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The Calcium-Sensing Receptor in the Breast

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Abstract

Normal breast epithelial cells and breast cancer cells express the calcium-sensing receptor (CaSR), the master regulator of systemic calcium metabolism. During lactation, activation of the CaSR in mammary epithelial cells downregulates PTHrP levels in milk and in the circulation, and increases calcium transport into milk. In contrast, in breast cancer cells the CaSR upregulates PTHrP production. A switch in G-protein usage underlies the opposing effects of the CaSR on PTHrP expression in normal and malignant breast cells. During lactation, the CaSR in normal breast cells coordinates a feedback loop that matches the transport of calcium into milk and maternal calcium metabolism to the supply of calcium. A switch in CaSR G-protein usage during malignant transformation converts this feedback loop into a feed-forward cycle in breast cancer cells that may promote the growth of osteolytic skeletal metastases.

Keywords

breast; mammary gland; PMCA2; PTHrP; skeletal metastasis; lactation; milk

The Calcium Sensing Receptor

The extracellular calcium-sensing receptor (CaSR) is a G protein-coupled, 7-transmembrane pass, cell surface receptor (GPCR) that binds calcium ions and mediates cellular responses to changes in the concentration of extracellular free calcium (Ca^{2+}_o) (1–3). It is a member of class C of the large family of GPCRs and is most closely related to the metabotropic glutamate receptors, GABA(B) receptors, several taste receptors and pheromone receptors. It is highly expressed in the parathyroid glands and in the kidney where it acts as the principal regulator of parathyroid hormone (PTH) secretion and renal calcium handling, respectively, in response to changes in the circulating concentrations of calcium (1–8). Therefore, it is absolutely critical to the proper regulation of systemic calcium and bone homeostasis.

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Since the discovery of the CaSR, it has become apparent that it is expressed widely within the body and its functions are not limited to the regulation of PTH secretion and renal tubular calcium handling (2, 3). Thus, the CaSR modulates cellular proliferation, cellular differentiation and ion transport in many different organs (2, 9, 10). In addition, mirroring its effects on PTH secretion, in many cells outside the parathyroid glands, it regulates the secretion of parathyroid hormone-related protein (PTHrP) in response to changes in Ca^{2+}_o (11). Other chapters in this volume delve into the structure and pharmacology of this remarkably versatile receptor in great detail. In this chapter, we will concentrate on the function of the CaSR in the breast. Before doing so, we will briefly review some aspects of mammary gland biology required for a full appreciation of the function of the CaSR in mammary epithelial cells.

Mammary Gland Development and Milk Secretion

The mammary gland forms initially during embryonic life as a bud-like appendage that grows down from the epidermis as a simple series of branched tubes embedded within a fatty stromal compartment known as the mammary fat pad (12, 13). Little subsequent development of the gland occurs until puberty, when hormones stimulate the growth and branching of the ductal system, which expands to fill the entire mammary fat pad (14, 15). Pubertal mammary development occurs primarily in response to the synergistic actions of estrogen and growth hormone (14–16). The adult virgin duct system consists of a simple bi-layered epithelium composed of basal myoepithelial cells and centrally located luminal epithelial cells. Further development and regression of the mammary gland occur during reproductive cycles. During pregnancy, the sequential actions of progesterone and prolactin stimulate the development of terminal ductules and the proliferation of luminal epithelial cells to generate grape-like, or alveolar spheres of cells that form an acinar structure with a central lumen connected to the ducts (14–17). Pregnancy also stimulates the alveolar epithelial cells to differentiate in order to form milk. Milk production requires the synthesis, transport and secretion of large amounts of triglycerides, immunoglobulins, lactose, milk proteins and ions such as calcium and phosphate (17). Bulk milk secretion does not start until parturition and is stimulated by the fall in progesterone levels and a further increase in prolactin secretion (15, 17). Milk secretion is sustained locally by suckling and systemically by prolactin secreted from the pituitary gland. Once lactation ceases, the mammary gland regresses through an intricately choreographed wave of cell death and tissue remodeling known as mammary gland involution (18). The end result of this process is a return to a simple ductal tree similar to that of the virgin female. Subsequent rounds of alveolar development and involution occur with each successive reproductive cycle, allowing for milk production with each pregnancy and lactation, but not in between.

Regulation of Calcium and Bone Metabolism During Lactation

Milk provides all the calcium and phosphorus required for rapid skeletal growth during the neonatal period. Nursing humans secrete 300 – 400 mg of calcium into milk each day (19–21). The extra demand for calcium stresses maternal calcium homeostasis and, as a result, lactation is associated with a series of adaptations involving calcium and bone metabolism (22). Suckling induces maternal hyperphagia and prolactin secretion, which stimulate the absorption of calcium by the gastrointestinal tract (23–25). Therefore, some of the extra calcium required to support infant growth comes from the maternal diet. During lactation, the kidneys retain calcium, and urinary calcium excretion declines to very low levels (26, 27). Thus, some calcium is reclaimed from the urine. Finally, lactation is associated with significant bone loss, and it has been assumed that much of milk calcium comes from the maternal skeleton (26, 27).

Bone mineral density (BMD) declines between 5–8% over 6 months of full-time nursing in humans. This decline occurs at an estimated rate of between 1–3% per month. The rate of decline in BMD following menopause by comparison, approximates 1–3% per year (26). Rodents, which typically nurse many more offspring than humans, lose up to 20–30% of their bone mass over 3 weeks of lactation (27, 28). Bone loss during lactation is associated with increased turnover. Thus, biochemical markers of bone resorption and markers of bone formation are elevated in nursing humans. In rodents, bone histomorphometry reveals that osteoclast numbers and activity are increased in lactating as compared to nulliparous females. Osteoblast numbers and bone formation rates are also elevated but are insufficient to offset the increased rates of bone resorption, thereby resulting in net bone loss (26–29). The mechanisms that drive the increased bone turnover and bone loss associated with lactation are only partly understood. Two key factors are low estrogen levels due to hypogonadotropic hypogonadism caused by the suckling reflex, and the secretion of PTHrP from the breast, both of which act synergistically to increase osteoclast numbers and activity. Finally, a characteristic feature of bone loss associated with lactation is its rapid and complete reversibility after weaning (26, 29). In rodents, skeletal mass is almost completely restored 4 weeks after the end of lactation. In humans, bone mineral density is restored to baseline within 6–12 months (26, 30–32). As a result, the skeleton can support multiple rounds of pregnancy and lactation, and repeated nursing does not predict future osteoporosis in women (22, 26).

Role of the CaSR in Normal Mammary Gland

Dr. Edward M. Brown's laboratory published the first report of CaSR expression in the human breast (33). A subsequent survey of CaSR expression in the mouse mammary gland revealed that CaSR mRNA levels are low during post-natal mammary gland development through late pregnancy, but increase to a peak during lactation (34). With the onset of mammary gland involution, CaSR expression again falls to nulliparous levels (34). To date, defects in mammary gland development or differentiation status have not been attributed to alterations in the CaSR. However, these are times of dynamic changes in mammary epithelial cell turnover and given the role of the CaSR in regulating cell proliferation and differentiation in other cell types, it is possible that the CaSR might participate in these processes in the mammary gland during puberty or pregnancy. Specific genetic manipulations designed to answer these questions have not yet been directed at these time periods. Instead, work to date has focused on the period with the highest levels of CaSR expression, lactation. (34–36).

The CaSR Regulates PTHrP Production in Mammary Epithelial Cells

In parathyroid chief cells, CaSR signaling represents the main mechanism by which the secretion of PTH is matched to the circulating concentration of calcium (37). The related autocrine/paracrine factor, parathyroid hormone-related protein (PTHrP, gene symbol *Pthlh*), which was first described as the tumor-derived factor responsible for many cases of humoral hypercalcemia of malignancy, has a much wider tissue distribution than PTH, and does not normally regulate systemic calcium homeostasis (11). However, PTHrP shares N-terminal homology and a common receptor (PTHrP1) with PTH that allow it to mimic the calcitropic actions of PTH in the kidney and bone (11). Normally, PTHrP acts in a paracrine/autocrine fashion and does not circulate. During lactation, however, the mammary gland secretes PTHrP into the circulation, where it acts in an endocrine fashion to mobilize skeletal calcium stores by activating bone resorption (11). The CaSR has been reported to regulate PTHrP expression and/or secretion in several cell types, such as astrocytes, ovarian epithelial cells, cytotrophoblasts, osteoblasts, prostate cancer cells, HEK293 cells (transfected with CaSR), and breast cancer cells (38). Therefore, we asked whether the

CaSR might regulate PTHrP expression and/or secretion by mammary epithelial cells during lactation. In contrast to its effect on most cell types studied, including breast cancer cells (39), we found that stimulation of the CaSR on normal mammary epithelial cells suppressed PTHrP production (34). The observation of an inverse relationship between CaSR stimulation and PTHrP secretion in normal breast cells has been supported by a series of pharmacologic and genetic studies in mice. Low dietary calcium-induced reductions in Ca^{2+}_o levels in wild-type mice (34), global haploinsufficiency of the CaSR (35), or mammary-specific deletion of the CaSR all resulted in increased *Pthlh* gene expression in the mammary gland and higher milk PTHrP concentrations. In calcium-deficient lactating mice, infusion of the calcimimetic compound NPS-R467 returned PTHrP production to levels approaching those in control, calcium-replete mice (34). Because global homozygous deletion of *Casr* is lethal (40), we recently studied a genetic model of mammary-specific CaSR insufficiency generated by breeding β -lactoglobulin-Cre mice (41) with *Casr*^{lox/lox} mice (42). In this model, exon 7 of the *Casr* gene is deleted specifically in mammary epithelial cells, but only during late pregnancy and lactation during which the β -lactoglobulin gene is switched on. Consistent with previous results, these mice demonstrated increased PTHrP production by mammary epithelial cells and increased milk PTHrP concentrations. Circulating immunoreactive PTHrP was also apparently elevated in these mice, although the difference did not reach statistical significance. Nonetheless, urinary cAMP levels, which serve as a sensitive bioassay for circulating PTH and PTHrP (43), were significantly higher in lactating BLG-Cre;PTHrP^{lox/lox} mice. Since PTH levels were suppressed in these mice, the elevated urinary cAMP levels indicate that bioactive PTHrP levels were elevated. In addition, Cao et al. showed that increasing dietary calcium in lactating WT or PTH^{-/-} mice reduced PTHrP concentrations in milk (44), presumably by activation of the mammary CaSR. The inverse relationship between circulating calcium and milk PTHrP also holds true in lactating 1 α (OH)ase^{+/-} and 1 α (OH)ase^{-/-} mice on either a high calcium diet or on a “rescue diet”, a high-calcium, -phosphorus, and -lactose diet that facilitates 1,25-dihydroxy-vitamin-D-independent calcium absorption (45). Together these observations support the conclusion that, in the normal mammary gland, Ca^{2+}_o acts on the CaSR to suppress PTHrP secretion both into milk and into the circulation.

The purpose of regulating the PTHrP content of milk and its dependence on the CaSR are unclear. However, we have observed that pups suckling dams with mammary-targeted deletion of the CaSR that were thus ingesting milk with reduced milk calcium content and elevated levels of PTHrP, had a significantly lower total body calcium content (46). Furthermore, when we engineered mice (β -lactoglobulin-Cre/PTHrP^{lox/-}) to produce milk with no PTHrP but normal Ca^{2+} concentrations, they had increased levels of total body calcium. These data suggest that milk PTHrP levels affect neonatal calcium accrual independently of the milk calcium concentration. Our data are also consistent with those of Cao et al. (44) and Ji et al. (45), who found that increasing dietary calcium in lactating mice suppressed milk PTHrP concentration and increased skeletal calcium accrual of the suckling pups. In both studies, dietary calcium supplementation also increased milk calcium levels, which likely had an additional effect on the neonatal skeleton (44, 45). Thus, it is possible that milk PTHrP levels are adjusted by the breast CaSR in an opposite manner as milk calcium levels in order to generate an integrated biochemical signal to program neonatal skeletal metabolism to the nutritional availability of calcium. Further testing of this hypothesis will require a model in which milk calcium can be adjusted independently of milk PTHrP.

The CaSR Increases Calcium Transport into Milk

Several studies have shown that CaSR signaling modulates milk calcium concentrations in response to changes in the circulating calcium level. Lactating mice on a low-calcium diet

had low serum calcium levels and produced milk with reduced calcium concentrations. Simultaneous treatment with the calcimimetic NPS R-467, however, partially restored milk calcium levels towards baseline (34). Genetic ablation of the CaSR, whether from global haploinsufficiency (35) or mammary-specific deletion of the CaSR (46), also reduced milk calcium concentrations. Furthermore, mammary-specific deletion of the CaSR dramatically reduced the clearance of calcium from the circulation into milk. In vitro, Ca^{2+}_o and calcimimetic compounds stimulated transport of ^{45}Ca in 3-dimensional cultures of differentiated mammary epithelial cells (34, 35). This resulted, in part, from changes in the enzymatic activity of a specific calcium pump, PMCA2. The plasma membrane calcium-ATPase 2 (PMCA2) is a P-type calcium pump expressed in the apical (milk-facing) surface of mammary epithelial cells. It is responsible for transporting 60–70% of the calcium found in milk (36, 47). In vitro and in vivo, CaSR stimulation or *Casr* gene deletion had no effect on PMCA2 protein levels or PMCA2 (*Atp2b2*) gene expression (36). Rather, CaSR activation stimulated the Ca^{2+} -pumping, ATPase activity of PMCA2 (36). Therefore, calcium stimulates its own transport into milk. In addition, calcium transport into milk is modulated by the CaSR-PMCA2 dyad, according to calcium availability in the maternal circulation. Still unknown are the intracellular signal transduction pathways linking the CaSR to PMCA2 activation.

In MCF-7 breast cancer cells, the CaSR activates Ca^{2+} entry through the canonical transient receptor potential ion channel, TRPC1 (48). However, the main calcium entry mechanism(s) in normal mammary epithelial cells during lactation have not been identified, and whether the CaSR might also regulate this step is unclear (49).

CaSR Signaling Pathways in Mammary Epithelial Cells

The CaSR binds Ca^{2+} and other polyvalent cations, aminoglycoside antibiotics, and amino acids as a homodimer (50). The CaSR undergoes a conformational change upon ligand-binding, which catalyzes GDP dissociation from a $\text{G}\alpha$ subunit of a heterotrimeric G protein complex, causing it to dissociate from the $\text{G}\beta\gamma$ subunits. As with many GPCRs, the CaSR interacts with multiple $\text{G}\alpha$ subtypes, and the downstream signaling pathways are highly divergent, and depend on the cellular context (50). The CaSR has been shown to stimulate the PLC/PKC pathway downstream of $\text{G}\alpha_q$ and to decrease cAMP downstream of $\text{G}\alpha_i$ (50). Both $\text{G}\alpha_q$ and $\text{G}\alpha_i$, as well as interaction with the scaffolding proteins filamin-A and caveolin-1, are thought to be involved in the regulation of PTH secretion by the CaSR in parathyroid chief cells (50). In growth plate chondrocytes, CaSR interaction with GABA-B receptor subunits induced signaling through PLC and ERK1/2 (51). MAPK signaling mediates CaSR-stimulated PTHrP secretion in some cell types (52). However, in normal mammary epithelial cells the MAPK/ERK pathway is not involved in the regulation of PTHrP production (53). Instead, the CaSR couples with $\text{G}\alpha_i$ to decrease cAMP production by adenylate cyclase, and subsequently PKA activation, without affecting phosphodiesterase activity (53). Lower cAMP production results in the inhibition of PTHrP expression, an effect that can be mimicked by inhibition of PKA, or reversed with forskolin or dibutyryl-cAMP (53).

The CaSR during Lactation: At the Crossroads of Milk Production, Calcium Metabolism and Skeletal Turnover

As reviewed above, genetic and pharmacologic evidence suggests the existence of a feedback loop (54) that helps to ensure a steady supply of calcium to the mammary gland to support milk production (see Figure 1). At the beginning of lactation, mammary epithelial cells produce PTHrP and secrete it into the maternal circulation where it acts on bone cells to stimulate osteoclastic bone resorption and release calcium into the circulation. Circulating

calcium, in turn, acts on the mammary gland CaSR to inhibit further PTHrP production and to stimulate the transport of calcium into milk. If the delivery of calcium to the gland lags behind calcium usage, the resulting lower systemic calcium levels then reduces CaSR stimulation, leading to a reduction in calcium transport and increase in PTHrP secretion by the mammary epithelial cells. In this way, the mammary gland directly adjusts its own calcium usage to match its supply. By secreting more PTHrP, it also influences calcium mobilization from the skeleton. Emerging evidence also suggests that by adjusting milk calcium and PTHrP content, the mammary gland CaSR entrains maternal and neonatal calcium and bone metabolism to respond in concert to changes in calcium availability. This system likely evolved when dietary calcium was only intermittently available to scavenging mammals and appears to be primarily designed to avoid severe or prolonged maternal hypocalcemia. By reducing the transport of calcium into milk and by increasing circulating and milk PTHrP levels, the CaSR attempts to restore maternal calcium levels by attenuating calcium usage and increasing skeletal calcium mobilization.

The Calcium Sensing Receptor and Breast Cancer

Breast cancer is the most common malignancy in women in western societies and affects approximately one in eight women in the US. In women with advanced disease, the skeleton is the main site of metastasis and up to 70% of patients have one or more bone metastases (55–57). These are most commonly osteolytic in nature and depend on the ability of the cancer cells to recruit and activate osteoclasts to resorb the surrounding bone. In turn, bone resorption releases growth factors and large amounts of calcium as well as phosphate into the microenvironment of the tumor cells, potentially stimulating osteolysis and tumor cell proliferation (55–57). This positive-feedback relationship that develops between breast cancer cells and factors released into the bone microenvironment is referred to as the vicious cycle of osteolysis and contributes to both the formation and progression of bone metastases. PTHrP is produced by many breast cancers and increases local osteolysis by bone metastases (55, 57, 58). Given that the CaSR regulates PTHrP production by normal mammary epithelial cells and given that breast cancer cells are exposed to high Ca^{2+}_o at sites of active bone resorption, it has been hypothesized that the CaSR promotes osteolytic bone metastases. To date, data to support or refute such a role for the CaSR are remarkably scant and largely based on studies in breast cancer cell lines *in vitro*. In the subsequent section, we review the current state of knowledge on the subject.

CaSR Expression in Breast Cancer

The first report of CaSR expression in breast cancer came from the Brown laboratory (8) when they demonstrated CaSR mRNA and protein expression in breast tissue samples from 6 different women; two were samples of normal breast tissue, two were samples of fibrocystic disease and two were samples from ductal carcinomas (33). A subsequent report from the same laboratory demonstrated CaSR expression in two standard human breast cancer cell lines, MCF-7 and MDA-MB-231 cells, an observation that has been confirmed (9, 39, 59, 60).

Although there has been no systematic or quantitative comparison of CaSR expression between normal breast tissue and breast cancer, Huang and colleagues demonstrated that the CaSR was expressed at much higher levels in fully transformed MCF-7 and MDA-MB-231 breast cancer cell lines than in two non-transformed breast epithelial cell lines, Hs 578Bst and MCF-10A (61). In addition, Mihai et al performed immunohistochemistry on 65 tumors from patients with metastatic breast cancer and found that 75% of the cancers expressed levels of CaSR that were graded 2+ or more on a semi-quantitative 0–5 score (62). In this study, the tumors from patients with bone metastases had higher levels of CaSR expression,

suggesting a role for the CaSR in modulating the formation of skeletal metastases (see discussion below). Very little is known about the mechanisms that control CaSR expression in breast cancer, although one interesting report demonstrated that CaSR gene expression could be modulated by BRCA1 in both MCF-7 and MDA-MB-231 cells (63).

In summary, the CaSR is expressed by many breast cancers, and it may be expressed at higher levels on malignant cells than on normal breast epithelial cells. In addition, primary tumors with higher levels of CaSR expression appear to be more likely to metastasize to bone. However, these conclusions are based on a small number of studies and many questions regarding the level and control of CaSR expression in breast tumors remain. It will be especially important to determine whether CaSR expression correlates with the molecular sub-type of breast cancer and whether levels of CaSR expression correlate with tumor size, stage, grade or clinical outcome in large-scale studies.

CaSR function in Breast Cancer

Effects on PTHrP production in Tumors

One clearly documented function of the CaSR in breast cancer cells is to regulate PTHrP production. As discussed in previous sections, in the intact normal mammary gland and in normal mammary epithelial cells in culture, CaSR activation decreases PTHrP mRNA levels and PTHrP secretion (34, 35, 53). However, in breast cancer cells, activation of the CaSR with Ca^{2+} , spermine, aminoglycoside antibiotics, or allosteric (type-II) calcimimetics has the opposite effect and increases PTHrP secretion (39, 53). This has been shown for several immortalized or fully transformed human or mouse breast cell lines, including Comma D cells, MCF-7 cells, and MDA-MB-231 cells (39, 53). In addition, Ca^{2+}_o acted synergistically with TGF- β to enhance PTHrP production in MCF-7 and MDA-MB-231 cells (39).

Our laboratory was interested in understanding why normal breast cells suppressed PTHrP production in response to CaSR activation whereas malignant breast cells stimulated PTHrP production in response to CaSR activation. We found that changes in PTHrP production were not explained by alterations in calcium transients or MAPK signaling downstream of the receptor, but rather correlated with divergent cAMP responses (53). In normal breast cells, activation of the receptor suppresses cAMP levels while in malignant cells activation of the receptor stimulates cAMP production. This results from a switch in G-protein usage. In normal cells the CaSR couples to $\text{G}\alpha_i$ but in malignant cells, it couples instead to $\text{G}\alpha_s$. The *PTHrP* gene is known to be sensitive to cAMP and the effects of CaSR activation on PTHrP production in these different cells could be mimicked by manipulating cAMP levels independently from CaSR activation (53). Thus, it would appear that malignant transformation alters downstream signaling, thereby explaining the paradoxical positive effect of extracellular calcium on PTHrP production in breast cancer cells. The alteration in PTHrP production may have important consequences for the pathophysiology of breast cancer, especially for the progression of bone metastases and will be discussed further below.

Evidence for pro-tumorigenic effects

There are conflicting reports regarding the effects of CaSR signaling on the proliferation of breast cancer cell lines. In the report by Sanders et al., Ca^{2+}_o levels between 0.5 and 10 mM had no effect on cell proliferation as assessed by cell number in MCF-7 or MDA-MB-231 cells (39). However members of the Ahidouch laboratory have reported that elevated Ca^{2+}_o (1.4–5.0 mM) stimulated the proliferation of MCF-7 cells by 25–30% (59, 64). The effect was mediated by the CaSR and depended on the activation of PLC, PKC, ERK and store-operated calcium entry through the calcium channel, TRPC1 (transient receptor potential

channel 1) (64). Activation of the CaSR in MCF-7 cells also induced membrane metalloproteinase (MMP) activity that released membrane-bound ligands of the epidermal growth factor receptor (EGFR) (59). Activation of the EGFR, in turn, promoted TRPC1 expression, ERK activation and cell proliferation. Transactivation of EGFRs by G protein-coupled receptors has been well documented and is referred to as “triple pass” signaling (65). The CaSR transactivates EGFR signaling in several cell types, suggesting that this may be a commonly utilized signaling pathway downstream of the receptor (66–69). EGFR activation contributes to breast cancer tumorigenesis, and these results suggest that the CaSR might promote the aggressiveness of breast cancers in an EGFR-dependent manner (70). Consistent with this idea, Saidak et al demonstrated that CaSR activation increases the migration of MCF7, T47D and MDA-MB-231 breast cancer cells at Ca^{2+}_o levels between 1.8 mM and 5.0 mM (60). Similar to Ca^{2+}_o -induced proliferation, Ca^{2+}_o -induced migration depended on the activation of PLC and ERK activity. In MCF-7 cells, activation of the CaSR also increased the transcriptional activation of the estrogen receptor and augmented effects of estradiol to increase the expression of estrogen receptor target genes such as the progesterone receptor (71, 72). Finally, Huang and colleagues reported that the CaSR promotes expression of choline kinase and the production of phosphocholine by MCF-7 and MDA-MB-231 cells (61). Increases in phosphocholine production occurred at Ca^{2+}_o levels between 1 and 3 mM and were reported to be mediated by coupling of the receptor to $\text{G}\alpha_{12}$ and Rho activation. Phosphocholine production is elevated in breast cancer and other tumors and may contribute to tumor progression (73, 74). Thus, these data suggest that CaSR expression promotes a more aggressive course in breast tumors.

Evidence for anti-tumorigenic effects

In contrast to the above studies suggesting that the CaSR contributes to the aggressiveness of breast cancer cells, the Chakrabarty laboratory has reported that CaSR activation inhibits proliferation and decreases malignant behavior in breast cancer cells (63, 75). These investigators assessed proliferation by directly counting cell number and compared low (0.2 mM) to physiological (1.4 mM) Ca^{2+}_o . Increasing Ca^{2+}_o concentrations over this range inhibited proliferation, invasion and anchorage-independent growth of MCF-7, MDA-MB-435 and MDA-MB-231 cells (75) and enhanced sensitivity to cell death in response to paclitaxel, a standard chemotherapeutic drug (63, 75). These effects were associated with the suppressed expression of the survivin gene, a well described anti-apoptotic factor in breast and other cancers (63, 75). These data suggest that the CaSR might inhibit the development and/or progression of breast cancer, similar to its purported role in colon cancer (9). This idea was supported by the finding that CaSR expression was increased by the wild-type BRCA1 gene in breast cancer cells and that the inhibitory effect of BRCA1 on survivin expression and positive effect on the paclitaxel response were mediated, in part, by the CaSR (63).

Is the CaSR a tumor promoter or tumor suppressor for breast cancer?

At present, it is difficult to reconcile these apparently conflicting data suggesting that the CaSR inhibits malignant behavior on the one hand or promotes it on the other, especially when different investigators have used the same cell lines. In essence, one can find reports suggesting that high Ca^{2+}_o stimulates, inhibits or has no effect on the proliferation of MCF-7 or MDA-MB-231 cells. One clear difference between the findings from the Ahidouch and the Chakrabarty laboratories was the Ca^{2+}_o concentration used (59, 63, 64, 75). However, Sanders et al used levels of Ca^{2+}_o that encompassed those used by both of the other laboratories and reported no effect of Ca^{2+}_o on proliferation (39). It seems likely that the differences reported reflect the complexity of downstream signaling pathways and the modifying impacts of other oncogenic or tumor suppressor pathways (53, 63, 76, 77). Whatever the explanation for these discrepancies, the conflicting results point to the need for

further studies of clinical samples and in animal models of breast cancer to more accurately assess the impact of CaSR function on tumor biology.

CaSR and Bone Metastases

The studies of Mihai and colleagues suggest a positive correlation between CaSR expression in primary tumors and the occurrence of bone metastases in patients (62). Therefore, although these findings need to be confirmed in other clinical studies, it is worth speculating on how the CaSR might favor the development of osteolytic bone metastases. As noted above, in order for a bone metastasis to grow in size, the surrounding mineralized tissue must be dissolved through the actions of peri-tumoral osteoclasts, which release high local concentrations of ionized calcium into the microenvironment (55–57). Although there is some disagreement in the literature on this point, some studies have suggested that tumor cells in close proximity to actively resorbing osteoclasts may be exposed to Ca^{2+}_o concentrations as high as 40 mM (78, 79). Therefore, it might be advantageous for a cancer cell to respond to Ca^{2+}_o in ways that would enhance growth and promote osteolysis. PTHrP has been shown to be an important factor through which breast cancer cells recruit and activate osteoclasts (55–58). Therefore, the ability of a malignant cell to sense high Ca^{2+}_o and increase PTHrP production would be clearly advantageous (53). In addition to increasing osteoclast activity via paracrine interactions with the microenvironment, PTHrP acts directly within breast cancer cells to increase proliferation through an intracrine pathway (11). Release of growth factors from the bone matrix as a result of bone resorption also stimulates the growth of bone metastases (55–58). Furthermore, as reviewed above, activation of the CaSR might directly stimulate proliferation of the breast cancer cells (59, 64). Most patients with bone metastases have ER-positive tumors and augmenting the transcriptional effects of the ER may promote the growth of the cells in bone (55, 57, 71, 72). Finally, in normal breast epithelial cells, activation of the CaSR stimulates the actions of the calcium pump, PMCA2 (36, 80). We have shown that PMCA2 can help protect breast cancer cells from cell death in response to high levels of Ca^{2+}_o (80). It is possible that a similar CaSR-dependent mechanism of enhanced intracellular Ca^{2+} clearance might also prevent cell death in the bone microenvironment *in vivo*. Therefore, there are several interconnected pathways through which CaSR expression on breast cancer cells would favor their growth and survival in the bone microenvironment and thus enhance progression of osteolytic bone disease. These pathways are highlighted in Figure 2. It must be emphasized that this figure is speculative, and the proposed pathways will need to be validated in patient samples and/or in animal models. Nevertheless, it is supported by existing evidence and manipulation of CaSR expression has been used successfully to alter the progression of skeletal metastases in a xenograft model of prostate cancer cells (81).

Summary

The CaSR is truly remarkable in its versatility. It signals in response to various ligands as well as biochemical changes in the microenvironment, such as alterations in pH or salinity. It forms homodimers, but can also heterodimerize with other receptor partners and it can signal differently in response to different ligands. Therefore, it is perhaps not surprising that it regulates biological processes in various organs. In the mammary gland, the receptor participates in key aspects of lactational physiology. It regulates mammary gland PTHrP production and the transport of calcium into milk. In essence the mammary CaSR serves as a key nutrient sensor to adjust the production of milk in response to calcium availability. As a result, it confers an ability to respond to alterations in systemic calcium concentrations in ways that allow the lactating mammary gland to actively participate in systemic calcium and bone metabolism. These effects may be exploited to alter rates of milk production or to modify its calcium content. In addition, manipulating rates of calcium uptake by the mammary gland or bone resorption might lead to improved treatments for milk fever in

dairy cattle. Interestingly, the biochemical signals generated by the CaSR appear to be altered by the process of malignant transformation, and emerging evidence suggests that the CaSR may influence the behavior of breast cancer cells. This may be especially important for the pathophysiology of osteolytic bone metastases, where breast cancer cells may be exposed to high concentrations of Ca^{2+}_o in the vicinity of resorbing osteoclasts. In this setting, the CaSR may reactivate some aspects of lactation-related calcium handling and PTHrP secretion in response to the bone microenvironment. If this is true, a greater understanding of the role of the receptor in both normal and malignant breast cells may provide clues to novel treatments for breast cancer in general and, perhaps, for bone metastases in particular.

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Research Agenda

- The roles of the CaSR in fetal and post-natal mammary gland development require further investigation.
- The intracellular signaling pathways by which the CaSR modulates PMCA2 activity should be explored.
- CaSR expression should be manipulated in xenograft models of breast cancer to determine its roles in tumor growth, with particular focus on the development and growth of skeletal metastases.

Figure 1.

During lactation, stimulation of the CaSR in mammary epithelial cells increases the transport of calcium into milk by affecting the activity of the calcium transporter, PMCA2. In addition, CaSR activation inhibits the release of PTHrP into milk and into the maternal circulation. Circulating PTHrP acts on the PTH/PTHrP receptor in the kidney to conserve calcium, and in bone, on the osteoblast (Ob), to increase osteoclast (Oc) development and activity through the RANK/RANKL pathway and mobilize skeletal calcium reserves. When the supply of calcium to mammary epithelial cells is abundant, the CaSR will promote its transport into milk, and limit renal conservation of calcium and mobilization of calcium from the skeleton by attenuating mammary PTHrP production. On the other hand, if calcium supplies decline, its transport into milk declines and systemic PTHrP rises, normalizing circulating calcium through its action on bone and kidney cells.

Figure 2.

In breast cancer cells, the CaSR increases PTHrP secretion, and could increase calcium export and/or import through regulation of the calcium export pump PMCA2 or the calcium channel TRPC1, respectively. In the setting of osteolytic bone metastasis, where breast cancer cells may be exposed to very high levels of extracellular calcium, the CaSR might facilitate a vicious feed-forward cycle by increasing PTHrP secretion, thereby promoting osteolysis to increase the local calcium concentration, which further increases CaSR stimulation. PMCA2 can protect breast cancer cells from calcium-mediated apoptosis, and its regulation by the CaSR might support the survival of breast cancer cells in the calcium-rich bone microenvironment.