Bone Marrow Mononuclear Cells Transplantation Promotes Functional Bone Healing Via Gap Junction-mediated Cell-cell Interaction With Accelerated Angiogenesis And Osteogenesis

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INTRODUCTION: The usefulness of CD34-positive bone marrow mononuclear cells (BM-MNC) in the treatment of bone fractures has been demonstrated in many studies and has been applied clinically1-3. However, the exact mechanism of their activation remains to be elucidated. In recent years, BM-MNCs including CD34-positive cells have been identified to stimulate angiogenesis in the cerebral circulation and metabolism via direct cell-cell interactions through gap junctions4. In this study, we postulated that gap junctions play a central role in the mechanism by which BM-MNCs promote fracture healing. The purpose of this study is to substantiate this relationship.

METHODS: In this in vivo study, transverse diaphyseal femoral fractures were induced in 10-week-old wild-type mice. Ten days after fracture, calcein-labeled mouse BM-MNCs were administered by tail vein. Peri-fracture tissues were removed 10 minutes after intravenous injection of calcein-labeled BM-MNCs. We then examined the interplay between angiogenesis, osteogenesis, and gap junctions by multi-fluorescent immunostaining of peri-fracture tissues for CD31, osteocalcin, and connexin43. To verify the results obtained in vivo, we performed in vitro experiments in which human umbilical vein endothelial cells (HUVECs) and mouse osteoblasts were co-cultured with calcein-labeled mouse BM-MNCs and observed whether calcein migrated.

RESULTS: In the in vivo study, accumulations of connexin43 were observed at the cell membrane of endothelial cells in the granulation zone, and calcein-positive cells were observed in the microvasculature around the connexin43-positive area. (Figure 1). In the woven bone zone, accumulations of connexin43 were observed on the cell surface of osteoblasts, and calcein-positive area was observed in osteoblasts around the connexin43-positive area (Figure 2). In the in vitro study, calcein migration from BM-MNC to HUVECs and osteoblasts were observed through gap junction.

DISCUSSION: CD34-positive cells in BM-MNC have been reported to promote fracture healing in the clinical setting 1. The mechanisms of this effect include direct differentiation of CD34+ cells into vascular endothelial cells and osteoblasts, and activation of vascular endothelial cells and osteoblasts by the paracrine effect of the release of cytokines such as VEGF1.2. In this study, in addition to these mechanisms, we elucidated a novel mechanism of direct cell-cell interaction via gap junction.

SIGNIFICANCE: Considering our findings, a novel mechanism for bone marrow mononuclear cell-mediated fracture healing promotion involves cell-cell interactions via gap junction.