

a genomic mechanism for independent developmental control of local growth of individual bones.

During these studies, the investigators also discovered 2 regulatory elements that controlled expression of BMP5 in nasal cartilage. These elements were distinct from those controlling BMP expression in ribs but like them mapped to locations within and 3' from the coding region of the gene as shown in the Figure. The authors suggested that the proposed mechanism may not be limited to regulation of BMP5 but common to other developmentally regulated genes that are involved in fine-tuning morphogenesis.

Guenther C, Pathalena-Filho L, Kingsley DM. Shaping skeletal growth by modular regulatory elements in the *Bmp5* gene. *PLoS Genetics*. 2008;4:1-13.

Editor's Comment: This investigation provides novel insight into the fine-tuning of skeletal development. It is interesting to speculate how subtle radiographic findings that allow experts to distinguish between similar bone dysplasias might reflect disturbances in these regulatory mechanisms.

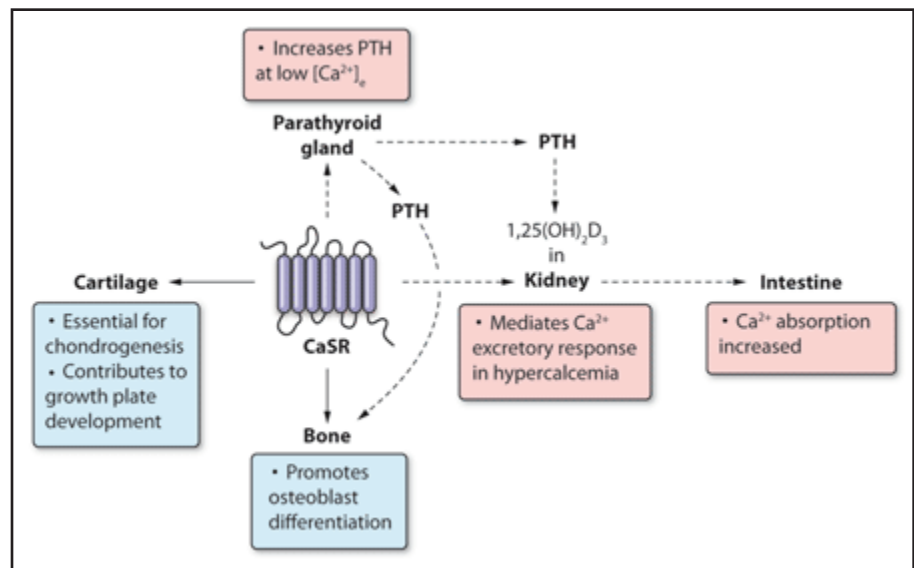
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Extracellular Calcium-Sensing Receptor: Modulator of Skeletal Development

The roles of the calcium-sensing receptor (CaSR) as a negative regulator of parathyroid hormone (PTH) synthesis and secretion and as an inhibitor of calcium reabsorption by the renal tubule are well documented; its functions in chondrocytes, osteoblasts, and osteoclasts have been less completely documented. In part, this has been due to inability to generate mice in which *Casr* has been ablated specifically in cartilage and bone cells and to the expression in these cells of an alternatively spliced, functionally active isoform of the CaSR.¹ *Casr* is expressed in differentiating osteoclasts, chondrocytes, and osteoblasts. Generalized ablation of *Casr* in mice results in a rachitic phenotype (increased width of the zone of hypertrophic chondrocytes, depressed and disordered calcification of the cartilage growth plate, and decreased rate of cartilage mineralization).²

The present investigators have developed strains of mice in which exon 7 of *Casr* (encoding the 7 transmembrane and 4 intracellular loops of the receptor protein) has been specifically "knocked-out" in parathyroid cells (PTC), growth plate chondrocytes (GPC), and osteoblasts (OB) rendering the *Casr* functionally inactive. Homozygous loss of *Casr* in PTC resulted in impaired growth and death within 2 weeks after birth. As anticipated, these mice had increased expression of *Pth* in their PTC. The skeletons of these mice had abundant matrix but were markedly undermineralized, and multiple fractures were present. There was substantially decreased expression of *Casr* in bone cells

and delayed OB differentiation. In mice in which *Casr* was specifically ablated in OB, the phenotype of growth retardation, skeletal undermineralization with increased osteoid formation, multiple fractures, and death by 3 weeks of age was observed. OB differentiation was severely impaired as was OB expression of *Igf1*. The rate of apoptosis of OBs was accelerated. The homozygous loss



Classic Ca^{2+} homeostasis (dashed arrows) and novel developmental functions (arrows) of CaSR have been revealed by cell type-specific null mutations of *Casr* in the mouse. CaSR is found in bone, kidney, and gut, which are the three main Ca^{2+} -mobilizing organs. The normal homeostatic signaling pathways between these organs and the parathyroid gland have been detailed previously.⁵ The functions performed by CaSR in each organ are outlined in boxes. To maintain normal Ca^{2+} homeostasis, CaSR in parathyroid cells (PTCs) senses alterations in $[Ca^{2+}]_e$. The release of parathyroid hormone (PTH) enables bone and kidney to respond in a manner to normalize $[Ca^{2+}]_e$, through the activation of key responses in kidney [production of $1,25(OH)_2D_3$ and reabsorption of Ca^{2+}], intestine [Ca^{2+} absorption through the increased abundance of $1,25(OH)_2D_3$], and bone matrix resorption through PTH (not shown). The direct role of CaSR in the intestine is questionable because an intestine-specific knockout of *Casr* has not been performed. Targeted knockout of *Casr* through the crossing of *Casr* floxed mice with mice expressing *Cre* under the control of tissue-specific promoters has identified novel functions for CaSR in skeletal development. Ablation of *Casr* in the parathyroid gland resulted in the expected phenotypes that occur in patients with inactivating mutations in *Casr*, such as hyperparathyroidism and hypercalcemia. Deletion of *Casr* in chondrocytes demonstrated a requirement for CaSR in early skeletal development, whereas a role for CaSR in promoting bone cell differentiation was determined by deletion of *Casr* in cells of the osteoblast lineage. Reprinted with permission Brown EM, Lian JB. *Sci Signal*. 2008;1; pe40. Copyright © AAAS 2008. All rights reserved.

of expression of *Casr* in GPC was lethal; affected embryos died before day 13 of embryonic life. Development of a mouse model in which “knock-down” of *Casr* expression in GPC at day 16 to 17 of embryonic life after treatment with an estrogen receptor agonist (tamoxifen) resulted in offspring with modestly decreased growth of long bones despite expansion of the hypertrophic zone of the growth plate, decreased differentiation to terminal chondrocytes, and decreased expression of *Igf1* and *Igf1r* by GPC.

The authors concluded that: (1) the elimination of a functional CaSR in PTC also depressed *Casr* expression in osteoblasts (hypothetically through hypercalcemia and increased signaling by the PTH receptor in bone); (2) the CaSR was innately essential for osteoblast differentiation, function, and survival; and (3) that partial and delayed loss of the CaSR in hypertrophic chondrocytes reduced chondrocyte differentiation in part through decreased IGF-1R signaling.

Chang W, Tu C, Chen TH, Bikle D, Shoback D. The extracellular calcium-sensing receptor (CaSR) is a critical modulator of skeletal development. *Sci Signa*. 2008;1: ra1\ [DOI:10.1126/scisignal.1159945]

Editor's Comment: *This research has demonstrated the individual importance of the CaSR in PTCs, OBs, and hypertrophic chondrocytes.³ Interestingly, “knock-out” of*

the CaSR in PTCs secondarily impaired expression of Casr in osteoblasts, demonstrating clearly the interdependence of the parathyroid-osteoblast axis. A study of the effect of overexpression of Casr in PTCs upon OB expression of Casr and bone morphology would be of interest. This manuscript also introduced a new feature of the electronic journal—Science Signaling—sponsored by the AAAS, that has until now published review articles and didactic materials on the subjects of intra- and intercellular communications.⁴ It will now publish original research articles as well.

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References

- Rodriguez L, Tu C, Cheng Z, et al. Expression and functional assessment of an alternatively spliced extracellular Ca²⁺-sensing receptor in growth plate chondrocytes. *Endocrinology*. 2005;146:5294-5303.
- Garner SC, Pi M, Tu Q, Quarles LD. Rickets in cation-sensing receptor-deficient mice: An unexpected skeletal phenotype. *Endocrinology*. 2001;142:3996-4005.
- Brown EM, Lian JB. New insights in bone biology: Unmasking skeletal effects of the extracellular calcium-sensing receptor. *Sci Signal*. 2008;1; pe40.
- Alberts B. Scientific publishing standards. *Science*. 2008;321:1271.
- Ho C, Conner DA, Pollak MR, et al. A mouse model of human familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Nat. Genet*.1995; 11:389-394.

Insulin Analogues...and Cancer?

Insulin analogues with different pharmacokinetics were created by inserting point modifications into the amino acid sequence of human insulin, particularly the C-terminus of its beta-chain which is not involved in binding to the insulin receptor (IR). However, these modifications may alter binding affinity for the closely related type 1 insulin-like growth factor (IGF) receptor (IGF1R). Thus, Weinstein et al asked the important question of how these analogues compare to insulin and IGF-I in eliciting IGF-I activities (namely, proliferation and protection from serum starvation-induced apoptosis) in cultured cancer cells.

They studied 2 long-acting insulin analogues (glargine [Lantus®] and detemir [Levemir®]) and 2 short-acting analogues (lispro [Humalog®] and aspart [Novolog®]) in 3 different cell lines: HCT-116 colorectal, PC-3 prostate and MCF-7 breast cancer cells. All experiments were conducted in vitro. Results are summarized in the Table.

HCT-116 cells showed a dose-dependent proliferative response to both glargine and detemir at 72 hours, but not IGF-I (all doses about +21%) nor insulin (all doses negligible effect). The authors then turned to signaling pathways that may underlie the hormonal effects in HCT-116 cells. Basal expression levels of IR and IGF1R were equivalent when measured by Western immunoblotting and immunofluorescent staining. After

Summary of effects on cell behavior in vitro.

Effect	Insulin	Glargine	Detemir	Lispro	Aspart	IGF-I
HCT-116 proliferation at 96 hrs (compared to untreated cells)	+0.4%	+22%	+17%	-	-	+24%
HCT-116 proliferation at 48 hrs ¹ (compared to untreated cells)	+7%	-	-	+20%	0	+22%
PC-3 proliferation at 72 hrs (compared to untreated cells)	+2%	+17%	+15%	-	-	+25%
MCF-7 proliferation at 72 hrs (compared to untreated cells)	0	+14%	+6%	-	-	+22%
HCT-116 % apoptotic cells at 12 hrs (control = 23%)	25%	15%	18%	-	-	17%
HCT-116 % apoptotic cells at 24 hrs (control = 30%)	30%	26%	25%	-	-	24%

¹ by MMT assay; all other proliferation experiments were measured by cell counts. Apoptotic cells were quantified via flow cytometry of Annexin V-FITC and Propidium Iodide labeled cells.