Role of Calcium-Sensing Receptor in Bone

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Inverse Sigmoidal Relationship Between Calcium and PTH

% Max. PTH response vs. Ionized Ca\(^{2+}\) (mM)

PTH release % of max. vs. Ionized Ca\(^{2+}\) (mM)
Evidences in Favor of Existence of a GPCR for Extracellular Ca$^{2+}$

- High calcium activates PLC
- High calcium inhibits PT-sensitive cAMP production
- Other polycations mimic such actions
CaR is a G-Protein Coupled Receptor

- CaR enables Ca\(^{2+}\) to act as extracellular first messenger
- Prevents body from hypercalcemic state
Diseases Caused by the Mutations of CaR Gene

Loss-of-function mutations

Heterozygous
* Familial hypercalcemic hypocalciuria (FHH)
  (Mild to moderate hypercalcemia, modest increases in set point)

Homozygous
* Neonatal severe hypercalcemia (NSHPT)
  (Severe hypercalcemia and marked increases in set point)
* CaR$^+/-$ and CaR$^-/-$ exhibit similar biochemical phenotypes of FHH and NSHPT, respectively.

Gain-of-function mutations

* Autosomal dominant hypocalcemia (ADH)
  (Moderate to severe hypocalcemia and reduction in set point)
CaR is Very Promiscuous!

Physiological Agonists (Type I):
- Ca$^{2+}$, Mg$^{2+}$, spermine, amino acids, Aβ

Pharmacological Modulators (Type II):

Calcimimetic (allosteric activator)

Calcilytic (antagonist)
Drugs Resets the ‘Calciostat’
Turnover Keeps Bone Alive

- Bone formation & resorption are continuous processes
- Up to 25% of spongy bones are turned over every year
Bone Remodeling Cycle & the CaR

Bone resorption site

High local Ca$^{2+}$

Pre-OBs

Chemotaxis

Proliferation

Differentiation to OB

OBs

Replace OCs

OCs

Pre-OBs

TGFβ

BMP-2

? CaR

High local Ca$^{2+}$
Evidences in Favor of CaR Functions in OBs

* High calcium augmented vitamin D-induced ALP production.

* High calcium induced nodule formation in rat chondrocyte cell line.

• One-third of the CaR immunoreactive mouse bone marrow cells stained for ALP. (*J. Bone Miner. Res.*, 1997)

• CaR is expressed in MC3T3-E1 cells and type I CaR agonists stimulate its proliferation and chemotaxis. (*J. Bone Miner. Res.*, 1998)

• Calcimimetic activates a Ca\(^{2+}\)-activated K\(^+\) channel in MC3T3-E1 cells (*Bone 2000*) & in MG-63 cells (*Am. J. Physiol.*, 2001)
Expression of CaR mRNA in Calvarial OB
Expression of CaR Protein in OBs

CaR  Cbfa/RUNX2

Merge

Rat femur  Human mandible

Endocrinology 2004  PNAS 2004
High Ca$^{2+}$ Stimulates OB Proliferation \textit{via} the CaR
CaR-Mediated OB Proliferation involves Multiple Pathways

- Among the MAPKs, JNK mediates CaR action
- Among the early genes, c-fos and egr-1
- Among the cyclins, cyclin D (D1-D3)
Other CaR-Mediated Functions in OBs

- Stimulates differentiation
- Stimulates *in vitro* mineralization
CaR in the Bone Forming Cells: The Bone of Contention!

*Can there be a different CaR in OBs?*

- Failure to detect CaR mRNA and protein in OB cell lines (*J. Bone Miner Res. 1999*)
- \( \text{Ca}^{2+} \) responsiveness of the OBs from CaR\(^{-/-}\) mice was comparable to that of CaR\(^{+/+}\) mice (*J. Biol. Chem., 2000*).
- A low affinity, GPRC6A could be an “OB CaR” (*J. Biol. Chem., 2005*)
Strontium Ranelate: A Dual Action Anti-osteoporosis Drug

Rx Proteol (Servier); Stronat (Glenmark)

Stimulates bone formation and inhibits bone resorption.

We hypothesized that \( \text{Sr}^{2+} \) acts by activating the CaR
Sr$^{2+}$ Induces Ca$^{2+}_i$ in CaR Transfected HEK-293 Cells

Biochem Pharmacol 2007
Sr$^{2+}$ Stimulates OB Proliferation via the CaR

% increase in proliferation over control

0  5.0  10.0  0  5.0  10.0  mM Sr$^{2+}$

β-gal  DNCaR

Biochem Pharmacol 2007
Conclusions from the Studies on Osteoblast

• Osteoblasts express parathyroid/kidney CaR

• CaR activation results in osteoblast proliferation by multiple mechanisms

• The effect of CaR on osteoblast differentiation and mineralization is contentious

• GPCR6A could be an “OB’-specific calcium sensor in addition to CaR
Is CaR Meant for Bench Jockeys When It Comes to Osteoblast?

• As CaR\(^{-/-}\) mouse is lethal, OB-specific CaR\(^{-/-}\) mouse needs to be developed to study its role in osteoblast.

• Could a calcilytic-derivative induce transient PTH secretion?

Make one and take over the business of Teriparatide!!!
Evidences in Favor of CaR Functions in Osteoclasts

• OCs are exposed to unusually high millimolar Ca$^{2+}$ concentrations

• Rise in cytosolic Ca$^{2+}$ is transduced into inhibition of resorption

• A type 2 ryanodine receptor is a possible calcium ‘sensor’ (Zaidi et al. J. Clin. Invest., 1995)

• A receptor operated $trp$ family cation channel (Bennett et al. Endocrinology, 2001)

• CaR immunoreactivity in bone marrow monocytic/macrophage cells (J. Bone Miner Res. 1997)

• CaR is expressed in J774 monocytic-macrophage cells and type I CaR agonists stimulate its proliferation and chemotaxis. (J. Bone Miner Res., 1998)

• CaR is expressed in osteoclast supporting, ST-2 stromal cells and type I CaR agonists stimulate its proliferation. (Endocrinology, 1998)
CaR Expression in Osteoclasts
CaR Promotes Osteoclast Differentiation
CaR is Involved in Osteocalst Apoptosis

- CaR stimulates OC apoptosis via PLC, NF-κB and caspase pathways
Constitutive Activity of OB-CaR Promotes Cancellous Bone Loss

- Mice with Act-CaR targeted to OB has normal serum Ca$^{2+}$ & PTH
- Reduced BV and trabecular density, network and connectivity with no change in mineral content
- Increased RANKL expression in the OBs

Constitutively active CaR stimulates bone loss that is secondary to increased RANKL secretion by OB

Endocrinology, 2007 (Shoback’s group)
Conclusions from the Studies on Osteoclast

- Osteoclasts express parathyroid/kidney CaR
- CaR is required for osteoclast differentiation
- CaR activation stimulates osteoclast apoptosis via PLC, NF-κB and caspase activation
- OBs with Act-CaR results in bone loss due to increased RANKL production
HHM, PTHrP & CaR

Various cancers metastasizes in bone

The major factor is PTHrP

Ca^{2+} via the CaR could contribute in HHM
Positive Relationship Between CaR & PTHrP in Endocrine Cancers

CaR activation stimulates PTHrP secretion in:

• Human breast cancer cells (*Endocrinology, 2000*)
  - MCF-7 (ER +ive) & MDA-MB-231 (ER –ive)

• Human prostate cancer cells (*Am. J. Physiol., 2001*)
  - PC-3 (AR +ive) & LnCaP (AR –ive)

• Rat Leydig tumor (H-500) cells (*Am. J. Physiol. 2003*)
  Xenotransplantable tumor in male Fisher rats
CaR Elicits Multiple Signaling in a HHM Model

- Calcimimetic promotes H-500 proliferation & survival via p38 MAPK and PI3K pathway (*Endocrinology* 2004)

- CaR stimulates expression of securin and VEGF (*Endocrinology* 2003)

- CaR stimulates iNOS expression and NO production (*Am. J. Physiol. 2005*)

- CaR stimulates PTHrP release via PKC, p38 MAPK, JNK and ERK pathways (*Am. J. Physiol. 2003*)

- Stimulation of PTHrP release by the CaR requires transactivation of EGF receptor (*Bone 2004*)
Summary of Regulation of PTHrP Secretion by the CaR

CaR → Gq/11 → Ras → MEK 1/2 → ERK 1/2 → PTHrP Synthesis/Secretion
Overall Summary

- CaR activation *in vitro* has dual action of promoting OB function and inhibiting OC function.

- *In vivo* data are lacking

- Dual action of Sr$^{2+}$ provides circumstantial evidence toward CaR’s role in anti-osteoporosis

- A CaR antagonist giving rise to transient PTH release holds immense therapeutic promise

- CaR in HHM setting aberrantly contributes to begetting hypercalcemic condition by stimulating PTHrP production in cancer cells
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CaR-Induced OB Proliferation is JNK Mediated

Endocrinology 2004
CaR-Stimulated Osteoclast Apoptosis is Mediated by NF-κB
Unanswered Question About CaR & HHM

CaR positive breast tumors are more likely to develop bone metastasis (Mihai et al. *Eur J Sur. Oncol.*, 2006)

• No direct evidence toward CaR’s role in tumor metastasis to bone?