle tissues to replace damaged muscle fibers for treatments is underway. However, effective cellular therapy for ECM-related CMDs rests on the ability of the therapeutic cells to secrete normal ECM proteins that can prevent muscle cell degeneration rather than on the potential of these cells to differentiate into muscle fibers.

The regenerative potential of BMSCs has been promising. Cells injected into cardiotoxin-damaged muscle were shown to engraft and incorporate into regenerating myofibers. Further trials in a transgenic murine model were also effective [36]. This demonstrates the effect of BMSC to repair damage following acute injury as well as in a degenerative model. Emerging results with ASCs point to the possibility of a similar therapeutic potential. Although bone marrow is the main source for MSC isolation, subcutaneous fat represents an alternative repository for stem cells and is currently a subject of intensive investigations [37]. Adipose cell lineage plays a positive role and is required for efficient muscle regeneration after acute injury. It would be interesting to examine whether an appropriate proportion of adipogenic cells is necessary for the normal growth and maintenance of the skeletal muscle. Another important question to address is at which level adipogenic cells regulate muscle regeneration. Conceivably, preadipocytes may interact directly with myogenic progenitors and regulate their differentiation.

ADSCs were demonstrated to possess myogenic differentiation capacity in vitro as well as in vivo [3, 9, 22, 38]. The in vitro myogenic differentiation potential was evident from the expression of characteristic markers and the formation of multinucleated myotubules. Degenerative diseases, on the other hand, are characterized by slow but progressive accumulation of damage. Cell-based therapies that may replenish the exhausted supply of satellite cells [36] may be particularly suited to prevent this decline. Vitali Alexeev et al. [39] and coworkers demonstrated that ADSCs cultured in vitro secrete a variety of ECM proteins, including collagen VI and, therefore, can provide therapeutic ECM proteins without cell differentiation in the muscle environment for the treatment of congenital muscular dystrophy. Significant work is needed to establish the methods necessary to treat progressive diseases; however, the demonstration that ASCs have myogenic potential both in vitro and in vivo is encouraging. Although these results were statistically significant, it remains to be seen whether they will result in clinically noticeable improvements.

Bone and Cartilage Defects

Both BMSCs and ADSCs have proven to be favorable candidates based on their osteogenic capacity in in vitro and in vivo studies [40–42]. Osteoblast differentiation represents a crucial event during skeletal tissue formation, bone repair, and bone remodeling. Craniofacial defects, in particular calvarial defects, have been of special interest in treatment with ADSC. Due to the restricted amount of BM available in patients with calvarial defects, ADSCs combined with milled autologous cancellous bone and fibrin glue were sued to

repair a large calvarial defect [43]. In efforts to utilize this potential for tissue-engineered bone repairs, many laboratories have begun seeding osteogenically differentiated ASCs onto various scaffolds and biomaterials. The biomaterials that rendered the most significant result are PGA [12], atelocollagen [44], and hydroxyapatite/tricalcium phosphate (HA-TCP) [45]. Although evidence to date suggests that ASCs may one day be useful in the treatment of difficult osseous repairs, further investigations are needed to determine their ultimate safety and efficacy in the clinic.

Cartilage, particularly articular, primarily serves a structural and mechanical function in the body. Clinical cartilage repair has remained an elusive goal for some time. Autologous [46] and allogeneic [47] chondrocyte transplants have been used successfully, but are limited by donor site morbidity and the slow repairs seen, respectively, with these approaches. Recognition of the chondrogenic differentiation potential seen in many stem cells has led to the exploration of an alternative source of cells. Chondrogenic potential, described in vitro in ASCs, includes evidence of cell condensation into nodules and the production of an extracellular matrix rich in proteoglycans and collagen type II [48–51]. It has been proved successful for a minimum period of 12 weeks when implanted with alginate constructs subcutaneously in nude mice [52]. A direct comparison of the in vitro chondrogenic potential of ASCs and BMSCs examined similarities in histological staining and gene expression. In vivo experiments using ASC spheroids had been identified and were successful at generating cartilage-like tissue [53]. Induced spheroids were implanted between two muscle bellies in immunodeficient mice. At 6 weeks, the implants were harvested and found to have produced a cartilage-like tissue consisting of cells within lacunae surrounded by a gel-like extracellular matrix in the absence of any fibrous network. Although this study demonstrates an in vivo potential for differentiation intramuscularly, no physiological models of cartilage repair have yet been tested. To this end, one laboratory is investigating what effects scaffold material [52, 53], oxygen tension [53], and media composition have on the biomechanical properties of ASC-seeded constructs [54]. Clear differences are seen depending on the combination of factors used; however, nothing yet approaches the mechanical properties of mature cartilage. Thus far, results suggest that future in vivo models may demonstrate a potential for ASCs to enhance the healing of debilitating osteochondral diseases. Repairs with the resilience necessary for weight-bearing joints, however, will probably be more difficult to develop. However, ASC seem to have a greater chondropotential effect, and further enhanced work on the same might improve engineering of cartilage in vitro as well in vivo.

Conclusion

Adipose tissue is an abundant, easily accessible, and reproducible cell source for musculoskeletal regenerative medicine applications, especially in conditions where BMSCs could not be obtained, as discussed in the previous chapter. The advances that have been observed with ASC have provided evidence of their great potential and applicability in cell therapy, as well as in the enhancement of healing process. Although its utility is gaining importance in preclinical studies and clinical trials, the factors that drive the repair processes and the details of underlying science continue to present major challenges.

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