Irisin Stimulates Osteoclast Differentiation and Function

Eben Estell
Phuong Le
Yosta Vegting
Hyeonwoo Kim
Roland Baron

See next page for additional authors
Authors
Eben Estell, Phuong Le, Yosta Vegting, Hyeonwoo Kim, Roland Baron, Bruce Spiegelman, and Clifford Rosen
Irisin Stimulates Osteoclast Differentiation and Function

Eben Estell, PhD1, Phuong Le1, Yosta Vegting 1, Hyeonwoo Kim, PhD2, Roland Baron, PhD3, Bruce Spiegelman, PhD2, Clifford Rosen, MD1

Maine Medical Center Research Institute, Scarborough ME1, Dana Farber Cancer Institute, Boston, MA2, Harvard School of Dental Medicine, Boston, MA3

Introduction

The myokine irisin, the cleaved product of the membrane protein FNDC5 expressed in skeletal muscle, is elevated in human serum after exercise.1

- Irisin injections increase mouse bone formation in vivo and exogenous irisin stimulates murine osteoclast differentiation in vitro.2
- FNDC5 knockout blocks resorption-driven bone loss after ovariectomy.3
- Irisin binds to αv integrins on osteocytes, reducing apoptosis and increasing expression of sclerostin and RANKL, key osteoclast factors.4
- FNDC5 forced expression in muscle suppresses bone formation and increases osteoclast differentiation.5

Hypothesis: Irisin directly stimulates osteoclastogenesis. By its direct actions on the osteoclast, osteoblast, and osteocyte, in addition to modulating paracrine signaling between these cell types, it plays a dynamic role in regulating coupled bone remodeling in response to exercise that is yet to be fully elucidated.

Methods

In Vitro Osteoclast Differentiation with Exogenous Irisin Treatment

8-week male C57BL/6J
MCSF/RANKL-induced osteoclast differentiation

- Osteoclast differentiation from primary bone marrow isolated hematopoietic precursors over 7-day culture with 30 ng/mL MCSF and 100 ng/mL RANKL.6
- Treatment: +/- 10 ng/mL Irisin (ISN), parallel untreated controls (CTL), +/- integrin αvβ5 antibody (ABS)
- TRAP staining & counting for osteoclast number (TRAP+, >3 nuclei/cell)
- Parallel wells for RNA isolation, gene expression via RNaseq & RT-qPCR
- Resorption assays via OsteoAssay (Corning)
- Statistical analysis via Student’s-t test (2 groups) or Two-Way ANOVA with Tukey’s Post-Hoc (>2 groups).

Irisin Stimulates Clastokine Production

Induced differential gene expression

Figure 8. Hierarchical clustering and principal component analysis from unbiased RNAseq analyses of RNA from osteoclasts treated with irisin (ISN) versus untreated controls (CTL). n = 2 biological replicates per group.

Conclusions

Irisin stimulates osteoclastogenesis
Exogenous irisin at physiologically relevant levels increases osteoclast differentiation (quantity and size)

Irisin effect dependent on αvβ5 integrin binding, confirming this receptor as previously established in the osteocyte6

Irisin induces differential gene expression that supports increased differentiation, fusion, resorption, and clastokine production

Irisin-induced clastokines may in turn stimulate osteoblast mineralization, suggesting a role in mediating coupled remodeling

By its direct actions on the osteoclast, osteoblast, and osteocyte, as well as paracrine signaling between the key cells in the bone remodeling unit, irisin demonstrates the potential to regulate bone formation and resorption independently, or through coupled remodeling, in differing contexts of dose, timing, and experimental model. Future work addressing this dynamic functionality (similar to PTH) will be crucial to elucidate the role of irisin in mediating muscle-bone crosstalk during exercise, and its potential role as a therapeutic to treat degenerative bone diseases.

References


Acknowledgments

NIH U19AG069917 & U54GM115516-01A1 NIDDK R01 DK121374, NIGMS 1P20GM121301