Osteoclast and gp130 signals that regulate bone metabolism
Bone remodelling determines bone mass

Activation

Resting → Resorption

Resorption → Reversal

Reversal → Formation

Formation → Activation
Bone Loss

Balanced BMU

Time

Resorption Osteoid Mineralization Quiescence

Loss due to excessive resorption

Loss due to impaired formation
Osteoblast: osteoclast communication

- Osteoclast precursor
- RANKL (RANK Ligand)
- RANK
- OPG (osteoprotegerin)
- 1,25D (vitamin D)
- PTH/PGE
- OSM
- VDR
- cAMP
- gp130

Osteoblasts

Osteoclast
The Osteoclast Niche

Mizoguchi et al, J Cell Biol 184:541, 2009
Signals from the osteoclast to the osteoblast

Osteoblasts

Osteoclast

IGFs

PDGF

TGFβ

FGF

BMPs
PTH treatment has two possible effects both mediated through a receptor in the osteoblast.

Understanding PTH therapy
Local role of PTHrP
Interesting paradox
Intermittent PTH stimulates RANKL expression

**A**

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**Fold Induction**

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**GAPDH**

**B**

PTH single injection time course for OPG and RANKL

RNA: Femoral Metaphysis


Transient activation of osteoclasts?
Rapid effect of PTH on osteoclasts

(Holtrop ME, et al. Calcif Tissue Int. 1979;27:129-135)
Is the osteoclast required for the anabolic action of PTH?

Evidence for osteoclast requirement: PTH + bisphophonates
Alendronate inhibition of osteoclasts reduces the anabolic effect of PTH

Tiludronate inhibition of osteoclasts blocks the anabolic effect of PTH

\[ \text{Bone formation rate} \quad (\mu m^3/\mu m^2/d) \]

\[ \Delta \text{Bone formation rate} \]

\[ \Delta \text{Activation frequency} \quad (\text{year}) \]

\[ p < 0.003 \quad p < 0.002 \]

\[ p < 0.002 \quad p < 0.005 \]

\[(\text{Delmas 1993})\]
Is the osteoclast an integral mediator of the anabolic actions of PTH?

Altering SDF-1, OPG, or c-fos but not c-src inhibited the anabolic action of PTH

Courtesy of L. McCauley
Does transient osteoclast inhibition reduce the anabolic action of PTH?
Calcitonin is a transient inhibitor of osteoclast activity.
Does calcitonin modify the bone-building effects of PTH?

Combine CT blockade of osteoclast activation with daily PTH treatment over 3 weeks.

- Vehicle
- PTH alone (30µg/kg)
- CT alone (0.5µg/kg)
- PTH + CT simultaneously

![Diagram showing daily injection and cull timeline from week 0 to week 3]
Unpublished data slide removed
Signals from the osteoclast to the osteocyte

- IGFs
- PDGF
- TGFβ
- BMPs
- FGF
- sclerostin
- Osteocyte

?
Finding osteoclast derived factors that influence osteoblasts and osteocytes....
gp130 plays a critical role in intercellular communication in bone.
gp130 mediates osteoclast formation in response to:

![Graph showing number of osteoclasts formed in response to different stimuli.](image)
gp130 knockout: low bone mass

- Many large osteoclasts
- Few osteoblasts

Coupling disrupted!

Balance shifted in favour of resorption - bone mass reduced

Almost identical phenotype observed in LIFR KO (Ware 1995)

Shin et al, Endocrinology 145:1376-1385, 2004
gp130-signalling cytokines

(Sims, Molecular and Cellular Endocrinology, In Press)
Human LIFR mutation - Stüve-Wiedemann / Schwartz-Jampel Type 2 Syndrome

**Bent-bone dysplasia** - death within the first few months of life - respiratory / swallowing difficulties, hyperthermic episodes

**Mild forms** - longer lifespan, progressive scoliosis, spontaneous fractures, flared joints, abnormal trabecular bone

(Dagoneau et al, Am J Hum Gen 2004)
LIFR expression in osteoblasts

(Allan J Cell Physiol 1990)
gp130: LIFR-signalling cytokines

Cardiotrophin-1

(Sims, Molecular and Cellular Endocrinology, In Press)
CT-1 is expressed by osteoclasts

CT-1 in bone  TRAP (OC marker)  CT-1 in cultured OC

Walker JBMR 2008
CT-1 KO phenotype (4 days)

![Wild type and CT-1 KO images]

Bar graphs showing the following parameters:
- BV/TV (%)
- Tb.N. (/mm)
- Tb.Th. (µm)
- ObS/BS (%)
- OcS/BS (%)
- CtgV/BV (%)
CT-1 KO phenotype (adult)

**Femoral BMD (mg/cm³)**

- **WT female**: 200 ± 10
- **KO female**: 250 ± 15
- **WT male**: 150 ± 10
- **KO male**: 300 ± 15

***, p<0.05; ***, p<0.001 vs wild type

**Trabecular BMD (mg/cm³)**

- **WT female**: 100 ± 5
- **KO female**: 150 ± 10
- **WT male**: 50 ± 5
- **KO male**: 200 ± 10

**, p<0.05; ***, p<0.001 vs wild type

**Cartilage Volume (% BV)**

- **WT female**: 10 ± 1
- **KO female**: 15 ± 1
- **WT male**: 5 ± 1
- **KO male**: 20 ± 1

**, p<0.05; ***, p<0.001 vs wild type

**Osteoclast Surface (% BS)**

- **WT female**: 10 ± 1
- **KO female**: 20 ± 1
- **WT male**: 5 ± 1
- **KO male**: 25 ± 1

*, p<0.05; **, p<0.01 vs wild type

**Walker JBMR 2008**
CT-1 KO phenotype (adult)

*, p<0.05; **, p<0.01; ***, p<0.001 vs wild type

Walker JBMR 2008
Many large osteoclasts from CT-1 KO BMM+RANKL

Bone marrow + RANKL/M-CSF, males only
7 day cultures; **, p=0.01 vs wild type
Data from >/=4 wells each of 4 expts
Large osteoclast phenotype in gp130 KOs

But LIFR not in osteoclasts!

(Ware 1995, Shin, Endocrinology 2004; Bozec, Nature 2008)
CT-1 stimulates mineralisation \textit{in vitro} and \textit{in vivo}

5 days consecutive treatment
Fluorochrome labelled
Calvarial bone

Mean±SEM, n=6/group, *, p<0.05; **, p<0.01
Walker JBMR 2008
CT-1 inhibits adipogenesis in vitro
CT-1 increases C/EBPδ expression in Kusa4b10 cells (qPCR)

N=3 independent experiments, *, p<0.05; ***, p<0.001
OsM stimulates osteoblast differentiation at the expense of adipogenesis.

Guiterrez 2002, Lane 1999
C/EBPδ activates osteocalcin transcription

Ducy MCB 1995; Shin J Mol Endocrinol 2006
CT-1 and OSM enhance osteocalcin promoter activity

CT-1 (10ng/ml)

OSM (10ng/ml)

time (hours)

Fold Change
CT-1 stimulates mineralisation *in vitro* and *in vivo*.

5 days consecutive treatment

Fluorochrome labelled

Calvarial bone

Mean+SEM, n=3, all CT-1-treated sig>ctrl, p<0.05

Walker JBMR 2008
Unpublished data slide removed
Influence of CT-1 on the BMU

CT-1 influences the BMU through interactions with LIFR and gp130. CT-1 stimulates osteoblast differentiation, while sclerostin, a negative regulator, is inhibited.

Walker JBMR 2008
Signals from the osteoclast to the osteocyte

- Cardiotrophin-1
- sclerostin
- Osteocyte
- IGfs
- PDGF
- TGFβ
- BMPs
- FGF
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