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The Role of the Calcium-Sensing Receptor in the Development and Progression of Cancer

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The calcium-sensing receptor (CaR) is responsive to changes in the extracellular Ca^{2+} (Ca^{2+}_o) concentration. It is a member of the largest family of cell surface receptors, the G protein-coupled receptors, and it has been shown to be involved in Ca^{2+}_o homeostasis. Apart from its primary role in Ca^{2+}_o homeostasis, the CaR may be involved in phenomena that allow for the development of many types of benign or malignant tumors, from parathyroid adenomas to breast, prostate, and colon cancers. For example, whereas the CaR is expressed in both normal and malignant breast tissue, increased CaR levels have been reported in highly metastatic primary breast cancer cells and breast cancer cell lines, possibly contributing to their malignancy and associated alterations in their biological properties. In these settings the CaR exhibits oncogenic properties. Enhanced CaR expression and altered proliferation of prostate cancer cells in response to increased Ca^{2+}_o have also been described. In contrast, colon and parathyroid cancers often present with reduced or absent CaR expression, and activation of this receptor decreases cell proliferation, suggesting a role for the CaR as a tumor suppressor gene. Thus, the CaR may play an important role in the development of many types of neoplasia. Herein, we review the role of the CaR in various benign and malignant tumors in further detail, describing its contribution to parathyroid tumors, breast, prostate, and colon cancers, and we evaluate how pharmacological manipulations of this receptor may be of interest for the treatment of certain cancers in the future. (*Endocrine Reviews* 30: 178–195, 2009)

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Abbreviations: Ca^{2+}_o, extracellular Ca^{2+}; CaR, Ca^{2+}-sensing receptor; EGF, epidermal growth factor; EGFR, EGF receptor; ER, estrogen receptor; FHH, familial hypocalciuric hypercalcemia; GABA, γ-aminobutyric acid; GPCR, G protein-coupled receptor; MMP, matrix metalloproteinase; NO, nitric oxide; $1,25-(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; PKC, protein kinase C; PTTG, pituitary tumor-transforming gene; RANK, receptor activator for nuclear factor κB; RANKL, RANK ligand; siRNA, small interfering RNA; TCF, T cell factor; VDR, vitamin D receptor.

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I. Introduction

A. General introduction

ONE OF THE greatest challenges facing scientists and the pharmaceutical industry today is the development of effective medications to prevent or delay the incidence of cancer as well as to treat those cancers that have already developed. The characteristic features of cancer, such as uncontrolled growth, loss of the capacity for apoptosis and senescence, and acquisition of the ability to invade, metastasize, and form new blood vessels (angiogenesis), have been attributed to mutations of various oncogenes and tumor suppressor genes coding for growth factors, tyrosine kinases, and transcription factors (1, 2). Mutations in the genes encoding the G protein-coupled receptors (GPCRs) have also been implicated in tumorigenesis. It has been shown that the α1B adrenergic receptor gene can act as a protooncogene. A mutation, resulting in constitutive activation of this receptor, leads to the acquisition of oncogenic properties by enhancing focus formation in an agonist-independent manner. Injection of Rat-1 or NIH-3T3 cells expressing the mutant receptor into

nude mice eventually results in increased tumor generation (3). It has also been reported that a point mutation in the chemokine receptor CXCR2, the closest homolog of the Kaposi's sarcoma herpesvirus-GPCR, involving the DRY sequence (D138V) in the second intracellular loop, which is normally conserved among GPCRs, leads to oncogenic transformation. This transformation is similar to that seen in cells transfected with the Kaposi's sarcoma herpesvirus-GPCR, which contains the VRY sequence, producing constitutive activation which then promotes proliferation (4).

However, it is currently believed that the principal process by which GPCRs contribute to cancer is through differential receptor expression in healthy *vs.* malignant cells, and the subsequent modifications in signaling, as has been demonstrated by numerous studies. For instance, the adenosine A2B receptor, neuropeptide receptors, metabotropic glutamate receptors, the chemokine CXC receptors, and the P2Y purinoceptors, such as the GPR87 receptor, are expressed at significantly higher levels in some cancers (5–7). The chemokine CXC receptors are well-known examples of GPCRs that play a role in cancer, having a role in growth, metastasis, and angiogenesis (8). For example, it has been shown that the chemokine CXCL12 acts as a potent chemoattractant for breast cancer cells expressing the CXCR4 receptor, thereby potentially contributing to their sites of metastasis (9). The TSH receptor is another example of a GPCR that plays a role in the development of tumors, namely in thyroid malignancies. Interestingly, changes in the functionality of this receptor that contribute to tumor development arise through various mechanisms. Not only does the loss of this receptor due to abnormal methylation of its gene contribute to a more aggressive phenotype in thyroid cancers (10–12), but mutations of the TSH receptor have also been demonstrated to lead to neoplastic transformation (13). Activating mutations of the TSH receptor, for example, are frequent in thyroid adenomas (14, 15) and also occur occasionally in thyroid carcinomas (16, 17). In this review we will discuss the role of another GPCR in neoplasia, the Ca^{2+}-sensing receptor (CaR), which, based on accumulating evidence from recent studies, plays a role in the development and progression of several types of benign and malignant tumors.

B. Structure and physiological function of the CaR

Ca^{2+}_o plays an essential role in numerous physiological processes, including blood clotting, neuromuscular excitability, and maintenance of skeletal integrity. The concentrations of Ca^{2+}_o are kept at nearly constant levels with the help of a complex homeostatic system, which includes the parathyroid glands and calcitonin-secreting C cells of the thyroid gland, kidneys, bones, and intestines (18, 19). It has been known for years that Ca^{2+}-selective ion channels enable Ca^{2+} to move across the cell membrane. However, the mechanism by which cells, such as the chief cells of the parathyroid glands, which are extremely sensitive to the slightest variations in Ca^{2+}_o, can sense Ca^{2+}_o was for many years unknown.

In 1993, a membrane-spanning, Ca^{2+}_o-sensing receptor, the CaR, containing 1085 amino acids was cloned from the bovine parathyroid, and it was shown to belong to the GPCR superfamily (20). The structure of the CaR, like that of other members of the GPCR superfamily, consists of seven transmembrane helices, an extracellular N terminal, and an intracellular C terminal (Fig. 1). The CaR belongs to family 3 (or C) of the GPCRs, which also includes the metabotropic glutamate receptors (mGluR1-8), γ-aminobutyric acid receptor subunits ($GABA_{B1}$ and $GABA_{B2}$), sweet and umami taste receptors (T1R1, T1R2, and T1R3), as well as pheromone and several orphan receptors (21), and is characterized by a large extracellular N-terminal ligand-binding domain that possesses structural similarity to the Venus flytrap domain motif of bacterial periplasmic binding proteins (18, 21). As is widely accepted for numerous GPCRs, the CaR also mainly exists in the form of a dimer (22, 23). The monomers within the dimeric, cell surface form of the CaR are covalently linked by disulfide bridges involving two cysteine residues (Cys129 and Cys131) within the Venus flytrap domain motifs (24).

The activated CaR is capable of binding to a number of different G proteins, with preferential activation of $G\alpha_{q/11}$ and $G\alpha_i$, which leads to a range of cellular responses, such as stimulation of phospholipase Cβ, production of inositol 1,4,5-triphosphate, release of intracellular Ca^{2+}, stimulation of MAPKs, and an inhibition of adenylate cyclase, causing a decrease in cAMP levels (18, 25).

When the CaR was first cloned from the bovine parathyroid (20), it was identified as the cation-sensing receptor that is responsible for the sensitivity of the parathyroid chief cells to changes in Ca^{2+}_o, and it was suggested to be the primary player in Ca^{2+}_o homeostasis. Adding to its importance in Ca^{2+}_o metabolism was the subsequent cloning of the same CaR from various other tissues that are also involved in maintaining constant Ca^{2+}_o levels, including the kidney (26, 27), thyroid C cells (28), the colon (29), osteoclasts (30, 31), and

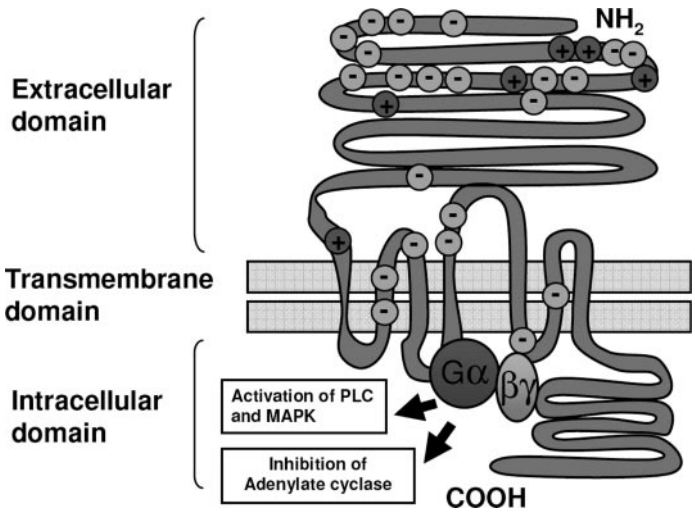

FIG. 1. A schematic representation of the human CaR, the human ortholog of the receptor that was initially cloned from the bovine parathyroid, demonstrating the localization of activating (+) and inactivating (−) mutations that were identified in various diseases of Ca^{2+}_o sensing, thereby producing either hypo- or hypercalcemia (see text). The CaR is a GPCR, consisting of seven transmembrane helices and a characteristically long N terminal. It preferentially couples to G proteins, especially the $G\alpha_i$ and $G\alpha_{q/11}$ families, leading to activation of various intracellular pathways or inhibition of adenylate cyclase.

recently also osteoblasts (32). However, it has been shown that the CaR is also expressed by various cells not involved in Ca^{2+}_o homeostasis, such as the brain (33), lens epithelial cells (34), the pancreas (35), and antral gastrin-secreting cells of the stomach (36), to name a few.

The CaR has recently also been documented to be expressed in a variety of benign tumors and malignancies, often at expression levels that differ from those in their healthy counterparts, as has been described in breast cancer (37, 38), prostate cancer (39), as well as in cancers originating from organs involved in Ca^{2+}_o homeostasis, including colorectal cancer (29) and parathyroid adenomas and carcinomas (40–43).

C. The role of CaR in Ca^{2+}_o homeostasis

Serum Ca^{2+} levels are maintained at constant levels, mainly through the concerted actions of PTH, calcitonin, and 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], the active form of vitamin D (44). The CaR has been demonstrated to play a vital and central role in the maintenance of Ca^{2+}_o homeostasis by modulating PTH secretion from the parathyroid (18, 20). In parathyroid cells, high Ca^{2+}_o levels activate the CaR, leading to inhibition of PTH secretion, PTH gene expression, and parathyroid cell proliferation (45). Although the precise mechanisms by which the CaR regulates PTH secretion remain elusive, they may involve modulation of intracellular cAMP and Ca^{2+} levels as well as activation of ERK1/2 and other kinases (46–48). In contrast, when a decrease in Ca^{2+}_o concentration is sensed by the CaR, the inhibition of PTH secretion is reduced, resulting in increased release of preformed PTH from the chief cells of the parathyroid. This is then followed within minutes by an increase in net PTH production as a result of a decrease in intracellular degradation (49, 50), and within hours, the transcription of the prepro-PTH mRNA is increased (51). The resultant increase in circulating PTH then normalizes the Ca^{2+}_o levels by its actions on the kidneys, bones, and indirectly, intestines (19, 52). Additional events in Ca^{2+}_o homeostasis that occur when changes in Ca^{2+}_o are detected and have been attributed to the CaR include direct regulation by the CaR of the renal synthesis of 1,25-$(OH)_2D_3$, and stimulation of calcitonin secretion from the C cells in the thyroid when Ca^{2+}_o levels are high (25, 28).

D. Genetic diseases of the CaR gene

Shortly after the CaR was cloned, it was recognized that mutations of the CaR gene cause several inherited disorders of Ca^{2+}_o sensing (Fig. 1). Disorders due to loss of function of the CaR include familial hypocalciuric hypercalcemia (FHH) (53) and neonatal severe hyperparathyroidism (53). These conditions are caused by inactivating mutations in the CaR gene, which right-shift the set-point for Ca^{2+}_o inhibition of PTH secretion and for stimulation of urinary Ca^{2+} excretion. FHH is usually caused by heterozygous inactivating mutations of the CaR, whereas neonatal severe hyperparathyroidism results from homozygous or compound heterozygous mutations (54, 55). Autosomal dominant hypocalcemia, in contrast, is a disorder caused by gain-of-function mutations of the CaR (56, 57), as is Bartter Syndrome type V (58). In addition to elucidating the pathobiology of the CaR, these conditions have provided formal proof of the importance of the receptor in Ca^{2+}_o homeostasis. Interestingly, alterations in the general risk of cancer have not been reported in patients suffering from these disorders, although the number of available cases for study is somewhat limited and a change in cancer risk may not have been specifically sought. As described in *Sections II–V*, numerous studies have reported firm evidence of the contribution of the CaR in different cancers.

II. The Role of the CaR in Prostate and Breast Cancers

A. Bone metastases

Breast and prostate cancers are the most frequent forms of cancer in women and men, respectively, and they are second only to lung cancer as cancer-related causes of death (59–61). Both breast and prostate cancer preferentially metastasize to bone. Approximately 75% of patients who develop advanced breast cancer will have secondary tumors in the bone, with the majority being osteolytic (62, 63). Prostate cancer almost exclusively metastasizes to bone, and about 90% of patients dying of advanced prostate cancer develop bone metastases, with the majority being blastic, although mixed blastic and lytic metastases also occur (63, 64). Both breast and prostate cancers are preferentially attracted to bones displaying high rates of bone turnover owing to active remodeling (64–66), such as the femur, pelvis, rib cage, skull, and humerus (67). Development of bone metastases leads to numerous adverse effects, including severe pain, spinal cord compression, higher risk of fractures and hypercalcemia, and greatly increased mortality rates (63, 68). Although in the case of prostate cancer it is possible to live for long periods of time with localized cancer, bone metastases can be life threatening (63, 64). Therefore, discerning the precise mechanisms that lead to the attraction of cancer cells to bone has been considered to be of vital importance in the search for more effective therapeutic agents combating the development of bone metastases.

The bone environment is believed to provide favorable conditions for the growth of certain types of cancer, such as breast, prostate, and lung, due to bone-derived factors released during bone turnover that attract the cancer cells and facilitate their metastasis and subsequent growth (69). This concept has been supported by numerous animal studies that have demonstrated that inhibitors of bone resorption, including osteoprotegerin, bisphosphonates (70–72), and inhibitors of cathepsin K (73), also decrease tumor growth in the bone in animal models of cancer metastasis. For example, zoledronic acid, a bisphosphonate that inhibits osteoclastogenesis and osteoclast activity, decreases the frequency and size of skeletal metastases in prostate cancer (65), as was shown in athymic mice inoculated with luciferase-tagged PC-3 prostate cancer cells (65). Additionally, increased bone turnover produced by PTH was shown to increase prostate cancer metastasis to bone (65), and moreover, increased bone resorption induced by dietary Ca^{2+} deficiency promotes the growth of bony metastases of breast cancer (74). Chemoat-

tractant factors such as TGFβ, IGF-I, IGF-II, and platelet-derived growth factor are believed to attract cancer cells to their metastatic location and increase their survival and proliferation (66, 75). TGFβ, IGF-I, and IGF-II are laid down in the bony matrix during bone formation and are released by bone resorption, thereby contributing to the propensity of breast and prostate cancers to metastasize to regions of active bone resorption.

An important contributor to the perpetuation of this vicious cycle initiated by bony metastases is PTHrP, which is believed to be a mediator in approximately 70% of malignant osteolysis in cancers such as breast and prostate (76). PTHrP binds to the same receptor as PTH, the type 1 PTH receptor (77), thereby activating bone turnover, including the formation and activity of osteoclasts. Although PTHrP was first isolated from renal, lung, and breast cancers, it also has numerous other roles in normal tissues, such as in the development of skin, teeth, and the mammary gland (78). In normal breast cells, similarly to PTH release, the secretion of PTHrP is inhibited by increases in the $Ca^{2+}{}_o$ concentration. Interestingly, in breast and prostate cancer cells, PTHrP release is augmented, rather than inhibited, by activation of the CaR (38, 79) (Fig. 2). Recently, it has been revealed that the change between inhibition and stimulation of PTHrP release by $Ca^{2+}{}_o$ occurs as a result of a switch in G protein activation by the cancerous cells (80). Thus, in normal mammary cells it was shown that the CaR couples to $G\alpha_i$, leading to inhibition of cAMP formation and, consequently, PTHrP release, whereas in cancerous cells, namely MCF7 and Comma-D cells, the CaR was shown to activate $G\alpha_s$, thereby promoting PTHrP release.

In the bone microenvironment, an important system regulating osteoclastogenesis exists, involving the receptor activator for nuclear factor κB (RANK), present on the surface of cells of the osteoclast lineage, and its ligand, RANKL, which is expressed by the preosteoblastic/stromal cells and/or released by these cells in a soluble form. Binding of RANKL to RANK leads to the fusion of preosteoclastic cells into multinucleated cells, and it also results in an increase in their activity and survival (81). PTHrP stimulates osteoclastogenesis by increasing the expression of RANKL on osteoblasts, thereby stimulating the formation and activity of mature osteoclasts (82). In this manner, PTHrP contributes to increased bone resorption and the further release of the chemoattractant factors noted earlier (*e.g.*, TGFβ, IGF-I, IGF-II, and platelet-derived growth factor), which are implicated in the induction and development of the vicious cycle. Another factor released during osteolysis is Ca^{2+}, whose concentrations range from 8–40 mM in the proximity of active osteoclasts to 2 mM near nonresorbing bone (83, 84). Elevated $Ca^{2+}{}_o$ levels, at concentrations of 0.8 or 0.9 mM higher than serum Ca^{2+} concentrations, activate the CaR, producing additional release of PTHrP from the cancer cells, thus feeding the vicious cycle. This phenomenon occurs in breast cancer cell lines (38), prostate cancer cell lines (39), oral squamous cancer cells (85), rat testicular cancer cells (86, 87), astrocytomas, and meningiomas (88).

Interestingly, metastatic breast cancer cells present in the bone have been shown to express higher PTHrP levels, compared with primary breast cancer cells or cells metastatic to nonskeletal sites (89–91), and there is a positive correlation between PTHrP expression by primary breast cancer cells

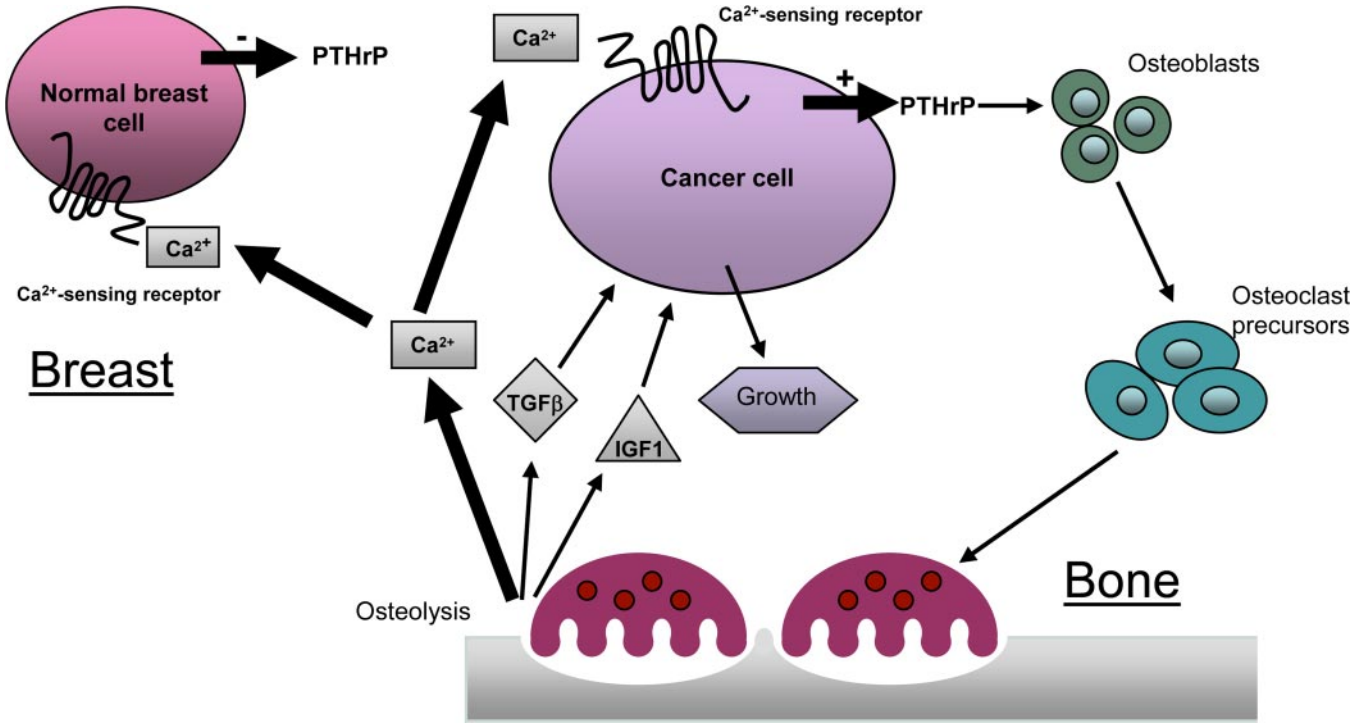

FIG. 2. The vicious cycle of metastatic breast cancer in bone. Factors released during osteolysis, such as Ca^{2+}, act on malignant cells in the microenvironment, promoting the release of agents that stimulate further bone breakdown and liberation of additional Ca^{2+} and other factors stimulating PTHrP secretion. This results in a vicious cycle. In contrast, stimulation of the CaR in normal breast cells leads to reduced PTHrP secretion.

and the risk of developing bone metastases (92). As has been shown by Guise *et al.* (93), injection of anti-PTHrP monoclonal antibody reduces skeletal metastases of MDA-MB-231 breast cancer cells in nude mice. Due to the role of PTHrP in bone metastasis and its functional interaction with the CaR, it is conceivable that the CaR represents a new pharmacological target in the treatment of bone metastases.

B. The CaR and prostate cancer

1. CaR-mediated PTHrP release in prostate cancer. Sanders *et al.* (39) initially demonstrated that CaR mRNA is expressed by the human-derived, prostate cancer cell line, PC-3. Upon stimulation by CaR agonists, including Ca^{2+}_o, neomycin, and spermine, these cells secreted PTHrP in a concentration-dependent manner. This effect was demonstrated to be mediated through the activation of the CaR because the introduction of a dominant-negative CaR into the cells by adenovirus-mediated infection suppressed the response. Interestingly, TGFβ, which is released from resorbing bone along with Ca^{2+}, produces a synergistic effect with Ca^{2+} on PTHrP secretion: pretreatment of the PC-3 cells with TGFβ increased both basal and Ca^{2+}_o-induced PTHrP secretion. This suggests that Ca^{2+} and TGFβ released into the bony microenvironment by PTHrP-induced osteolysis may, in turn, synergistically increase PTHrP secretion, leading to further bone resorption and contributing to the vicious cycle noted above (39). Yano *et al.* (79) have discerned more precisely the mechanism underlying CaR-mediated PTHrP release in prostate cancer cells. They showed that the activated CaR transactivates the epidermal growth factor (EGF) receptor (EGFR), a phenomenon that has been shown to occur for other GPCRs in earlier studies (94, 95), which is then followed by ERK1/2 activation and PTHrP release. This was demonstrated by showing that the stimulation of PTHrP release from prostate cancer cells by Ca^{2+}_o, as well as the associated ERK1/2 activation, was abolished by preincubation with an EGFR kinase inhibitor or an EGFR neutralizing antibody. A matrix metalloproteinase (MMP) inhibitor also produced a decrease in ERK1/2 activation, implying that the transactivation is mediated by the activation of MMP. This is consistent with previous studies that have suggested that transactivation of EGFRs by GPCRs occurs via the activation of MMPs, which then cleave proheparin-bound (HB)-EGF, thereby releasing HB-EGF (94, 96).

2. Role played by the CaR in prostate cancer bone metastasis. In a recent study, Liao *et al.* (97) investigated the role of the CaR in prostate cancer proliferation and metastasis. They have demonstrated that elevated Ca^{2+}_o concentrations increase the proliferation of the PC-3 and C4-2B prostate cancer cell lines, which have a high metastatic potential, but not the LNCaP prostate cancer cells, which do not metastasize to bone. The CaR-induced proliferation of the PC-3 and C4-2B cells was correlated with higher CaR expression. In this study, *in vitro* cell proliferation and *in vivo* metastatic progression were reduced by a knockdown of the CaR by RNA interference, showing the requirement of this receptor for these effects. Additionally, Ca^{2+}_o was shown to stabilize cyclin D, a key regulator of the G_1 transition from the G_1 to the S phase, and to increase PC-3 cell attachment in *in vitro* assays. The participation of the CaR in prostate cancer progression noted by Liao *et al.* is in accordance with previous microarray data that have also suggested that the CaR contributes to prostate cancer metastatic potential (98). Taken together, these studies suggest that Ca^{2+}_o, acting through the CaR, is a key mediator of prostate cancer metastasis to bone, enabling malignant prostate cells to proliferate in the bony environment.

C. The CaR and breast cancer

1. CaR expression in normal and malignant breast cells. The breast is an organ with a physiological function of vectorial transport of Ca^{2+} during milk production, resulting in the generation of milk containing approximately 200 mg Ca^{2+} daily in lactating mothers (99). Therefore, it is not surprising that the CaR is expressed in healthy and malignant breast cells and that it regulates diverse functions in this tissue. VanHouten *et al.* (100) have described that during lactation the breast participates in Ca^{2+}_o homeostasis by monitoring Ca^{2+}_o concentrations through the CaR and adjusting PTHrP secretion and milk production accordingly. Elevated PTHrP release increases skeletal Ca^{2+} secretion and renal Ca^{2+} retention. The resultant increased blood Ca^{2+}, by activating the CaR, promotes transport of Ca^{2+} from blood to milk (101). CaR expression in the breast was confirmed in a recent clinical study by Mihai *et al.* (102), who showed that whereas the CaR is expressed in both normal and malignant breast cells at both mRNA and protein levels (37, 38), elevated levels are expressed in highly metastatic breast cancer cells. Among breast cancer patients with strong CaR expression, 13 of 15 had bone metastases, whereas only two of 23 patients with a normal bone scan expressed high CaR levels, suggesting that the CaR may contribute to the preferential metastasis of breast cancer cells to the bone. Additionally, it has been shown in earlier studies that the growth and differentiation of cells derived from the human mammary gland are increased in the presence of elevated Ca^{2+}_o concentrations (103, 104), further supporting the role of Ca^{2+}_o, and possibly the CaR, in the modulation of events that may lead to cancer.

2. Dietary Ca^{2+} reduces breast cancer risk. Indirect evidence supporting the involvement of the CaR in breast cancer progression has been provided by epidemiological studies, most of which have shown that high dietary intake of Ca^{2+} decreases the risk of developing breast cancer (105, 106), although no effect of dietary Ca^{2+} on breast cancer development has also been reported (107). A recent study by Zheng *et al.* (74) showed that dietary Ca^{2+} deficiency elevated the level of PTH in serum, enhancing bone turnover and producing an associated increase in breast cancer tumor growth in bone in mice. In contrast, concurrent treatment with osteoprotegerin, a naturally occurring inhibitor of osteoclast formation and activity, resulted in greatly decreased bone resorption and completely abolished the increased lytic region area, tumor area, and cancer cell proliferation observed in Ca^{2+}-deficient mice, suggesting that increased Ca^{2+} intake and osteoprotegerin may decrease bone metastasis. These findings are consistent with clinical observations showing that breast cancer patients often have low dietary Ca^{2+} intake

and high bone turnover and that newly diagnosed cancer patients have a higher risk of developing bone metastasis if they have high bone turnover (108, 109).

3. Synergistic effect of Ca^{2+}_o and TGFβ on PTHrP release. Similarly to Ca^{2+}_o, TGFβ also up-regulates the production of PTHrP by some breast cancer cells (110), and previous studies have shown that osteolysis is reduced in mice injected with MDA-MB-231 breast cancer cells expressing a dominant-negative type II TGFβ receptor compared with control cells (75). As has been described above for prostate cancer (39), a synergistic effect of the combination of Ca^{2+}_o and TGFβ on PTHrP release has also been demonstrated in breast cancer cells (38). In TGFβ-pretreated MCF7 and MDA-MB-231 breast cancer cells, PTHrP release was augmented upon Ca^{2+}_o treatment, demonstrating a synergism between these two bone-derived factors and their contribution to the vicious cycle (38).

4. A protective G protein polymorphism in breast cancer bone metastasis. An analysis of 500 breast cancer patients has revealed that a 825C>T polymorphism in the GNB3 gene, encoding the G protein β3-subunit, is protective against bone metastasis (111). The frequency of the double mutant, GNB3 825 TT, was shown to be significantly lower among patients with bone metastases (3.1%) compared with those with other metastases (12.8%) or no metastases (13.3%). Although breast cancer cells express numerous GPCRs that are involved in bone metabolism, such as the PTH and calcitonin receptors, the CaR may be one of the receptors whose signaling is altered when polymorphisms occur in the genes encoding the G protein subunits. The 825C>T polymorphism in the GNB3 gene, which leads to increased G protein activation (112), has been suggested by previous studies to be associated with cancer (113–115). However, this polymorphism is not associated with the general risk of breast cancer *per se* (116).

5. Interaction of the estrogen receptors and the CaR. The estrogen receptors (ERs), ERα and ERβ, are known to have an important function in breast cancer pathogenesis, having roles in cell growth and differentiation. These receptors are expressed in approximately 70% of breast cancers (117, 118). Recently, the functional association between the CaR and the ERs has been examined by Journe *et al.* (119). Ca^{2+}_o was shown to modulate the function of ERα, and at 20 mM Ca^{2+}_o, similar to the concentrations encountered by cancer cells in the bone microenvironment, down-regulation of ERα was detected and ERα transcriptional activity was increased in MCF7 cells, possibly through the activation of the CaR. At 3 mM Ca^{2+}_o, increased expression of the progesterone receptor was also observed. The calcimimetic, NPS R-467, enhanced the effects of Ca^{2+}_o, whereas a CaR antagonist partly suppressed the Ca^{2+}_o-induced effects, supporting the participation of the CaR. Interestingly, Mg^{2+}, another CaR agonist, had no effect, whereas 17β-estradiol produced effects similar to those of Ca^{2+}_o. An ER antagonist, ICI 182780, also abolished the effects of Ca^{2+}_o, suggestive of a weak estrogenic effect of Ca^{2+}_o in breast cancer cells (119). This study suggests that through its interaction with the ERα, the CaR may contribute to breast cancer progression, especially in the bony microenvironment where these cells are exposed to very high Ca^{2+}_o concentrations in the vicinity of resorbing osteoclasts.

III. The CaR and Parathyroid Tumorigenesis

As has been described in *Section I*, the primary role of the CaR is in the regulation of PTH secretion from the chief cells of the parathyroid gland. Therefore, it is not surprising that considerable research has been dedicated to the quest for the role of the CaR in diseases of the parathyroid (53, 56), including cancer. Although cancers of the parathyroid gland are rare, the CaR is now known to participate in the development and/or progression of benign and malignant parathyroid tumors.

A. Inhibitory effect of the CaR on parathyroid cell proliferation

Under normal physiological conditions, one of the functions influenced by the CaR in the parathyroid is cellular proliferation. Patients with inactivating mutations of the CaR, especially those homozygous for such mutations, as well as homozygous CaR knockout mice, demonstrate parathyroid hyperplasia, indicating an inhibitory effect of the CaR on parathyroid cellular proliferation (120–122). Thus the CaR in the parathyroid can be thought of as serving as a tumor suppressor gene by virtue of its ability to suppress parathyroid cell growth. A recent report has described a decrease of 55 and 41% in the expression of CaR mRNA and protein, respectively, in hyperplastic parathyroid glands of uremic rats, and, additionally, it was shown by immunohistochemistry that CaR expression was decreased primarily in areas of active cell proliferation (122). However, as described briefly above, the decrease in cellular proliferation in response to CaR activation that is observed in the parathyroid is not a feature common to all cell types. Indeed, in some cell types, such as fibroblasts, osteoblastic, stromal, monocyte-macrophage, and prostate cancer cells, activation of the CaR leads to increased proliferation (97, 123–125), in some cases involving activation of the c-Src kinase and MAPK pathways (126). In these cells, the receptor functions more as a protooncogene.

B. Decreased CaR expression in parathyroid tumors

Previous histological studies have shown that expression levels of CaR mRNA and protein are also decreased in parathyroid adenomas, which is consistent with the weak inhibition of PTH release induced by Ca^{2+}_o in these cells, resulting in hyperparathyroidism, and their abnormal control of proliferation by Ca^{2+}_o. However, this proliferative effect normally has an upper limit, and parathyroid adenomas can be stable for years (40–42, 127, 128). In the study by Farnebo *et al.* (127), CaR mRNA and protein expression were reduced in parathyroid adenomas, demonstrating 64% of the expression level of normal patients. This was corroborated in a previous report by Gogusev *et al.* (40), where an even greater reduction was seen in CaR mRNA expression in adenoma cells, with levels at around 29–36% those of normal cells. Furthermore, in yet another study, a 60% reduction in CaR

protein immunostaining was detected in parathyroid tumors compared with normal parathyroid tissue from the same patients (41, 127). Despite these data documenting reduced CaR expression in parathyroid adenomas, there was no association between adenoma weight and CaR mRNA levels (42, 127). Additionally, Haven *et al.* (43) have shown that a strong down-regulation of the CaR also occurs in parathyroid carcinomas with a high proliferative index, a reduction that was even greater than that observed in adenomas and hyperplasias, suggesting a possible role of the CaR in parathyroid cancers.

C. CaR expression and parathyroid cell proliferation

Although it is now clear that a relationship exists between CaR down-regulation and parathyroid hyperplasia, the precise nature of this association is not fully understood. It still remains to be determined whether increased parathyroid cell proliferation leads to decreased CaR expression or vice versa. It is likely that decreased CaR levels are responsible for the increased PTH secretion set-point; however, serum PTH levels are also greatly influenced by the parathyroid cell mass. Some of the possible explanations for the reduced CaR mRNA expression levels in parathyroid adenomas that have been offered previously include less stable CaR mRNA (129) or the loss of one of the CaR alleles on chromosome 3q (130). So far these theories have not been supported, and Farnebo *et al*. (42) have shown no loss of heterozygosity in the region of the CaR gene in 11 parathyroid tumors.

D. Lack of CaR mutations in parathyroid tumors

Moreover, whereas mutations in the CaR gene have been shown to be associated with abnormal PTH secretion, leading to either hyperparathyroidism (53) or hypoparathyroidism (56), it seems that mutations and deletions of the coding region of the CaR gene are not involved in the pathogenesis of parathyroid tumors (131, 132). In the study by Hosokawa *et al.* (131), no mutations were identified in the coding regions of the CaR in 44 parathyroid tumors, including adenomas, carcinomas, and primary hyperplasias. In contrast, a variety of nonsense, missense, deletion, frame shift, insertion, and splice site mutations have been described in FHH (53). These findings were also supported by Cetani *et al.* (132), who also did not detect any mutations in the CaR gene in 20 parathyroid adenomas. Furthermore, despite the presence of reduced receptor expression levels, Corbetta *et al.* (128) have shown that the CaR acts in a similar way in parathyroid adenomas as in normal parathyroid cells, producing similar modulation of intracellular signaling pathways. However, apart from reduced CaR levels, other factors must be involved in the abnormal $Ca^{2+}{}_o$-sensing in parathyroid adenomas, because some adenoma cells express normal CaR levels, while demonstrating reduced *in vitro* sensitivity to $Ca^{2+}{}_o$ (128). One reason that may account for the defective $Ca^{2+}{}_o$ sensing in these cells despite normal CaR expression levels is the lower content of the G protein, G_q, in some parathyroid tumors (128).

E. Differential expression of exon 1A in normal parathyroid cells vs. parathyroid tumors

The human CaR gene, which is encoded by seven exons, was shown to have two promoters and two 5′ untranslated exons (exons 1A and 1B), and the alternative utilization of exons 1A and 1B leads to different mRNAs (133). It has been reported that multiple CaR mRNAs are present in both normal parathyroid cells and parathyroid adenomas, and they are expressed at different levels (129, 133). The expression of exon 1A, containing TATA and CAAT boxes, is reduced in parathyroid adenomas and is expressed at levels only 60% of those in normal glands, whereas the expression of exon 1B is not different between adenomas and normal glands. The reduced expression of exon 1A in parathyroid tumors compared with normal parathyroid cells demonstrates an alteration that may contribute to tumorigenesis.

F. Role of 1,25-$(OH)_2D_3$ in parathyroid tumors

The CaR is not the only contributor to the control of serum Ca^{2+} levels. As mentioned in *Section I*, 1,25-$(OH)_2D_3$ also plays a role by negatively regulating PTH synthesis and parathyroid cell proliferation through activation of the vitamin D receptor (VDR) (121). Previous studies have shown that PTH secretion is negatively correlated with VDR expression (134, 135). Yano *et al.* (136) have demonstrated that in addition to decreased CaR levels, VDR expression levels are also significantly lower in parathyroid adenomas compared with normal parathyroid cells. However, as was described for the CaR, mutations in the VDR are not believed to contribute to parathyroid tumor development (137). Because the two CaR promoters contain vitamin D response elements (138), it has been proposed that CaR expression levels are regulated by 1,25-$(OH)_2D_3$ (139), which was shown to up-regulate CaR mRNA levels in parathyroid, kidney, and thyroid in rat (138). Interestingly, the CaR up-regulates the VDR, and the CaR and VDR each up-regulate their own receptors. Therefore, it is not unexpected that a strong positive relationship between VDR and CaR protein expression levels exists (136), and it has been hypothesized that the reduced CaR expression present in parathyroid tumors may be secondary to the reduced VDR expression.

However, conflicting findings have been described by Rogers *et al.* (140), suggesting that CaR mRNA expression levels are not regulated by either $Ca^{2+}{}_o$ or 1,25-$(OH)_2D_3$ in rats. Furthermore, the above-mentioned CaR down-regulation in parathyroid tumors was suggested to be more closely associated with proliferative activity than the decrease in VDR expression. In fact, the decrease in CaR expression was suggested to be associated with high proliferation in a manner independent of the VDR (121). Therefore, further investigation is still necessary to determine the precise nature of the functional relationship between the CaR and VDR.

G. Possible mechanisms of CaR-induced reduction in parathyroid cell proliferation

It is not fully understood how activation of CaR leads to reduced parathyroid cell proliferation, and the precise intracellular pathways involved in this event still remain to be

elucidated. Cyclin D1 is one possible candidate, and in parathyroid tumors it behaves as an oncogene, having a role in parathyroid cell growth and dysregulated PTH secretion (141). The cyclin D1 gene is under the influence of the regulatory region of the PTH gene in occasional parathyroid tumors as a result of a chromosomal translocation and, as a result, is overexpressed (142). It is overexpressed more commonly without translocation in other parathyroid adenomas due to uncertain mechanisms (141), and overexpression of cyclin D1 in a mouse model of primary hyperparathyroidism reduces CaR expression levels, associated with a rightward shift in the Ca^{2+}_o-PTH set-point (143–145). In a recent study, Corbetta *et al.* (146) have demonstrated that EGF- and FGF-induced increases in cyclin D1 expression and ERK1/2 phosphorylation were inhibited by CaR agonists in parathyroid adenomas, demonstrating that in parathyroid tumor cells cyclin D1 expression is modulated by CaR activation in the presence of growth factors mimicking normal physiological conditions (146). These CaR-induced effects were not observed in the absence of growth factors. The differential effect of CaR activation on cyclin D1 expression, depending on the presence or absence of growth factors, is strongly suggestive of a transactivation between the CaR and growth factor receptors, a phenomenon that has been reported in other cell lines (79). Taken together, these studies show that activation of the CaR may play an inhibitory role in parathyroid tumorigenesis through its effects on cyclin D1. Due to down-regulation of the CaR in many parathyroid tumors, the normal inhibitory effect of CaR activation on cellular proliferation of the parathyroid cells is not able to proceed, leading to detrimental events promoting the development of parathyroid tumors.

IV. The Function of the CaR in Colon Cancer

A. Dietary Ca^{2+} has preventive effect in colon cancer

The chemopreventive effects of Ca^{2+}_o in colon cancer have been described in numerous previous studies (147–150). Most epidemiological studies have shown that the incidence of human colorectal carcinoma is inversely related to dietary Ca^{2+} consumption (149–153). However, a minority have reported no influence of dietary Ca^{2+} on the risk of colon cancer (154). It is not completely understood how this protective effect occurs. A possible explanation that has been previously proposed is that low Ca^{2+} reduces the amount of insoluble Ca^{2+} salts formed from otherwise carcinogenic bile acids in the lumen of the intestine (155). However, even in *in vitro* studies, it has been shown that when human colon carcinoma cells are kept in Ca^{2+}-free medium, the cells remain loosely attached to the substratum and to one another and proliferation is increased. Conversely, elevated Ca^{2+}_o decreases the rate of growth, and the cells take on a flattened appearance and behave as a cohesive epithelial unit (156). Additional studies conducted by Cross *et al.* (157, 158) have also demonstrated that the proliferative potential of intestinal Caco-2 cells is inversely related to the Ca^{2+}_o concentration. It is now, therefore, well established that Ca^{2+}_o is a direct modulator of colonocyte proliferation and differentiation.

B. The CaR is expressed by colon epithelial cells and is responsible for Ca^{2+}_o-mediated effects

Caco-2 cells (159), as well as normal colonic epithelium, have been shown to be able to sense variations in Ca^{2+}_o through the CaR, leading to the regulation of proliferation and differentiation (29, 160). The expression of the CaR in the colon is not unexpected because, apart from diet-derived Ca^{2+}, numerous other CaR agonists occur naturally in the colon, such as polyamines, suggestive of a physiological function of the CaR. In the healthy gut, a gradient of CaR expression exists in the colonic crypts, and rapidly proliferating epithelial cells at the bottom of the crypt do not express the CaR, whereas cells in the middle and top of the crypt do express this receptor, with the highest level of expression at the top (161) (Fig. 3). The cells present in the colonic crypts have been shown to acquire CaR expression as they differentiate and move toward the apex of the crypt (161). A Ca^{2+}_o concentration gradient has also been postulated to exist in the colonic crypts, with the highest concentrations at the apex of the crypt, where CaR expression is the highest and cells are fully differentiated, and decreased Ca^{2+}_o levels at the bottom of the crypt, with high cellular proliferation (161). This Ca^{2+}_o concentration gradient was hypothesized to be responsible for the differential CaR expression, as well as the enhanced differentiation, and decreased proliferative activity of the cells from the bottom to the top of the crypt.

C. CaR expression is reduced in colon cancer

It has been reported in numerous recent studies that CaR expression is reduced in colon cancer tissue compared with normal colonic mucosa (29, 160, 161); the greater the decrease in CaR expression, the greater the progression of malignancy. CaR expression is decreased in differentiated carcinomas that exhibited glandular-tubular structures, but very little or no CaR was shown to be expressed in undifferentiated, invasive carcinomas, with only isolated CaR-positive cells (29, 160). Because elevated Ca^{2+}_o concentrations decrease proliferation and increase differentiation of colon epithelial cells, it has been hypothesized that the loss or disruption of normal functionality of the CaR may lead to abnormal differentiation and proliferation, greatly contributing to the malignant pro-

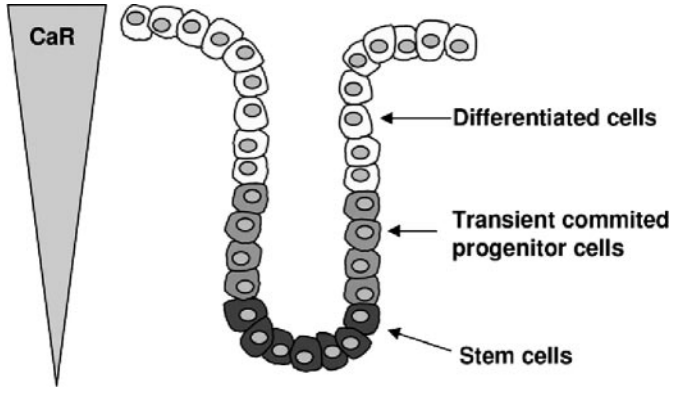

FIG. 3. The gradient in CaR expression in the colonic crypts. The CaR is expressed at the lowest levels by the stem cells present at the bottom of the colonic crypts. These cells acquire CaR expression as they differentiate and move to the apex of the crypt.

gression of colon cancer. Additionally, Bhagavathula *et al.* (162) have demonstrated in CBS carcinoma cells that down-regulation of the CaR by small interfering RNA (siRNA) leads to a lack of Ca^{2+}_o-induced inhibition of proliferation and an induction of morphological changes. Cells lacking a functional CaR continue to proliferate and do not flatten or form cell-cell contacts as the parental cells normally do in the presence of Ca^{2+}_o (162). Therefore, the chemopreventive effects of dietary Ca^{2+} normally exerted on the colonic epithelium may not take place in colon cancer cells with reduced or absent CaR. Thus, colon cancers resemble stem cells of the colon, where Ca^{2+}_o is also unable to inhibit cell proliferation and induce differentiation (163). An additional argument in the support of the involvement of the CaR in the protective effect of Ca^{2+}_o in colon cancer is the fact that Gd^{3+}, a cell membrane impermeable CaR agonist, mimics the chemopreventive effects of Ca^{2+}_o, and *in vitro*, proliferation of Caco-2 colon cancer cells decreases in response to Gd^{3+} (164). The chemopreventive effects of Ca^{2+}_o in colon cancer depend on the stage of progression of the malignancy. Ca^{2+}_o inhibits the proliferation of well-differentiated colon cancer cells, but not poorly differentiated cells (165), which is consistent with the fact that CaR expression is lost in undifferentiated cells, so Ca^{2+}_o cannot induce its effects. The mechanism by which CaR expression is reduced in colon cancer is not yet fully understood. Some possibilities include the loss or mutation of the CaR gene, or the lack of expression may simply be the result of down-regulation due to unknown mechanisms.

D. The role of E-cadherin and the β-catenin/TCF4 pathway in CaR-mediated effects

In a study by Chakrabarty *et al.* (160), it was shown that Ca^{2+}_o, acting on the CaR, promotes the expression of E-cadherin in several human colon carcinoma cell lines, including FET, SW480, MOSER, and CBS cells, leading to suppression of their malignant properties. E-cadherin belongs to the superfamily of intercellular adhesion molecules expressed by intestinal epithelial cells, and it is a tumor suppressor, having a role in various epithelial cancers (166–172). Dysregulation of E-cadherin plays a role in the transition from adenoma to carcinoma, which involves the acquisition of an aggressive phenotype (173, 174), leading to increased metastasis and invasiveness (175, 176). Consistently, E-cadherin is expressed at very low levels in rapidly proliferating epithelial cells, but it is up-regulated during differentiation (173). The precise mechanism of the CaR-induced increase in E-cadherin expression still needs to be elucidated. However, some recent evidence has shown that in CBS colon carcinoma cells, ERK1/2 may be involved in the up-regulation of E-cadherin upon CaR activation, thereby leading to reduced growth and the onset of differentiation (156). This was demonstrated by treating the cells with the ERK1/2 inhibitor, U0126, which abolished E-cadherin up-regulation (156).

The tumor-suppressing effects of E-cadherin are believed to be produced through its interaction with β-catenin, a protooncogene (177) that is a member of the Wnt pathway family (178). β-Catenin is known to play a role in colon cancer, and the expression of this protein is dysregulated at different stages of carcinogenesis. E-cadherin forms a complex with β-catenin, which is then linked to the actin-based cytoskeleton (179). Only when both β-catenin and E-cadherin are present at the cell surface, and are functional, do cells within a given tissue act as a cohesive unit. Otherwise single cells are able to move and invade surrounding tissues (180). E-cadherin controls the function of β-catenin by sequestering this protein from the cytoplasm to the cell membrane, limiting its availability for signaling, thus antagonizing its functions (181). Activation of unsequestered β-catenin leads to its accumulation in the nucleus, where it interacts with the lymphoid enhancer factor-T cell factor (TCF) family of transcription factors, leading to expression of various growth-inducing genes, thereby promoting the malignant phenotype (182). Conversely, suppression of this pathway leads to differentiation of colon epithelial cells. Activation of the CaR by Ca^{2+}_o, as well as Gd^{3+}, was shown to lead to decreased binding of β-catenin to TCF4 and suppression of this malignancy-promoting pathway (160). Recently, it has also been demonstrated that in CaR-siRNA-transfected CBS cells, Ca^{2+}_o failed to induce E-cadherin production or the shift of β-catenin from the cytoplasm to the cell membrane that was observed in normal cells, thus allowing the progression of pro-proliferative effects (162). These findings suggest that induction of E-cadherin expression and suppression of the β-catenin/TCF4 pathway may contribute to the chemopreventive action of Ca^{2+}_o in colon cancer through activation of the CaR (160). Although it was initially believed that the primary adverse effect of dysregulated β-catenin in colon cancer was through its disruption of cell-to-cell adhesion, thus promoting invasion and metastasis, it is now clear that it also produces important effects on cell proliferation and differentiation (173).

Interestingly, a downstream event following the activation of β-catenin/TCF is the stimulation of the protooncogenes, c-*myc* and cyclin D1 (182, 183). Bhagavathula *et al.* (162) have demonstrated a relationship between activation of the CaR and cell surface β-catenin localization on the one hand, and reduced expression of *c-myc* and cyclin D1 on the other, suggesting that the quiescence induced by Ca^{2+}_o may be produced through inhibition of the β-catenin/TCF/c-*myc*/cyclin D1 sequence of events. It was also shown that in CaR-siRNA transfected cells, c-*myc* and cyclin D1 were not down-regulated by Ca^{2+}_o treatment; however, they were down-regulated in nontransfected cells, confirming that CaR activation has a suppressive effect on colonic epithelial cell proliferation and malignant progression (162). Interestingly, Kallay *et al.* (159) have also shown that the c-*myc*-mediated stimulation of cell proliferation in the colon was transduced along the protein kinase C (PKC) pathway, whereas others (184, 185) have shown that PKC activation exerts a negative effect on cell proliferation in the colon, suggesting that PKCα signaling is associated with cyclin D down-regulation (184). The differentiation of intestinal cells by Ca^{2+}_o was also shown to involve activation of the PKC pathway by phospholipase C, which is consistent with the reduced PKC activity in colon cancer cells compared with normal colon cells, in parallel with their decreased levels of differentiation (185, 186). The exact role of PKC in colon cancer needs to be clarified in future studies.

Apart from c-*myc* and cyclin D1, the association of β-catenin with the TCF family of transcription factors also leads to increased expression of MMP7, urokinase plasminogen activator receptor, and γ-catenin, all of which have roles in the development and progression of colon cancer (182). Previous studies have also demonstrated that β-catenin plays a role in the up-regulation of nuclear factor-κB, possibly through the p38 MAPK pathway (187), which has previously been suggested to be associated with the induction of differentiation of intestinal cells. In fact, phosphorylated forms of p38 are present predominantly in the nuclei of differentiated intestinal cells (188). Additionally, $Ca^{2+}{}_o$ has been shown to activate the cyclin-dependent kinase inhibitors p21 and p27 (161), which potently induce differentiation in intestinal epithelial cells (189, 190). This is consistent with the observation that the expression of p21 and p27 is lost in the early stages of colon tumorigenesis, along with the reduction of CaR expression (191).

E. The chemopreventive effects of 1,25-$(OH)_2D_3$ in colon cancer

In addition to $Ca^{2+}{}_o$, vitamin D has also been shown to have chemopreventive effects in colon cancer (149, 154). The majority of epidemiological studies have suggested that an inverse correlation exists between vitamin D intake or sunlight exposure and the occurrence of colon cancer (155, 192), but no effect of vitamin D on colon cancer development has also been reported (193). The protective effect of vitamin D may be partly due to its proapoptotic effects on colon cancer cells through up-regulation of the proapoptotic protein BAK (BCL2 antagonist/killer) (194), but perhaps is also due to its role in increasing the absorption of Ca^{2+} from the gut, which is its primary physiological function. Consequently, high VDR expression levels have previously been associated with a favorable prognosis in colon cancer (195, 196), and accordingly, VDR levels are significantly reduced in late stages of colon carcinogenesis (197).

As mentioned earlier, the two CaR promoters contain a vitamin D response element (138). Chakrabarty *et al*. (161) have shown that an enhanced response occurs in the stimulation of CaR promoter activity in CBS colon carcinoma cells when $Ca^{2+}{}_o$ and 1,25-$(OH)_2D_3$ are administered together, leading to enhanced CaR protein expression. This may partially account for the elevated CaR expression as the cells move up to the top of the crypt because $Ca^{2+}{}_o$ and 1,25-$(OH)_2D_3$ have a greater opportunity there to stimulate CaR expression and colon cell differentiation. Therefore, it is likely that a functional relationship exists between the CaR and vitamin D in numerous cellular events. It has been shown that just like $Ca^{2+}{}_o$, 1,25-$(OH)_2D_3$ also promotes differentiation of colon carcinoma cells via the promotion of the E-cadherin pathway (161, 198). The study by Chakrabarty *et al*. (161) showed that $Ca^{2+}{}_o$ and 1,25-$(OH)_2D_3$ can each increase the expression of E-cadherin individually, but that 1,25-$(OH)_2D_3$ was less effective in producing this effect than $Ca^{2+}{}_o$, whereas a combination of the two agents was more effective at stimulating E-cadherin expression than either alone. Additionally, the ligand-activated VDR competes with TCF4 for β-catenin binding, thereby reducing the promalignant effects of the β-catenin/TCF4 interaction (198) in a manner similar to $Ca^{2+}{}_o$. It was also shown that upon treatment with 1,25-$(OH)_2D_3$, β-catenin, γ-catenin, and zonula occludens-1, a key regulator of tight junction formation, are translocated from the nucleus to the plasma membrane, and they are thus unable to produce their promalignant effects. Moreover, 1,25-$(OH)_2D_3$ treatment also leads to reduced expression of c-*myc* (198). Additionally, in a similar manner to $Ca^{2+}{}_o$, 1,25-$(OH)_2D_3$ was also shown to produce a strong induction of p21 and p27 (161) as it does in parathyroid cells (199). Therefore, it seems that 1,25-$(OH)_2D_3$, acting on the VDR or through the regulation of the CaR gene, may produce its beneficial effects in colon cancer by increasing E-cadherin signaling and inhibiting downstream promalignant pathways, thus modulating cancer progression (161).

V. The Function of the CaR in Other Cancers

From the studies described above, it is clear that the CaR has a role in many types of cancer. In addition to the above noted findings, recent studies have revealed that pituitary, testicular, pancreatic, and brain cancers may be influenced by the CaR. This receptor is expressed in the human pituitary, in both normal cells and in pituitary adenomas (200), as well as in normal and malignant mouse and rat pituitary cells (201, 202). In human pituitary adenomas, elevated $Ca^{2+}{}_o$, neomycin and Gd^{3+} were shown to produce an increase in intracellular Ca^{2+}, due to Ca^{2+} mobilization, and an increase in cAMP levels. Because pituitary adenomas are often characterized by differential hormone secretion compared with normal pituitary cells, Romoli *et al*. (200) investigated the effect of CaR activation on GH secretion. Treatment with CaR agonists did not result in an increase in GH secretion from GH-secreting adenomas; however, an amplification of GH secretory response to GHRH was observed, showing that the CaR may contribute to the increased GH secretion by pituitary adenomas (200). Supporting the role of the GHRH in pituitary adenomas, a study by Levy and Lightman (203) previously described localized and elevated levels of GHRH mRNA in somatotroph adenomas. A separate study by Zhang *et al.* (204) investigating pituitary malignancies demonstrated that a positive correlation exists between the expression of the pituitary tumor-transforming gene (PTTG) and the degree of pituitary tumor invasiveness. PTTG is a putative oncogene overexpressed in most cancers (205–207), and it is normally associated with cell proliferation and angiogenesis (208). The findings by Zhang *et al.* (204) are interesting in the light of recent evidence suggesting that activation of the CaR by $Ca^{2+}{}_o$ leads to up-regulation of PTTG mRNA in rat testicular Leydig H-500 cancer cells in a concentration-dependent manner (206). Therefore, it is tempting to speculate that this may also be true for other cell types, such as pituitary cells, offering a possible direction for future investigation. The $Ca^{2+}{}_o$-induced effect on PTTG expression in testicular cancer cells was abolished by overexpression of a dominant-negative CaR, confirming the involvement of this receptor (206). Additionally, elevated $Ca^{2+}{}_o$ concentrations also produced an up-regulation of VEGF, a growth factor involved in angiogenesis, a process known to occur robustly in testicular

cancer, because the rapid proliferation of these cells requires an adequate blood supply (206).

An additional contributor to testicular cancer is nitric oxide (NO), which is produced by testicular cells and acts as a negative regulator of steroidogenesis while also influencing processes such as proliferation, apoptosis, and angiogenesis (209). Chronically high NO levels play a role in carcinogenesis, producing a mutagenic effect on testicular cells. Using Leydig H-500 cancer cells, it has been shown that Ca^{2+}_o, acting through the CaR, regulates the production of NO by modulating the levels of expression of the mRNA and protein for inducible NO synthase (210). Moreover, as has been described above for breast and prostate cancer, Ca^{2+}_o was shown to stimulate PTHrP secretion in testicular Leydig H-500 cancer cells through activation of the CaR (86, 87, 211). Other CaR agonists, such as Mg^{2+} and neomycin, also increased PTHrP production in a concentration-dependent manner (86). The intracellular events that are thought to precede the increase in PTHrP release upon CaR activation include the PKC, ERK1/2, p38 MAPK, and JNK pathways (48, 211). The effects of high Ca^{2+}_o on both NO production and PTHrP secretion were confirmed to be CaR-mediated by overexpression of a dominant-negative CaR mutant, which abolished these effects (210, 211).

The CaR has also been shown to be expressed in normal human pancreas (35) and in rat pancreatic islets (212, 213), where it is believed to have a physiological role in the regulation of the Ca^{2+} concentration in the pancreatic juice (212). Recently, Ca^{2+}_o has been suggested to contribute to the pathogenesis of several types of endocrine pancreatic cancers, including insulinomas (214), gastrinomas (215), and vasoactive intestinal polypeptide-secreting tumors as well as carcinoid tumors, which resemble pancreatic endocrine tumors (216–218). In gastrinomas, Ca^{2+}_o stimulated the secretion of gastrin and modulated the growth pattern of the cells through activation of the CaR (215). One of the mechanisms of action of Ca^{2+}_o in pancreatic cancer has been suggested to involve voltage-gated Ca^{2+} channels, leading to Ca^{2+} influx into these cells (219). However, in insulinoma cells, an inhibitor of voltage-dependent Ca^{2+} channels was shown to be unable to block all the effects of hypercalcemia, suggesting that another Ca^{2+}_o sensor must be involved, such as the CaR (214). Komoto *et al.* (220) have demonstrated that in human pancreatic islets and insulinoma cells, elevated Ca^{2+}_o concentrations produced an increase in cytosolic free Ca^{2+}, and this response was greater in human insulinoma cells compared with normal islets. An inhibitor of phosphatidylinositol-3 kinase abolished the response in insulinoma cells but not in islets, suggestive of differential intracellular signaling in healthy *vs.* malignant cells in response to Ca^{2+}_o, possibly involving the CaR.

The CaR was shown to be expressed in several regions of the rat central nervous system, including the striatum (33), the hippocampus (221, 222), and perivascular sensory nerves (223) as well as oligodendrocytes (224). In the human brain, the CaR was detected in primary embryonic astrocytes, the astrocytoma tumor cell line, U87, and meningiomas (88, 225), and it was shown to modulate the activities of Ca^{2+}-activated K^+ channels and nonselective cation channels as well as other cellular events, such as proliferation (224–228) and the secretion of PTHrP (88). Of note in this regard, PTHrP is an important mediator of astrocytic differentiation in rat brain (229, 230). Due to the role of the CaR in numerous cellular events, including proliferation, differentiation, and PTHrP secretion, it seems possible that this receptor may modulate the malignant progression of brain-derived cells. However, this topic is currently still in its early stages of investigation, and further research clearly needs to be carried out to support these speculations.

VI. Future Perspectives/Clinical Developments

Despite intensive efforts, few effective treatments that slow or abolish the development and progression of cancer currently exist. Additional therapeutics are therefore required to overcome this widespread and frequently deadly disease. Determining the molecular changes that underlie cancer development may enable specific targeting of the malfunctioning molecules and pathways to achieve more effective cancer therapies. In the case of cancers with modified CaR expression levels or signaling, this receptor may be a potential target. Allosteric modulators of the CaR have recently been identified that interact with the transmembrane domain of the CaR, changing the conformation of the receptor and thus the affinity of the CaR for its agonists. The calcimimetics enhance CaR agonist activity, resulting in a left-shift in the Ca^{2+}_o concentration-response curve, whereas CaR antagonists, so-called calcilytics, decrease the activity of CaR agonists. These compounds have proved to be of substantial therapeutic utility in diseases where CaR dysregulation occurs, such as various forms of hyperparathyroidism. The calcimimetic, cinacalcet, has been approved by the U.S. Food and Drug Administration for the treatment of patients with hyperparathyroidism arising from chronic kidney disease who are receiving dialysis therapy, as well as in parathyroid cancer (231). Although effective in reducing serum Ca^{2+} concentration in patients with mild primary hyperparathyroidism, cinacalcet has not yet been approved for use in this setting (232).

Due to the diverse functionality of the CaR in various cancers, the potential for clinical use of allosteric modulators of the CaR is still unclear. In cancers characterized by reduced CaR levels, such as parathyroid and colon cancers, the use of calcimimetics might be beneficial. In contrast, calcilytics, such as NPS 2143, may have a role in the treatment of cancers where elevated CaR signaling poses a problem, such as breast and prostate cancer (233). The effect of these compounds on cancer progression still remains to be tested in *in vivo* studies. However, as a result of the ubiquitous expression of the CaR and its role in numerous important physiological functions, the systemic use of allosteric agonists of the CaR might be associated with unacceptable side effects. Organ- or tissue-specific targeting methods need to be developed to mitigate any major adverse effects.

Other approaches that might theoretically produce more specific effects on the modulation of CaR function include the use of dominant-negative CaR constructs and siRNA silencing, the latter producing reduced CaR levels and thus signaling (234). Although these methods are widely used in research, further investigation into their clinical applications is needed because their utilization would greatly aid in pro-

TABLE 1. The role of the CaR in different cancers

	Oncogenic role of the CaR	Tumor suppressor role of the CaR
Breast cancer	↑ Cell proliferation (103, 104) ↑ PTHrP secretion (38) ↑ Bone metastasis in highly CaR-expressing cancer cells (102)	↓ Risk with high dietary Ca^{2+} (105, 106)
Prostate cancer	↑ Cell proliferation (97) ↑ PTHrP secretion (39, 79) ↑ Bone metastasis in highly CaR-expressing cancer cells (97)	
Parathyroid tumors		↓ Cell proliferation (120–122) ↓ CaR expression in parathyroid tumors (40, 41, 43, 127)
Colon cancer		↓ Risk with high dietary Ca^{2+} (149–153) ↓ Cell proliferation of normal colon epithelial cells and in early stages of cancer through the activation of the CaR (156–158) ↓ CaR expression in colon cancer. Absent CaR in late stages of colon cancer, thus a lack of chemopreventive effects of Ca^{2+}_o (29, 160, 161) ↑ Differentiation of colon epithelial cells (160)

↑, Increased; ↓, decreased.

ducing therapeutic effects that are highly specific for the gene in question. It is probable that the precise role of CaR activation will have to be determined for each specific cancer type, due to the heterogeneity in the cell types involved, with differential receptor and G protein expression levels, which may influence numerous signaling events. In addition, some type of targeting of the siRNA and dominant-negative CaR constructs to malignant tissue would be necessary to avoid systemic side effects resulting, for example, from reduction of CaR expression in the parathyroid. The relevant parameters of the CaR's structure and function impacting the receptor's therapeutic potential remain to be investigated but might run the gamut from the choice of a heterodimerization partner (the CaR heterodimerizes with both the metabotropic glutamate receptors and $GABA_B$ receptors) to intracellular pathway activation or receptor transactivation. For example, GPCRs are known to transactivate growth factor receptors, such as EGFR, an event that has been implicated in various cancer types, including breast, colon, lung, and prostate (94, 95, 235). Indeed, the CaR has been shown to transactivate the EGFR in prostate cancer, thereby stimulating PTHrP release (79). Therefore, there are numerous potential targets for modulation of CaR signaling. The precise contributors to the development and progression of different cancers involving the CaR still remain to be determined.

VII. Conclusion

Apart from its primary role in the maintenance of constant blood Ca^{2+} levels, the CaR plays diverse roles in the control of numerous other physiological functions, potentially including the development and progression of a wide range of benign and malignant tumors (Table 1). The numerous mechanisms by which the CaR may contribute to tumorigenesis present challenging problems in terms of determining how manipulating this receptor may be advantageous in specific types of cancer. Due to its importance in normal physiological functions, especially in Ca^{2+}_o homeostasis, great emphasis should be placed on the development of drug-targeting methods to modulate the activity of the CaR solely in tissues where its function is dysregulated and thus to avoid potentially major adverse side effects.

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