RANKL subcellular trafficking in osteocytes OMasashi Honma, Yuki Ikebuchi, Yoshiaki Kariya, Madoka Hayashi, Naoki Hayashi, Shigeki Aoki and Hiroshi Suzuki (Department of Pharmacy, The University of Tokyo Hospital) Background Conventional concept (~2011) Purpose RANK Elucidating how osteocytic Bone remodeling maintains bone quantity and **RANKL** is presented to RANK in Oc quality, and receptor activator of the NF-kB expressed in osteoclast ligand (RANKL) is the central player in the regulation of osteoclastogenesis. RANKL Soluble form ? **Osteoblastic RANKL is essential Bone remodeling** in Ob for osteoclastogenesis. Bone Osteoblast Osteoclast or matrix Molecular (Ob) (Oc) mechanism ? Bone formation Bone resorption **Novel hypothesis Direct interaction ?** Sema4d from osteoclasts pushes by Ob tro by Oc

RANK

in Oc

RANKL

in Ocy

Osteocyte

(Ocy)

osteoblasts away, leading to the absence of cell-cell interactions.

2 Deletion of RANKL in osteocytes

Osteocytes act as the

major source of RANKL

osteoclastogenesis.

led to the drastic suppression of

Nat Med. 2011,17(11):1473-80.

Nat Med. 2011,17(10):1231-34. Nat Med. 2011,17(10):1235-41.

Scheme

Co-culture system of Oc with Ocy

- 1. Establishing a co-culture system of osteocytes and BMMs to mimic the physiological situation 2. Revealing RANKL signal delivery mechanism
 - soluble form ? or direct interaction? –
- 3. Examining the roles of regulatory machineries of RANKL traffic

stem of primary Osteocyte

resorption

formation

Formation

Formation

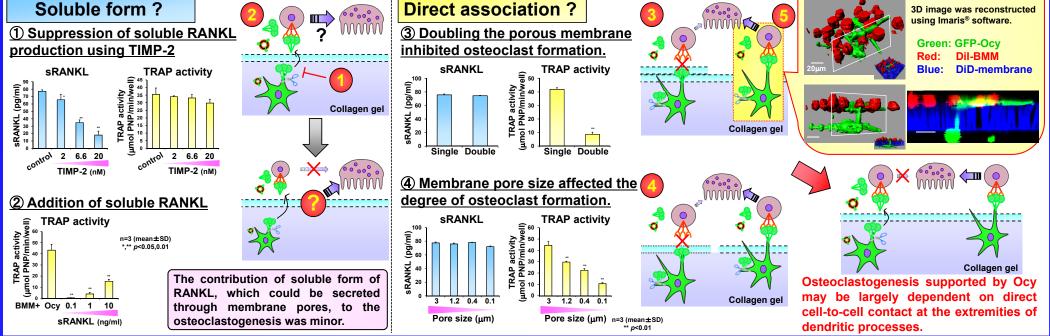
Osteoporosis

Resorption

Resorption

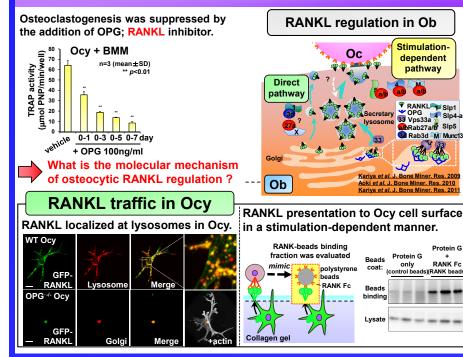
Normal

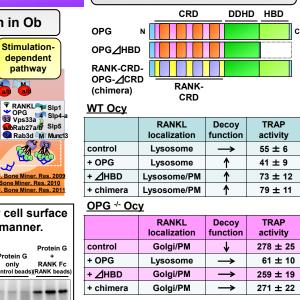
1 TRAP activity 2 gene expression Preparation of Ocy-rich population Porous Cathepsin K Marker genes expression mRNA Ocy 60 1 Ocy+BMM membrane 50 Ocys were isolated from calvarias of 🔷 вмм Ocy+BMM 10 ate Oc erly Ocy E11 mRNA level Ccy C57BL6 mice and the purity was expres SOST Osteocalci 30 elative Осу confirmed by immuno-staining. 🔶 вмм 1.5 2D n=3 (mean±SD) *,** p<0.05,0.01 📃 3D collagen %) 0.5 Ocy population (Ocy/Ob marker) 🔲 Ob gel Ocy population population 30 (+/-) 3 multinucleation (blank Ab) Relative (+/+) n=3 Ob population Formation TRAPof (-/+) an±SD Ob population p<0.05 positive multinuclear (-/-) (blank Ab) p<0.01 osteoclasts was observed dendritic processes Cell in the novel co-culture **Cell morphology** system of BMMs with Culture condition of primary Ocys TRAP primary osteocytes. Reconstructed 3D images 2D culture (Imaris®) of Ocys Green: GFF (collagen-coated plate) Bone marrow macrophage |(BMM) as Oc precursor | Membrane with 3µm pores to Ocy collagen gel prevent the interaction of BMMs Collagen gel-embedding 3D with osteocyte cellular bodies. **3D** culture culture condition was suitable (collagen-gel embedded) for examining Ocy functions. Interaction between Osteocyte and Osteoclast **5** Dendritic processes of Ocy directly contacted with BMMs Soluble form ?



_ regulatory mechanisms

OPG as a traffic regulator





pathway

OPG determines basal-level cell surface presentation of RANKL in Ocys as observed in Obs.

Summary

- 1. The novel co-culture system of Ocys with BMMs using collagen matrix and porous membrane was established.
- 2. Osteoclastogenesis may be largely supported by direct cell-to-cell contact between Ocys and BMMs at the Ocy dendritic processes.
- 3. OPG functions as a traffic regulator of RANKL in Ocys as well as in Obs.

