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

Yoshihito Suda[1,2], Akihiko Taguchi[2], Tomoyuki Matsumoto[1], Yuka Okinaka[2], Shinya Hayashi[1], Masanori Tsubosaka[1], Tomoyuki Kamenaga[1], Yuichi Kuroda[1], Naoki Nakano[1], Kensuke Anjiki[1], Kemmei Ikuta[1], Yuma Onoi[1], Shotaro Tachibana[1], Kensuke Wada[1], Akira Saito[1], Takuma Maeda[1], Ryosuke Kuroda[1]

[1]Department of Orthopaedic Surgery, Kobe University School of Medicine, Kobe, Japan, [2]Department of Regenerative Medicine Research, Biomedical Research and Innovation, Kobe, Japan
Email of Presenting Author: y.suda834@gmail.com

Disclosures: All authors (N)

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 clinically[1-3]. 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 junctions[4]. 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 [3]. 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 VEGF[1,2]. 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.

REFERENCES: **1**. Matsumoto T et al. *Am. J. Pathol* 2006. **2**. Matsumoto T et al. *J. Cell. Physiol* 2007. **3**. Kuroda R et al. *Stem Cell Transl Med* 2014. **4**. Kikuchi-Taura A et al. *Stroke* 2020.

Figures:

Figure 1

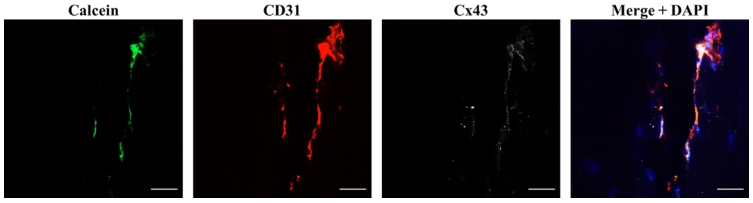

Figure 2