Inhibition of miR-92a Enhances Fracture Healing via Promoting Angiogenesis in Mice

Koichi Murata1, Hiromu Ito2, Hiroyuki Yoshitomi3, Koji Yamamoto4, Moritoshi Furu2, Masahiro Ishikawa4, Hideyuki Shibuya4, Shuichi Matsuda4.

1Hospital for Special Surgery, New York, NY, USA, 2The Department of the Control for Rheumatic Diseases, Kyoto University Graduate School of Medicine, Kyoto, Japan, 3The Center for Innovation in Immunoregulative Technology and Therapeutics, Kyoto University Graduate School of Medicine, Kyoto, Japan, 4The Department of Orthopaedic Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan.


Introduction: MicroRNAs (miRNAs) are endogenous small non-coding RNAs regulating the activities of target mRNAs and cellular processes. Increasing evidence suggests that miRNAs regulate chondrocyte, osteoblast, and osteoclast differentiation, implying important roles in fracture repair. miRNAs are also present in human plasma called as circulating miRNAs in a remarkably stable form, protected from endogenous RNase activity. Here we investigated the circulating miRNA in patients with fractures and the role of miR-92a in the fracture healing of mice.

Methods: Ethical approval for this study was granted by the ethics committee of Kyoto University Graduate School and Faculty of Medicine. Informed consent was obtained from all participants. All animal studies were conducted in accordance with principles by Kyoto University Committee of Animal Resources, based on International Guiding Principles for Biomedical Research Involving Animals. The plasma concentrations of 134 miRNAs in four patients with trochanteric fractures and four healthy controls (HCs) were compared using real-time quantitative PCR, and the plasma levels of dysregulated miRNAs were measured in 27 patients with bone fracture and HCs. Among these miRNAs, miR-92a levels were significantly changed. Antimir-92a, designed using locked nucleic acid technology to inhibit miR-92a, were administered to mice with a femoral fracture. Fracture healing was evaluated by plain X-ray, μCT, real-time PCR and histological analysis.

Results: Among the miRNAs analyzed, plasma miR-92a levels were significantly decreased after 24 hours following fracture, compared to HCs. In patients with a trochanteric fracture or a lumbar compression fracture, the plasma concentrations of miR-92a were lower on days 7 and 14, but had recovered on day 21 after the surgery or injury. Systemic administration of antimir-92a to fractured mice twice a week increased enhanced fracture healing radiographically (Figure 1A). Total volume and bone volume of the callus was 84% and 45% larger in the antimir-92a group than in the control group on postfracture day 14 (Figure 1B, 1C). Histological analyses on postfracture day 14 showed there was a larger area of cartilage at the fracture junction in the control group, indicating accelerated remodeling in the antimir-92a group (Figure 1D). The expression of Col1a in the callus was significantly increased in the antimir-92a group than in the control group on postfracture day 14 (Figure 1E). Enhancement of fracture healing was also observed after local administration of antimir-92a. Primary osteoblasts were isolated from neonatal calvariae and transfected with antimir-92a or control LNA and incubated in differentiation medium for 14 days. Inhibition of miR-92a did not affect the expressions of ALP, OPN and osteocarcin. miR-92a inhibition did not affect the differentiation of ATDC5 cells, neither. Vessel volume visualized by μCT and the number of CD31-positive vessels was increased in mice treated with antimir-92a (Figure 1F, 1G). The expression levels of VEGF-A and ANGPT1 in the fracture callus were significantly increased by the treatment of antimir-92a (Figure 1H).

Discussion: miR-92a targets ITGA5 and MKK4 and is considered to inhibit angiogenesis (1,2). Various growth factors in the angiogenic cascade, including vascular endothelial growth factor, fibroblast growth factor, and platelet-derived growth factor, are potential targets for upregulation and direct administration to accelerate bone repair. However, there are no reports describing a miRNA-based approach in treating bone fracture. miRNAs are considered to be an attractive target for therapeutic manipulation, compared with the growth factors, due to the fact that one miRNA can regulate dozens of genes and can thus act as an amplifier (3). Optimization of the dosage, volume, timing, and delivery methods to an injured tissue, and evaluation of the side effect are future concerns. In conclusion, plasma miR-92a plays a crucial role in bone fracture healing in human and that inhibition of miR-92a enhances fracture healing through angiogenesis and has therapeutic potential for bone repair.

Significance: We have firstly showed the role of miRNA in bone fracture, especially inhibition of miR-92a enhances fracture healing through angiogenesis and has therapeutic potential for bone repair.

Acknowledgments: None


ORS 2014 Annual Meeting
Poster No: 0047