

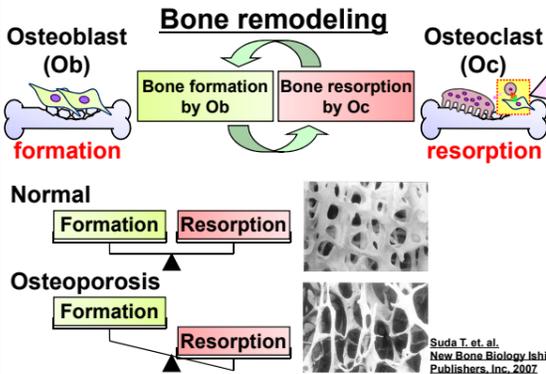
RANKL subcellular trafficking in osteocytes

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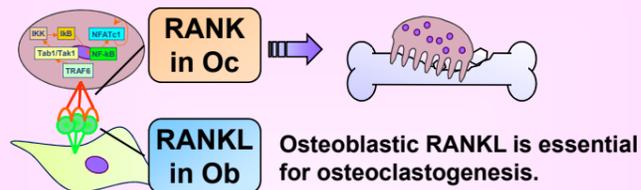
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Background

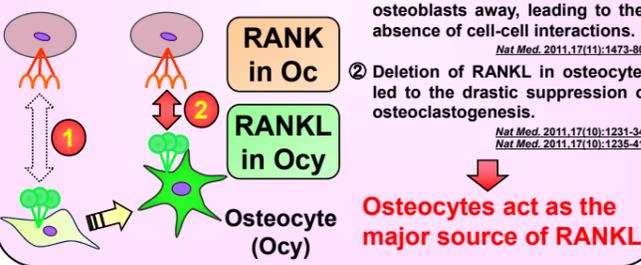
Bone remodeling maintains bone quantity and quality, and receptor activator of the NF- κ B ligand (RANKL) is the central player in the regulation of osteoclastogenesis.



Conventional concept (~2011)



Novel hypothesis



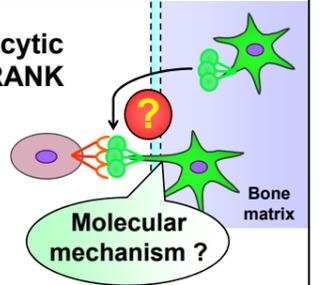
Purpose

Elucidating how osteocytic RANKL is presented to RANK expressed in osteoclast

Soluble form ?

or

Direct interaction ?



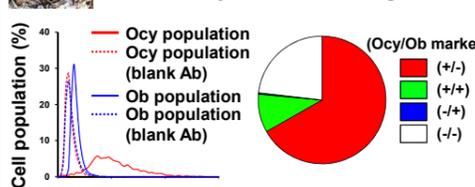
Scheme

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

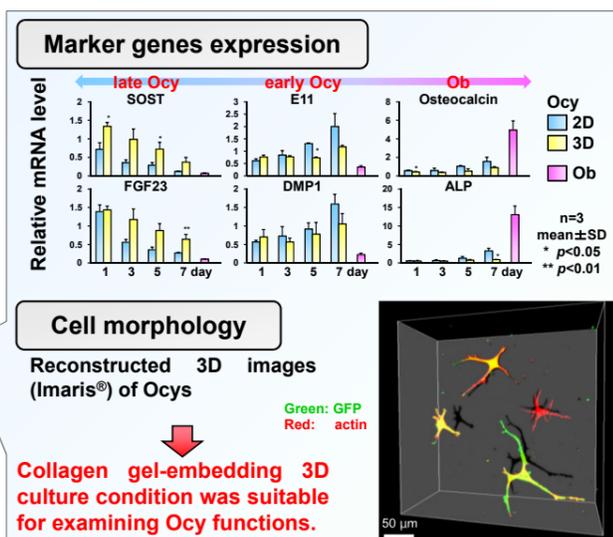
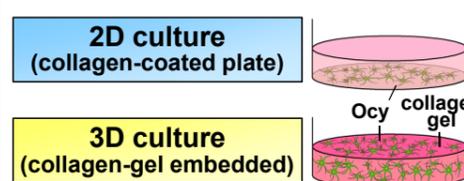
1. 3D culture system of primary Osteocyte

Preparation of Ocy-rich population

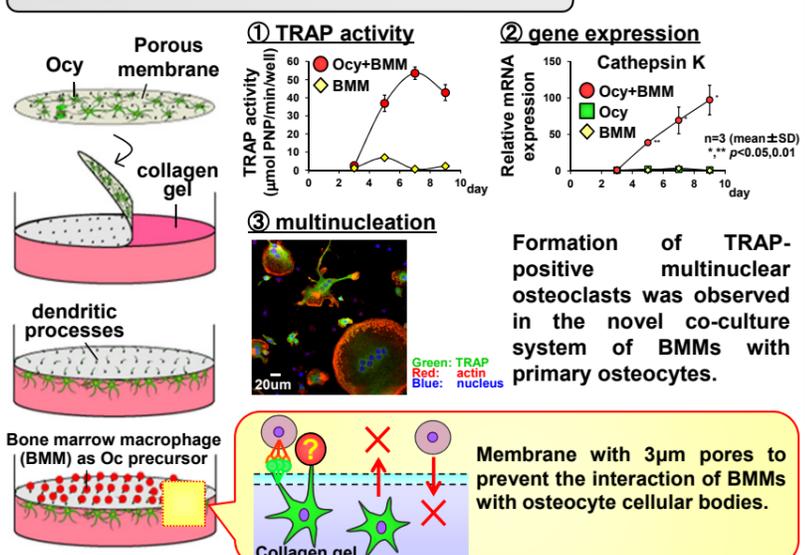
Ocys were isolated from calvarias of C57BL/6 mice and the purity was confirmed by immuno-staining.



Culture condition of primary Ocys



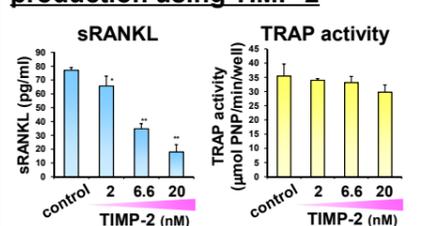
Co-culture system of Oc with Ocy



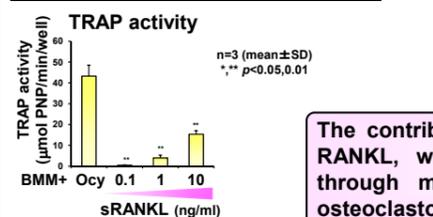
2. Interaction between Osteocyte and Osteoclast

Soluble form ?

① Suppression of soluble RANKL production using TIMP-2

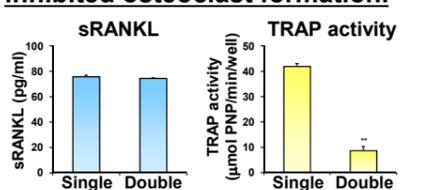


② Addition of soluble RANKL

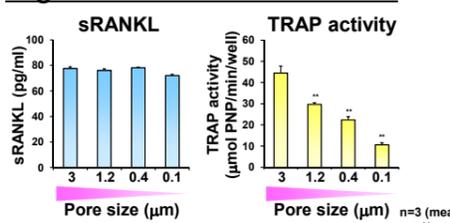


Direct association ?

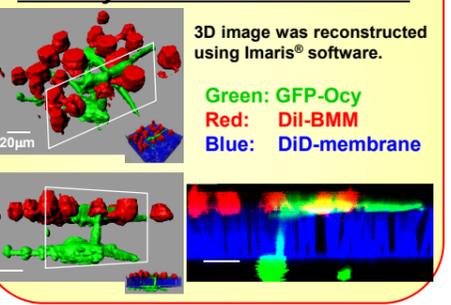
③ Doubling the porous membrane inhibited osteoclast formation.



④ Membrane pore size affected the degree of osteoclast formation.



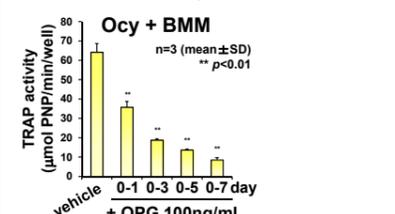
⑤ Dendritic processes of Ocy directly contacted with BMMs



Osteoclastogenesis supported by Ocy may be largely dependent on direct cell-to-cell contact at the extremities of dendritic processes.

3. RANKL regulatory mechanisms

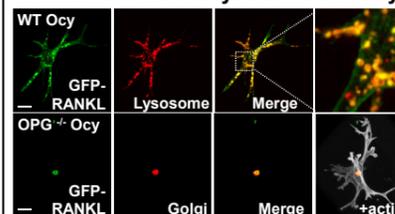
Osteoclastogenesis was suppressed by the addition of OPG; RANKL inhibitor.



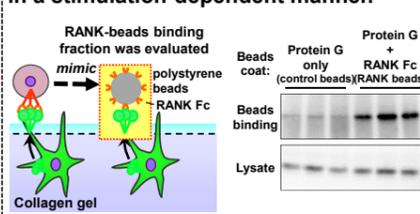
What is the molecular mechanism of osteocytic RANKL regulation ?

RANKL traffic in Ocy

RANKL localized at lysosomes in Ocy.



RANKL presentation to Ocy cell surface in a stimulation-dependent manner.



OPG as a traffic regulator



WT Ocy	RANKL localization	Decoy function	TRAP activity
control	Lysosome	→	55 ± 6
+ OPG	Lysosome	↑	41 ± 9
+ Δ HBD	Lysosome/PM	↑	73 ± 12
+ chimera	Lysosome/PM	↑	79 ± 11

OPG ^{-/-} Ocy	RANKL localization	Decoy function	TRAP activity
control	Golgi/PM	↓	278 ± 25
+ OPG	Lysosome	→	61 ± 10
+ Δ HBD	Golgi/PM	→	259 ± 19
+ chimera	Golgi/PM	→	271 ± 22

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.

