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Ligament and Tendon Repair through Regeneration Using Mesenchymal Stem Cells

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Abstract: One of Nature's gifts to mankind is mesenchymal stem cells (MSC's). They are multipotent in nature and are present literally in every tissue. Since, they possess certain characteristics of stem cells such as self-renewal and differentiation they are known to be one of the key players in normal tissue homeostasis. This novel function of mesenchymal stem cells has been explored by scientists in the field of regenerative medicine. This review gives an insight of the various sources of mesenchymal stem cells available for tissue engineering with regard to tendon and ligament and the mechanism involved during regeneration.

Keywords: Mesenchymal stem cells, tendon and ligament.

[#]**Author's Profile:** Following my PhD in biophysics and biochemistry in USA, I have acquired multidisciplinary experience and expertise in the areas of bacteriophages, retrovirology, immunology, immunogenetics, neuro-AIDS, hematopoiesis in HIV infection, humanized SCID animal models, cancer, stem cell biology and regenerative medicine. I have secured funding for projects and produced numerous publications.

INTRODUCTION

The first scientist more than a century ago to have established the presence of stem cells (non-hematopoietic) in the bone marrow which played a significant role in wound healing was Cohneim [1]. Later the work of Friedenstein and colleagues established that certain cells were capable of differentiating into bone tissue and also reconstitute a hematopoietic microenvironment in subcutaneous transplants. The above findings were further substantiated by various scientists that certain stem cells present in human bone marrow were capable of differentiating *in vitro* into different mesenchymal lineages [1, 2-5]. Earlier studies have shown that MSCs originate from neural crest and mesoderm. During a study of the development potential of Quail Neural crest, it was observed that neural crest progenitor cells possessed stem cell characteristics which were observed even in their differentiated cells suggesting a plausible potential role in tissue regeneration [6]. MSCs are found to be present in almost every tissue. One of the important characteristics of MSCs is their ability to self-renew and differentiate into multiple cell types (Fig. **1**). This property of MSCs play a crucial role during normal tissue homeostasis, wherein there is regeneration of new tissues whenever there is internal damage due to inflammation, fracture, trauma and tumors [7]. Whenever there is injury or damage to the cells, the body sends signals which activates the MSCs thus recruiting them to the site of injury or damage, wherein the micro environment which encompasses many factors such as cytokines (TNF-α,IL-1,IFN-α), toxins from infectious agents, and hypoxia condition activates the MSCs to release certain growth factors [8-11]. These growth factors in turn play a vital role during the formation of fibroblasts, endothelial cells and progenitor cells which are the key players which contribute to tissue regeneration [12]. Recent studies have shown that Stro-1 positive cells derived from bone marrow were found to differentiate into multiple lineages of mesenchymal stem cells such as hematopoietic stromal cells and also adipocytes, osteoblasts and chondrocytes [13].

The Mesenchymal stem cells derived from bone marrow were characterized based on the cytokine expression profile. They were also further characterized by the expression of certain markers such as, CD105, CD90, CD73 and adhesion molecule CD166. Conversely, the hematopoietic markers were absent [14-16].

SOURCES OF MESENCHYMAL STEM CELLS

It is quite evident, since the late nineteenth century that pre-clinical and clinical research depended solely on bone marrow derived mesenchymal progenitor cells. However, mesenchymal stem cells have been isolated from several different connective tissues namely adipose tissue, muscle, umbilical cord matrix, liver and dental pulp [17]. A growing body of evidence suggests that MSCs can be isolated from neonatal tissues such as placenta, amnion and umbilical cord blood (UCB). These neonatal tissues also known to harbor a variety of embryonic or premature cell population inclusive of MSCs, endothelial stem/ progenitor cells and hematopoietic stem cells. They exhibit superior cell biological qualities such as improved proliferation, life span as well as differential potential as compared to bone marrow derived

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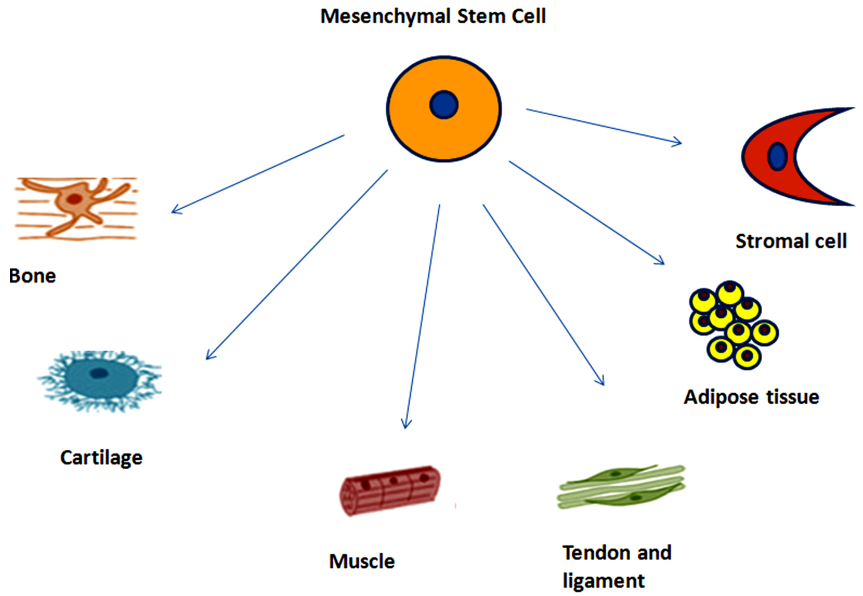

Fig. (1). Mesenchymal stem cells are characterized by their multi-lineage differentiation potential.

MSCs [5]. Placental membrane can be a good source of MSCs which can be used in regenerative medicine [18]. Reports suggests that endothelial cells can also be a source of mesenchymal stem cells, since they can be transformed through overexpression of ALK2 or its activation by TGFβ2 or BMP4 [19].

Earlier studies have reported that induced pluripotent stem cells (iPSCs) derived MSCs have similar characteristics as that of MSCs derived from adult sources [20]. They also had the potential to differentiate into mesenchymal lineages hence can be used for clinical research [21, 22]. Recent studies on MSCs derived from sheep (ovine) fetal tissues suggested as a good source for tissue regeneration [23]. Studies have also shown that human embryonic stem cells can be an excellent source of human mesenchymal stem cells which can be used in regenerative medicine [24].

A very recent study on the mesenchymal stem cells of dental origin showed that a considerable population of mesenchymal stem cells is involved in the regeneration process of the tooth and it was found that these cells are derived from peripheral nerve associated glia. These glial cells are known to generate multipotent mesenchymal stem cells that produce pulp cells and odontoblasts [25]. Encapsulated dental MSCs were developed for the investigation of their ability to differentiate into tendon tissues [26]. The results showed a marked increase in expression of gene markers related to tendon regeneration they are Scx, DCn, Tnmd and Bgy. The *in vivo* results showed ectopic neo-tendon regeneration in subcutaneous transplanted MSC-alginate constructs. Together their findings suggest that the above encapsulated MSCs could be a considered for tendon regeneration in clinical studies [26].

Recently, allogeneic adipose derived mesenchymal stem cells (ASC) along with platelet rich plasma (PRP)were used for the treatment of horses with superficial digital flexor (SDF) tendonitis, the results showed that after a follow up of 24 months, 89.5% horses returned to the competition with 10.5% re-injury. This treatment did not show any side effects such as acute of chronic adverse tissue reactions nor any formation of abnormal tissues in the long term thus, suggesting that the above treatment could be considered as a safe and effective approach in the treatment of SDF tendonitis in horses [27]. It was also observed that autologous bone marrow mesenchymal stromal cells were used for the treatment or regeneration of injured equine ligaments and tendons. The results showed that there was no adverse effect of the treatment with MSCs and thirteen out of eighteen treated horses returned to their sports activity, thus proving the safety of the MSC treatment at clinical trials [28].

SIGNALING PATHWAYS INVOLVED DURING REGENERATION OF TISSUE REPAIR

A growing body of evidence suggests that mesenchymal stem cells in the human body are like store house for regeneration of cells, thus playing a vital role in tissue homeostasis. As discussed above, apart from the injuries/ inflammations, cytokines, tissue microenvironment and growth factors, which regulate the fate of MSCs, there are signaling molecules or pathways which play a significant role in differentiation of MSCs which ultimately leads to tissue repair. Some of the important signaling pathways are Wnt/ β-Catenin, BMP, TGF-β, Nell-1 and Notch.

Wnt signaling is one of the key players during development encompassing around nineteen Wnt receptors and coreceptors that have been identified throughout seven families of proteins. Wnt signaling through both canonical (β-catenin dependent) and non-canonical (β-catenin independent) pathways has established proosteogenic and antiadipogenic activities. The β-catenin dependent pathway initiates with the binding of extracellular Wnt ligands to the seven-pass transmembrane frizzled receptors (Frz) expressed at the cell surface. Ultimately, β-catenin dependent Wnt signaling elicits gene transcriptional activity to influence MSC lineage determination. Canonical Wnt signaling has well-established effects on bone mass in both animal models and human patients. A direct role for β-catenin in regulating osteoblast and osteoclast activity has been reported. SHH activity may be a

key in stimulating osteoblastogenesis only during early stages of cell differentiation. An earlier result suggests that HH signaling promotes MSC osteogenic differentiation primarily *via* Gli transcriptional factor activity [29]. The Wnt-coreceptor LRP6 (low-density lipoprotein receptor related protein) is very important for the Wnt 3a/ β-catenin signaling and is also known to regulate human mesenchymal stem cell differentiation into adipogenic lineage [30].

The secreted molecule NELL-1 (NEL-like protein 1) was first discovered to have osteoinductive properties by its over expression during premature bone formation in human sporadic coronal craniosynostosis [31, 32]. NELL-1 is expressed during both intramembranous and endochondral bone formation. Overexpression of Nell-1 was known to play a significant role in bone development. Runx2 is known to be upstream of Nell-1and it was found that both the proteins were found to be expressed at the same levels and in significant amounts during skeleton development [33-35]. NELL-1 is known to promote osteogenesis accompanied by activation of MAPK, canonical Wnt and HH signaling [36-38]. This activation of MAPK signaling is associated with Runx2 protein phosphorylation [36]. In addition, NELL-1 induced MAPK activity is accompanied by activation of phosphate transporters Pit1 and Pit2 to increase pre-osteoblast mineralization. NELL-1 induction of Wnt signaling has been observed in both osteoblastic and osteoclastic cell types and is associated with its proosteogenic and antiosteoclastic effects.

Bone morphogenetic proteins (BMPs), are extracellular cytokines originally isolated from bone extract and found to induce of ectopic chondrogenesis and osteogenesis. BMPs are responsible for numerous cell regulatory processes, including the differentiation and patterning of bone and cartilage [39]. Over 20 different BMPs have been identified, of which BMP-2, -4, -7, -9, and -13 are most commonly studied in the context of MSC differentiation [40, 41]. BMPs produce their effects through interaction with two serine-threonine kinase cell surface BMP receptors (BMPRs). Type II BMPRs initiate signaling upon binding to a BMP ligand, following which recruitment, phosphorylation, and activation of type I BMPRs occurs. While there are several different type I BMPRs, only a few are involved in MSC differentiation, including BMPR-IA and BMPR-IB. Smad1/5/8 signaling transduction is the most significant to MSC differentiation, as it is principally through the Smad-protein complexes that transcriptional regulation of osteogenic programming is regulated [29]. Yu Dong *et al.*, [42] showed that tendons wrapped by BMP2- transfected bMSCs had the potential to improve tendon-bone healing.

Transforming growth factor-β signaling plays a crucial role in developmental biology. This pathway gets activated *via* ligand induced oligomerization of serine /threonine receptor kinases and phosphorylation of cytoplasmic signaling molecules such as Smad2 and Smad3 by TbRI [43]. Human BMSCs was induced to migrate towards sites of bone resorption by active TGF-β1 and this process is mediated through a SMAD signaling pathway [44]. Thus, TGF-β- TbRI- SMAD pathway was proposed as a novel signaling pathway involved in MSC migration. Therapeutic potentials of MSCs rely on migration of MSCs from the bone marrow or other residing niches to distant injured tissues, where they participate in repair and regeneration. Thus migration of the MSCs is controlled by complicated signal networks [45].

Notch Signaling: The human Notch signaling is yet another pathway which is a key player during developmental process comprising of cell fate determination, proliferation and apoptosis. Notch signaling gets activated once the receptor from one cell interacts with the ligand such as Delta-like or Jagged from the neighboring cell, resulting in two successive cleavages that liberates the cytoplasmic portion of Notch (Notch –IC) [46]. The Notch C –terminal translocates to the nucleus and recruits CSL protein which activates the downstream target genes like Hes1 [47]. Notch signaling is known to cross talk with other osteogenic pathways, which further leads to the regulation of osteoclasts [48]. Studies have shown, that silk proteins serve as nutritional source inducing the Notch signaling which in turn plays a vital role in pro-osteogenic effects and regeneration of bone during fractures and osteoporosis [49].

Studies have shown that Rho GTPase as an important mediator in mesenchymal stem cell migration and plays a crucial role during development of tendon and ligament and this occurs when they are subjected to mechanical stretching. Signaling molecules such as RhoA/ROCK and FAK play a role in hMSC differentiation [50].

LIGAMENT AND TENDON REPAIR USING MESENCHYMAL STEM CELLS

A growing body of evidence suggests that Mesenchymal stem cells along with various growth factors and differentiating factors are a great source of therapeutic value in terms of tendon and ligament regeneration. Studies have shown that MSCs transduced with two genes involved during development of tendon during embryogenesis namely Scleraxis [51] and membranes type1-matrix metalloproteinase (MT1-MMP) [52] showed improved healing of tendons.

Tissue engineering was officially defined in 1988 [53]. Tendon healing takes place over due course of time and involves three stages [54]. During the beginning of tendon healing process involves an inflammatory stage where there is hematoma formation, infiltration of white blood cells, release of cytokines and growth factors. Fibroblast begin to appear in this phase and macrophages will remove any debris, the second stage involves proliferation, where fibroblasts are producing mostly type III collagen and there is formation of new blood vessels. The final stage is the maturation, where the collagens are cross-linked and tissue becomes more organized. The tendon will get back to its original strength at 3-4 weeks and its maximum at 6 months [55].

In vivo studies with NOD/SCID mice showed that MSCs were used to heal segmental defects in various animals [56, 57]. Reports showed that children suffering from osteogenesis imperfecta were treated by infusing bone marrow cells which are a valuable source of MSCs, eventually restored back to normalcy [58, 59]. MSCs exhibit paracrine signaling which in turn releases growth factors such as TGF-beta, VEGF, FGF and other signaling factors essential for tissue repair [60]. A great body of evidences suggests that hypoxic conditions are required for stem cell maintenances. Studies with regard to human tendon stem cells also prove that hy-

poxic condition is an important niche factor that is essential to regulate stemness of hTSCs [61].

The BMP2 and SMAD8ca signaling pathways play a role in the synthesis of secreted matrix components that are essential to form the highly ordered collagen structure and extracellular matrix (ECM). Along with the above signaling pathways, the interplay between matrix metalloproteases (MMP) and tissue inhibitor metalloproteinase (TIMPs) play a key role in the formation of collagen structure of tendons and ligaments. Human induced pluripotent stem cells (IPS-MSC) were found to exhibit remarkable characteristic such as inhibition of Natural killer cell proliferation and cytolytic function by decreasing the levels of activation markers and ERK1/2 signaling.

IPS-MSC was found to be more resistant to pre-activated Natural killer (NK) cells than bone marrow derived MSCs. Thus IPS-MSC can be a valuable source for regenerative medicine and may play an important role in preventing allograft rejection [62]. MSCs induce immune tolerance by activating the type 1 regulatory T-like cells. BM-derived MSCs have been established to suppress immune responses *in vitro* by downregulating activated and cytotoxic T cells. They are known to promote the generation of regulatory T cells (Tregs) and modulate the maturation or function of dendritic cells [63]. Reports have shown that treatment with the infusion of autologous and allogenic MSCs has been effective in tissue regeneration and disease modulation [64].

CONCLUSION

Mesenchymal stem cells are thus a valuable source for tissue regeneration and in particular to ligament and tendon repair. The fact that human tendon stem cells (hTSC) require hypoxic condition as a niche factor for maintenances of stemness, this factor can be used to expand the hTSC *ex vivo*, so that it can be used for regenerations of various tissue injuries with respect to tendon and ligament. Recent research does state that IPS-MSCs can be a great source of immunoregulatory cells, however in terms of future research directions they can be a valuable source for various disease ailments, since they have the potential to prevent allograft rejection.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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