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Extracellular vesicles released from mesenchymal stromal cells stimulate bone growth in osteogenesis imperfecta

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Abstract

Background. Systemic infusion of mesenchymal stromal cells (MSCs) has been shown to induce acute acceleration of growth velocity in children with osteogenesis imperfecta (OI) despite minimal engraftment of infused MSCs in bones. Using an animal model of OI we have previously shown that MSC infusion stimulates chondrocyte proliferation in the growth plate and that this enhanced proliferation is also observed with infusion of MSC conditioned medium in lieu of MSCs, suggesting that bone growth is due to trophic effects of MSCs. Here we sought to identify the trophic factor secreted by MSCs that mediates this therapeutic activity. *Methods.* To examine whether extracellular vesicles (EVs) released from MSCs have therapeutic activity, EVs were isolated from MSC conditioned medium by ultracentrifugation. To further characterize the trophic factor, RNA or microRNA (miRNA) within EVs was depleted by either ribonuclease (RNase) treatment or suppressing miRNA biogenesis in MSCs. The functional activity of these modified EVs was evaluated using an *in vitro* chondrocyte proliferation assay. Finally, bone growth was evaluated in an animal model of OI treated with EVs. *Results.* We found that infusion of MSC-derived EVs stimulated chondrocyte proliferation in the growth plate, resulting in improved bone growth in a mouse model of OI. However, infusion of neither RNase-treated EVs nor miRNA-depleted EVs enhanced chondrocyte proliferation. *Conclusion.* MSCs exert therapeutic effects in OI by secreting EVs containing miRNA, and EV therapy has the potential to become a novel cell-free therapy for OI that will overcome some of the current limitations in MSC therapy.

Key Words: *cellular therapy, chondrocytes, extracellular vesicles, growth, mesenchymal stromal cells, osteogenesis imperfecta*

Introduction

Mesenchymal stromal cells (MSCs) have been applied as cell therapy for patients with various diseases as found at ClinicalTrials.gov [1–3]. MSCs can be isolated from multiple tissues/organs such as bone marrow, adipose tissue, umbilical cord and dental pulp [4–7]. Since MSCs have been demonstrated to have multipotency to differentiate, at least *in vitro*, into a variety of cell types including osteoblasts, chondrocytes and adipocytes, they have been used in regenerative therapy with the expectation of replacing target tissues with the administered MSCs [6,8,9]. However, despite their demonstrated therapeutic benefit, engraftment of MSCs as target tissue cells appears to be insufficient to account for the observed effects, especially when MSCs are systemically infused [10,11]. A growing body of literature suggests that, in addition to differentiating into target tissue cells, MSCs provide trophic effects by secreting growth factors and cytokines, which can activate and support endogenous cells, even though the detailed mechanism of these therapeutic effects has not been fully elucidated [12,13].

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Recently, it has been shown that MSCs release extracellular vesicles (EVs), including exosomes and microvesicles [12,14–16]. Exosomes are derived from early endosomes and consist of an endosomal membrane with diameters of 40–100 nm. On the other hand, microvesicles are larger with 100–1000 nm diameters and are shed from plasma membrane [14,17]. Both exosomes and microvesicles are known to contain bioactive molecules, such as proteins and RNAs, and deliver signals for intercellular communication [15,18]. Indeed, it has been demonstrated that MSC-derived EVs play an important role in the therapeutic effects observed in the treatment of several diseases [16]. Although the underlying mechanism still needs to be further identified, MSC-derived EVs have the potential to serve as a cell-free therapy equivalent to MSC therapy and thereby bypass many of the limitations of cell-based therapies, including variable therapeutic potency between MSC donors, failure of freshly thawed cryopreserved MSCs to exert therapeutic effects and safety concerns.

We have previously conducted clinical trials of MSC therapy for patients with osteogenesis imperfecta (OI) [10]. OI is a genetic bone disorder most commonly caused by autosomal-dominant mutations in one of the two genes that encode type I collagen [19–21]. Major clinical symptoms of OI are bone fractures, skeletal deformity, osteopenia and short stature [19,22]. At this time, there is no cure for OI. Current medical treatments for OI are mostly anti-catabolic to prevent bone loss using drugs such as bisphosphonates; however, there is no reliably effective treatment for short stature. Consistent with others' findings [23,24], systemic infusion of MSCs stimulated bone growth in our clinical trial of MSC therapy for OI patients [10]. Even though infused MSCs were detected in bones, the engraftment in bone was exceedingly low (<1% in osteoblasts) and unlikely to account for the growth acceleration [10]. Rather, our further investigations using animal models of OI identified a novel multi-step mechanism; MSC infusion induces the production of a factor into the serum from an undetermined tissue, which then stimulates chondrocyte proliferation in the growth plate where bone growth occurs (Figure 1) [25]. Additionally, we verified that even infusion of conditioned medium from MSC cultures can initiate this multi-step pathway and stimulate chondrocyte proliferation (Figure 1) [25]. These findings indicate that this MSC activity resides in MSC trophic factor(s) released by MSCs into the conditioned medium, and that novel cell-free therapy for OI growth deficiency can be developed by identifying this MSC trophic factor. In this study we sought to identify the MSC trophic factor that stimulates bone growth in OI to move toward development of a novel cell-free therapy for OI.

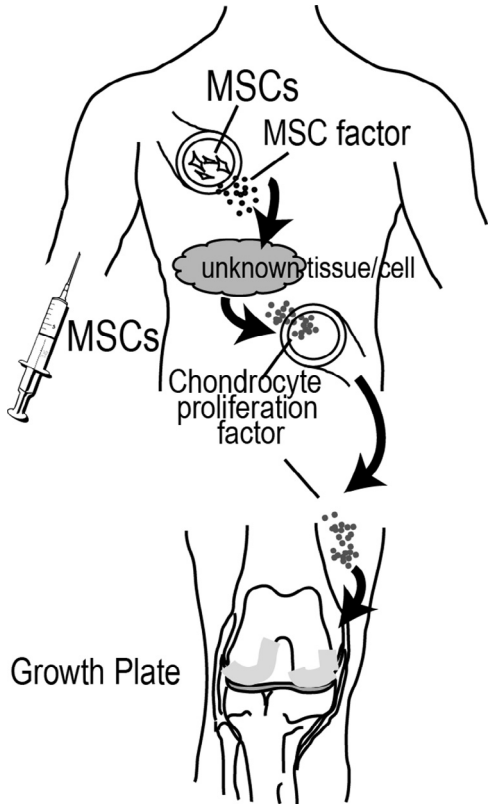

Figure 1. Hypothetical mechanism of bone growth after MSC infusion. Secreted MSC factor induces the production of chondrocyte proliferation factor, which acts on the growth plate chondrocyte.

Methods

MSC culture and isolation of EVs

Murine bone marrow–derived MSCs, which were established from C57BL/6 mice (The Jackson Laboratory) and described in our previous report [25], were used in this study. Human MSCs were isolated from bone marrow of healthy donors by density centrifugation with lymphocyte separation medium (MP Biomedicals, LLC) as previously described [26]. These MSCs were cultured and expanded in Dulbecco's modified Eagle medium (DMEM; Mediatech Inc.) supplemented with 10% fetal bovine serum (FBS) in 15-cm dishes. At approximately 90% confluency, cells were washed three times with phosphate-buffered saline (PBS) and the culture medium was replaced with 10 mL of FBS-free DMEM. After 3 days of incubation, conditioned medium was harvested and clarified by centrifugation at 500*g* for 5 min, followed by supernatant filtration through a 0.22 μm filter (EMD Millipore Corp). The filtered conditioned medium was stored in a -80°C freezer. To use equivalent amounts of EVs throughout the experiments, each preparation