RANKL subcellular trafficking in osteocytes

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

1. 3D culture system of primary Ocy

Preparation of Ocy-rich population
Ocys were isolated from calvarias of C57BL6 mice and the purity was confirmed by immuno-staining.

Culture condition of primary Ocys
Collagen gel-embedding 3D culture condition was suitable for examining Ocy functions.

2. Interaction between Osteocyte and Osteoclast

Soluble form?
1. Suppression of soluble RANKL production using TIMP-2
2. Addition of soluble RANKL

Direct association?
1. Samed from osteoclasts pushes osteoblast away, leading to the absence of cell-cell interactions.
2. Deletion of RANKL in osteocytes led to the drastic suppression of osteoclastogenesis.

OPG as a traffic regulator

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

Purpose
Elucidating how osteocytic RANKL is presented to RANK expressed in osteoclast

Conventional concept (~2011)

RANKL is essential for osteoclastogenesis. Osteocytes act as the major source of RANKL.

Novel hypothesis

Bone remodeling maintains bone quantity and receptor activator of the NF-κB (RANKL) is the central player in the regulation of osteoclastogenesis. Bone remodeling maintains bone quantity and receptor activator of the NF-κB (RANKL) is the central player in the regulation of osteoclastogenesis.

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.