Muscle-Derived Stem Cells for Bone and Cartilage Repair

Xueqin Gao[1,2], Johnny Huard[1,2]*

[1]Department of Orthopaedic Surgery, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, Texas.

[2]Steadman Philippon Regenerative Institute, Center for Regenerative Sports Medicine.

Corresponding Author: Johnny Huard, Department of Orthopaedic Surgery, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, Texas, **Email:** Johnny.Huard@uth.tmc.edu

Citation: Huard J and Gao X. (2018). Muscle-Derived Stem Cells for Bone and Cartilage Repair. M J Orth. 3(1): 023.

ABSTRACT

Adult muscle derived stem cells (MDSCs) are postnatal stem cells that can be isolated from skeletal muscle using a preplate technique. These cells can undergo multipotent differentiation in vitro, and when genetically modified to express bone morphogenetic proteins (BMPs), they can efficiently regenerate new bone and improve healing of focal chondral defects. Promoting angiogenesis preferentially enhances MDSC-mediated bone formation, whereas blocking angiogenesis preferentially potentiates cartilage regeneration. Genetic modification of human MDSCs to express BMP2, utilizing a lenti-BMP2 viral vector, has been shown to have similar efficacy as lenti-BMP2 transduced human bone marrow mesenchymal stem cells (BMMSCs), and therefore, MDSCs represent a very promising source of adult stem cells for bone and cartilage repair.

KEYWORDS

Muscle Derived Stem Cells; Gene Therapy; Bone Repair; Cartilage Repair.

INTRODUCTION

Myoblasts was first isolated from rat skeletal muscle tissues by David Yaffe using preplate techniques and could be maintained in vitro for many months in a state of continuous multiplication and retain the capacity to fuse and differentiate into postnatal multinuecleated myofibers. He found virtually all the cells in this cell line have potential to differentiate into myofibers[1]. Later, Dr. Yaffe established a C2 myoblast cells line from injured thigh muscle of two month old C3H mice [2]. Blau et al recloned C2 cells and expanded the cells to C2C12 myoblast cell line[3]. Furthermore, Blau's group isolated primary myoblasts use the preplating method and found that these cells are primarily myogenic, both in vitro and in vivo [4]. Subsequently, Huard group isolated a subpopulation of muscle stem cells termed muscle-derived stem cells (MDSCs, preplate 6), using the modified preplate technique which were found to be multipotent in vitro and in vivo [5-8]. Based on these pioneer researches, much more progress had been made in using MDSCs for various tissue engineering. The purpose of this review is to highlight recent advances in the use of MDSCs for bone and cartilage repair.

MUSCLE-DERIVED STEM CELLS FOR BONE REPAIR

Murine MDSCs for bone repair

Our team demonstrated that after transduction with BMP2 using an adenoviral vector, murine MDSCs could efficiently regenerate new bone within a critical size defect created in the calvaria of mice [6]. In addition, we also demonstrated that in vivo transplantation of allogenic BMP4-transduced murine MDSCs formed new bone both intramuscularly and within critical size calvarial bone defects in normal mice [9]. We proposed at this time that angiogenesis played an important role in MDSC mediated bone regeneration and validated this hypothesis by performing angiogenesis gain and loss of function experiments. We showed that the co-transplantation of VEGF (a strong inducer of angiogenesis) transduced MDSCs with BMP transduced MDSCs enhanced the formation of new bone; whereas, the co-delivery of sFlt-1(a VEGF antagonist) transduced MDSCs with BMP transduced MDSCs decreased bone formation [10, 11].

Though BMP transduced MDSCs can efficiently form new bone, bony overgrowth often occurs during the regenera-

tion process; hence preventing this overgrowth is also important. During the natural process of bone healing, there exist a number of positive and negative environmental cues that fine-tune and regulate bone formation. We predicted that the co-delivery of noggin, a BMP antagonist, could improve the quality of the new bone. Indeed, our team demonstrated that the co-transplantation of BMP-transduced MDSCs and noggin-transduced MDSCs into a critical size calvarial defect could prevent excessive new bone formation, which resulted in well integrated bone formation in the defect area [12]. Moreover, we recently found that, in addition to the direct differentiation of BMP4 transduced MDSCs toward an osteogenic lineage(osteoblasts and osteocytes), they also participate in paracrine signaling via the secretion of MCP1, VEGFα, FGF2, IGF2, PDGF, and TGFβ1 into the microenvironment which favorably interact with the host cells such as inflammatory cells, endothelial cells. [13]. The quality of the bone formed is also dependent on the scaffold used to deliver the cells. We have shown that fibrin sealant (an absorbable scaffold) promotes the integration of the new bone with the existing host bone [14].

The capacity of MDSCs to promote new bone formation is also affected by the sex of the donor and recipient. Corsi et al. demonstrated murine male MDSCs were more efficient than female MDSCs at promoting osteogenic differentiation in vitro and new bone formation in vivo. Male MDSCs were found to contain more osteogenic progenitor cells than female MDSCs. Furthermore, male recipients produced more ectopic bone than female recipients, regardless of the sex of the donor cells which further indicated that the sex of the host animal also played a role in MDSCs-mediated bone regeneration [15]. However, this difference in bone formation between male and female recipients does not appear to be related to their differing concentrations of circulating hormonal factors [16]. However, there are still more mechanisms regarding the sex difference of MDSCs mediate bone regeneration to explore.

Human MDSCs for bone repair

To facilitate the application of MDSCs in clinical practice, researchers have isolated human muscle-derived cells using different techniques. Earlier times, researchers in Dr. Huard's group have isolated human muscle-derived cells that contained myogenic cells and fibroblast and found they were capable of enhancing the healing of critical size calvarial defects in immunodeficient mice (i.e., SCID mice) when they were transduced to express BMP2 using adeno and retro viral vectors. The cells mainly served as gene delivery vehicle in this case [17]. Another group from Italy, isolated human muscle-derived cells using a similar technique used to isolate

BMMSCs. The cells that were allowed to adhere to the plates for 72 were found to be similar to bone marrow stromal cells [18]. They found that the human muscle-derived cells were multipotent in vitro and were capable of producing new bone in vivo after subcutaneous implantation [18]. More recently, we have successfully isolated MDSCs from human skeletal muscle tissues using the modified preplate technique [19] and termed these cells human muscle-derived stem cells (hMDSCs). Further characterization of hMDSCs indicated that more than 95% of these cells expressed CD73, CD90, CD105, CD44, CD56, and CD146, but were negative for UEA and CD45. These cells were found to be capable of undergoing adipogenesis, chondrogenesis, osteogenesis, and myogenesis in vitro, thus confirming their multipotency. These cells were found to be very similar to BMMSCs in terms of their multipotent differentiation capacities and marker profiles. In vivo, hMDSCs could efficiently regenerate bone in a critical size cavarial bone defect created in mice when transduced with lenti-BMP2 [20]. Moreover, we also found that hMDSCs were as efficient as human BMMSCs in term of their ability to promote bone defect healing when transduced with lenti-BMP2. Both hMDSCs and human BMMSCs require BMP2 to regenerate bone [21]. Interestingly, hMDSCs do not have the disadvantage of bony over growth seen in murine MDSCs mediated bone regeneration.

Using the prospective isolation method designed by Zheng et al [22], we were able to identify a population of myogenic endothelial cells (CD56+CD34+CD144+CD45) capable of promoting new bone formation when retro virally transduced to express BMP4 [22, 23]. An independent study by Jackson et al. demonstrated the isolation of mesenchymal stem cells from traumatically injured human skeletal muscle which also exhibited multipotent characteristics in vitro [24]. These aforementioned studies showed that stem cells isolated from human skeletal muscle are a very promising stem cell resource for bone tissue repair and engineering.

MUSCLE DERIVED STEM CELLS FOR CARTILAGE REPAIR

Although, it has been well-documented that both murine MDSCs and human MDSCs can significantly enhance new bone formation, only murine MDSCs have been studied with respect for cartilage regeneration. Our group has shown that murine MDSCs genetically transduced with retro-BMP4 can differentiate into chondrocytes in pellet culture and promote healing of focal cartilage defects in the femoral condyles of rats. The repair process is mediated by direct differentiation of murine MDSCs into chondrocytes and hypertrophic chondrocytes. Similar to its importance in bone healing, BMP4 transduction is also crucial for murine MDSC-mediated cartilage repair [25].

In contrast to the beneficial effects that angiogenesis has on murine MDSC mediated bone formation, it has been demonstrated that angiogenesis is actually detrimental to MDSC-mediated cartilage regeneration. In one study, it was demonstrated that BMP4-transduced murine MDSCs promoted cartilage regeneration much more efficiently when co-transplanted with sFlt-1 transduced murine MDSCs, which is a potent inhibitor of angiogenesis [26]. This concept was supported in a subsequent study which demonstrated that monoiodoacetate damaged cartilage (a model for osteoarthritis), regenerated much more effectively when BMP4 transduced murine MDSCs were co-transplanted with sFlt-1 expressing murine MDSCs [27].

Similar to the findings of MDSC-mediated bone formation, the capacity of murine MDSCs to regenerate cartilage is also influenced by the sex of the cells. More specifically, male BMP4 expressing MDSCs formed larger chondrogenic pellets with a richer cartilage extracellular matrix compared to female BMP4 expressing murine MDSCs in vitro. Moreover, male murine MDSCs repaired articular cartilage more efficiently than female BMP4 transduced MDSCs [28]. As a potential method to improve cartilage regeneration in clinical practice, we are currently exploring the use of ex vivo genetically modified hMDSCs for cartilage repair. We are also investigating the use of injectable biomaterials to deliver BMP2 and sFlt1 into the micro-milieu of the joint to repair damaged cartilage.

CONCLUSION

In summary, MDSCs represent an abundant source of stem cells that have great potential for bone and cartilage repair. Human MDSCs are equal to human BMMSCs in their efficacy for regenerating new bone after being transduced with BMP2 [21]. Human MDSCs can also be expanded in vivo for up to 20 passages without affecting their cellular function; on the other hand, human BMMSCs can only be expanded for approximately 10 passages before the onset of functional decline. In the setting of trauma, injuries to bone and/or cartilage, are almost always accompanied by injuries of muscle; therefore, when surgical management is chosen for these patients, hMDSCs can be easily obtained and utilized intraoperatively by orthopaedic surgeons. New developments in this area include stem cell banking, whereby previously obtained stem cells can be retrieved and expanded at a later date when they are needed. This strategy allows for the transplantation of autologous stem cells in a single-stage procedure when the need arises. As we continue to expand our knowledge about MDSCs, their potential use as an adjunctive treatment for patients with musculoskeletal injuries could soon become a reality.

REFERENCES

1. Yaffe D. (1968). Retention of differentiation potentialities during prolonged cultivation of myogenic cells. *Proc Natl Acad Sci U S A.* 61(2): 477-483.

2. Yaffe D and Saxel O. (1977). Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. *Nature.* 270: 725-727.

3. Blau HM, Chiu CP and Webster C. (1983). Cytoplasmic activation of human nuclear genes in stable heterocaryons. *Cell.* 32(4): 1171-1180.

4. Rando TA and Blau HM. (1994). Primary mouse myoblast purification, characterization, and transplantation for cell-mediated gene therapy. *J Cell Biol.* 125(5): 1275-1287.

5. Torrente Y, Tremblay JP, Pisati F, Belicchi M, et al. (2001) Intraarterial injection of muscle-derived CD34(+)Sca-1(+) stem cells restores dystrophin in mdx mice. *J Cell Biol.* 152(2): 335-348.

6. Lee JY, Musgrave D, Pelinkovic D, Fukushima K, et al. (2001). Effect of bone morphogenetic protein-2-expressing muscle-derived cells on healing of critical-sized bone defects in mice. *J Bone Joint Surg Am.* 83-A(7): 1032-1039.

7. Qu-Petersen Z, Deasy B, Jankowski R, Ikezawa M, et al. (2002). Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. *J Cell Biol.* 157(5): 851-864.

8. Qu Z, Balkir L, van Deutekom JC, Robbins PD, et al. (1998). Development of approaches to improve cell survival in myoblast transfer therapy. *J Cell Biol.* 142(5): 1257-1267.

9. Wright V, Peng H, Usas A, Young B, et al. (2002). BMP4-expressing muscle-derived stem cells differentiate into osteogenic lineage and improve bone healing in immunocompetent mice. *Mol Ther.* 6(2): 169-178.

10. Peng H, Wright V, Usas A, Gearhart B, et al. (2002). Synergistic enhancement of bone formation and healing by stem cell-expressed VEGF and bone morphogenetic protein-4. *J Clin Invest.* 110(6): 751-759.

11. Peng H, Usas A, Olshanski A, Ho AM, et al. (2005). VEGF improves, whereas sFlt1 inhibits, BMP2-induced bone formation and bone healing through modulation of angiogenesis. *J Bone Miner Res.* 20(11): 2017-2027.

12. Peng H, Usas A, Hannallah D, Olshanski A, et al. (2005). Noggin improves bone healing elicited by muscle stem cells expressing inducible BMP4. *Mol Ther.* 12(2): 239-246.

13. Gao X, Usas A, Proto JD, Lu A, et al. (2014). Role of donor and host cells in muscle-derived stem cell-mediated bone repair: differentiation vs. paracrine effects. *FASEB J.* 28(8): 3792-3809.

14. Usas A, Ho AM, Cooper GM, Olshanski A, et al. (2009). Bone regeneration mediated by BMP4-expressing muscle-derived stem cells is affected by delivery system. *Tissue Eng Part A.* 15(2): 285-29.

15. Corsi KA, Pollett J B, Phillippi JA, Usas A, et al. (2007). Osteogenic potential of postnatal skeletal muscle-derived stem cells is influenced by donor sex. *J Bone Miner Res.* 22(10): 1592-1602.

16. Meszaros LB, Usas A, Cooper GM and Huard J. (2012). Effect of host sex and sex hormones on muscle-derived stem cell-mediated bone formation and defect healing. *Tissue Eng Part A.* 18(17-18): 1751-1759.

17. Lee JY, Peng H, Usas A, Musgrave D, et al. (2002). Enhancement of bone healing based on ex vivo gene therapy using human muscle-derived cells expressing bone morphogenetic protein 2. *Hum Gene Ther.* 13(10): 1201-1211.

18. Mastrogiacomo M, Derubeis A R and Cancedda R. (2005). Bone and cartilage formation by skeletal muscle derived cells. *J Cell Physiol.* 204(2): 594-603.

19. Gharaibeh B, Lu A, Tebbets J, Zheng B. (2008). Isolation of a slowly adhering cell fraction containing stem cells from murine skeletal muscle by the preplate technique. *Nat Protoc.* 3(9): 1501-1509.

20. Gao X, Usas A, Lu A, Tang Y. (2013). BMP2 is superior to BMP4 for promoting human muscle-derived stem cell-mediated bone regeneration in a critical-sized calvarial defect model. *Cell Transplant.* 22(12): 2393-2408.

21. Gao X, Usas A, Tang Y, Lu A, et al. (2014). A comparison of bone regeneration with human mesenchymal stem cells and muscle-derived stem cells and the critical role of BMP. *Biomaterials.* 35(25): 6859-6870.

22. Zheng B, Cao B, Crisan M, Sun B, et al. (2007). Prospective identification of myogenic endothelial cells in human skeletal muscle. *Nat Biotechnol.* 25(9): 1025-1034.

23. Zheng B, Li G, Chen WC, Deasy BM, et al. (2013). Human myogenic endothelial cells exhibit chondrogenic and osteogenic potentials at the clonal level. *J Orthop Res.* 31(7): 1089-1095.

24. Jackson WM, Lozito TP, Djouad F, Kuhn NZ, et al. (2011). Differentiation and regeneration potential of mesenchymal progenitor cells derived from traumatized muscle tissue. *J Cell Mol Med.* 15(11): 2377-2388.

25. Kuroda R, Usas A, Kubo S, Corsi K, et al. (2006). Cartilage repair using bone morphogenetic protein 4 and muscle-derived stem cells. *Arthritis Rheum.* 54(2): 433-442.

26. Kubo S, Cooper GM, Matsumoto T, Phillippi JA, et al. (2009). Blocking vascular endothelial growth factor with soluble Flt-1 improves the chondrogenic potential of mouse skeletal muscle-derived stem cells. *Arthritis Rheum.* 60(1): 155-165.

27. Matsumoto T, Cooper GM, Gharaibeh B, Meszaros LB, et al. (2009). Cartilage repair in a rat model of osteoarthritis through intraarticular transplantation of muscle-derived stem cells expressing bone morphogenetic protein 4 and soluble Flt-1. *Arthritis Rheum.* 60(5): 1390-1405.

28. Matsumoto T, Kubo S, Meszaros LB, Corsi KA, et al. (2008). The influence of sex on the chondrogenic potential of muscle-derived stem cells: implications for cartilage regeneration and repair. *Arthritis Rheum.* 58(12): 3809-3819.