Biochimica et Biophysica Acta 1833 (2013) 1732–1744

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Calcium sensing receptor signalling in physiology and cancer ☆

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ARTICLE INFO

Article history:
Received 28 September 2012
Received in revised form 10 December 2012
Accepted 12 December 2012
Available online 23 December 2012

Keywords:
Parathyroid adenoma
Colorectal cancer
Breast cancer
Prostate cancer
Systems biology
Ligand-biased signalling

ABSTRACT

The calcium sensing receptor (CaSR) is a class C G-protein-coupled receptor that is crucial for the feedback regulation of extracellular free ionised calcium homeostasis. While extracellular calcium (Ca^{2+}_o) is considered the primary physiological ligand, the CaSR is activated physiologically by a plethora of molecules including polyamines and L-amino acids. Activation of the CaSR by different ligands has the ability to stabilise unique conformations of the receptor, which may lead to preferential coupling of different G proteins; a phenomenon termed 'ligand-biased signalling'. While mutations of the CaSR are currently not linked with any malignancies, altered CaSR expression and function are associated with cancer progression. Interestingly, the CaSR appears to act both as a tumour suppressor and an oncogene, depending on the pathophysiology involved. Reduced expression of the CaSR occurs in both parathyroid and colon cancers, leading to loss of the growth suppressing effect of high Ca^{2+}_o. On the other hand, activation of the CaSR might facilitate metastasis to bone in breast and prostate cancer. A deeper understanding of the mechanisms driving CaSR signalling in different tissues, aided by a systems biology approach, will be instrumental in developing novel drugs that target the CaSR or its ligands in cancer. This article is part of a Special Issue entitled: 12th European Symposium on Calcium.

1. Introduction

The calcium ion (Ca^{2+}) is crucial for the control of many important cellular functions such as proliferation, differentiation, and fluid secretion. Many organisms express cell-surface sensors for extracellular Ca^{2+} (Ca^{2+}_o). Ca^{2+}_o is the primary physiological ligand of a G protein-coupled receptor (GPCR), called the calcium sensing receptor (CaSR). The receptor is expressed primarily within the chief cells of the parathyroid glands where, when activated by an excess of Ca^{2+}_o, it decreases the release of the calcium-retaining hormone, parathyroid hormone (PTH) [1] to maintain Ca^{2+}_o within the physiological range (1.1–1.3 mM). Vice versa, in the case of hypocalcemia, the CaSR is inactive and PTH is released, an event which restores normocalcemia by acting on the kidneys, to enhance renal Ca^{2+} reabsorption; the intestine to, increase intestinal Ca^{2+} absorption and bone, to mobilise skeletal Ca^{2+} [1]. A large number of studies have shown that the CaSR is expressed in many other tissues in the body, which do not play an obvious role in Ca^{2+}_o homeostasis, such as the breast, blood vessels, liver, and placenta [1]. Altered CaSR expression and/or activity is associated not only with disorders of the parathyroid glands, but also with other conditions like osteoporosis, vascular calcification and cancer.

The CaSR is involved in the regulation of a number of diverse processes, such as hormone secretion, gene expression, ion channel activity, modulation of inflammation, proliferation, differentiation, and apoptosis, depending on cell type, and therefore represents a key molecule in physiology [1]. The CaSR is also able to respond to a variety of ligands, including polyvalent cations and amino acids.

Abbreviations: APC, adenomatous polyposis coli; bFGF, basic fibroblast growth factor; Ca^{2+}_i, intracellular calcium; Ca^{2+}_o, extracellular calcium; CaSR, calcium sensing receptor; CDX-2, caudal type homeobox 2; EGF, epidermal growth factor; ERK, extracellular-signal-regulated kinase; GEF, guanine nucleotide exchange factor; GPCR, G protein-coupled receptor; IP_3, inositol 1,4,5 tris-phosphate; MAP, mitogen-activated protein; MEK, MAP kinase kinase; pHPT, primary hyperparathyroidism; PKC, protein kinase C; PLC, phospholipase C; PTH, parathyroid hormone; PTHrP, parathyroid hormone related peptide; NFκB, Nuclear factor-kappaB; RANK, receptor activator of NFκB; RANKL, receptor activator of NFκB ligand; RGS5, regulator of G protein signalling 5; Ror2, receptor tyrosine kinase-like orphan receptor 2; SDF-1, stromal cell-derived factor 1; STAT, Signal Transducers and Activators of Transcription; SP, Specificity Protein 1/3; TGF-β, transforming growth factor beta; VDRE, vitamin D responsive element; Wnt, wingless

☆ This article is part of a Special Issue entitled: 12th European Symposium on Calcium.

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http://dx.doi.org/10.1016/j.bbamcr.2012.12.011

Furthermore, changes of pH and ionic strength affect the activity of the receptor, making the CaSR uniquely capable of integrating several metabolic signals. Activation of the CaSR by different ligands may stabilise the CaSR in unique activation states allowing preferential stimulation of different signalling pathways — termed "ligand-biased signalling" [2].

Altered CaSR signalling is associated with a number of pathophysiological states. In cancer, CaSR expression either becomes reduced or even lost, or signalling pathways become activated that are different from those activated in the respective normal tissues. The signalling may differ dependent on the type of cancer, stage, grade, etc. Therefore, there is need for a better understanding of how individual ligands affect CaSR-mediated signalling, and how this will in turn benefit development of novel pharmaceutical therapies targeting the CaSR.

This review article aims to provide an overview of the signalling mechanism mediated by the CaSR under normal physiological conditions and in cancer.

1.1. CaSR signalling in physiology

The CaSR is a pleiotropic GPCR that is extremely sensitive to very small deviations in plasma Ca^{2+}_o (i.e. less than 10%) within the physiological range. There is a very steep, inverse sigmoid relationship between Ca^{2+}_o and PTH release, with the steepest part of the curve being centred around the physiological serum Ca^{2+}_o of 1.2 mM, at which concentration PTH secretion is already suppressed by ~25% of its maximal value [1].

Evidence gathered since the late 1980s showed that CaSR activation leads to inhibition of PTH via signalling mediated by the trimeric G protein, $G\alpha_{q/11}$. CaSR activation in the parathyroid glands and many other cellular systems promotes phosphoinositide turnover through activation of membrane-bound phospholipase C (PLC), producing inositol 1,4,5 tris-phosphate (IP_3) and diacyl glycerol. These, in turn, promote release of Ca^{2+} from intracellular, IP_3-sensitive stores [3] and protein kinase C (PKC) activation. Indeed, in mice lacking both $G\alpha_q$ and $G\alpha_{11}$ PTH levels are greatly elevated [4]. Genetic mutations of the CaSR highlight the importance of this receptor for maintaining divalent ion homeostasis [5–7].

The CaSR is also expressed in the colon where it plays an important role in nutrient sensing and intestinal fluid transport. Hebert et al., using isolated crypts from rat [8,9] and the $CaSR^{-/-}$::$GCM2^{-/-}$ knock out mouse model, demonstrated that intestinal fluid secretion involves CaSR-dependent degradation of cyclic nucleotides by phosphodiesterases [9], reversing the electrolyte secretory effect of cholera toxin and enterotoxin. Further, it has been suggested that the presence of the CaSR in enteric nerve cells, which interact with smooth muscle along the colon, could indicate involvement of the CaSR in intestinal motility [10].

Activation of the CaSR can also induce intracellular calcium (Ca^{2+}_i) oscillations, which have been linked to inhibition of proliferation in colonic epithelial cells [11]. Such CaSR-mediated Ca^{2+}_i oscillations have been observed in a number of CaSR-expressing cells [12], including parathyroid cells [13,14] and CaSR-expressing HEK293 cells (HEK-CaSR) [15]. Recent work has examined how variations in CaSR-mediated Ca^{2+}_i oscillation frequency and amplitude are physiologically significant in a variety of cell types. For example, in human colonic epithelial cells the CaSR can induce two separate oscillatory pathways: while CaSR-mediated high frequency (~3 to 4 min^{-1}) sinusoidal Ca^{2+}_i oscillations induce inhibition of proliferation, low frequency (~1.5 min^{-1}) transient Ca^{2+}_i oscillations do not [11]. Thus, the mechanisms that control Ca^{2+}_i oscillation frequency and amplitude are critical for various CaSR-dependent biological responses.

In HEK-CaSR cells, the sinusoidal oscillations (~4 min^{-1} at 37 °C) [16,17] arise from the dynamic phosphorylation and dephosphorylation of T888 [18–20], the primary PKC phosphorylation site of the CaSR [21]. Thus, receptor-induced activation of PKC leads to phosphorylation of T888 to uncouple the receptor from $G\alpha_{q/11}$-induced PLC activation and Ca^{2+}_i mobilisation [21,22]. T888 is then dephosphorylated by protein phosphatases to restore receptor coupling to PLC and Ca^{2+}_i mobilisation [20]. Interestingly, T888 phosphorylation exhibits a bi-phasic, 'bell-shaped' profile in which phosphorylation of T888 peaks at Ca^{2+}_o concentrations around 2–3 mM [20]. Further increases in Ca^{2+}_o lead to decreases in T888 phosphorylation, and Ca^{2+}_o concentrations ≥ 4.0 mM elicit sustained elevations in Ca^{2+}_i rather than Ca^{2+}_i oscillations [20]. Disruption of the T888 PKC phosphorylation site produces an increase in Ca^{2+}_o sensitivity and recently the mutation T888M was identified in a case of Autosomal Dominant Hypocalcemia (ADH) [23], demonstrating that the T888 residue and its regulation by PKC is critical for physiological CaSR function *in vivo*.

In addition to phosphoinositol turnover, the CaSR also couples to a number of other signalling pathways. For example, in thyroid C cells activation of the CaSR is linked to calcitonin-secretion through the activation of voltage-gated calcium channels [24], while in kidney cells, CaSR activation is coupled to the metabolism of arachidonic acid by cytochrome P450 (CYP450) and cyclooxygenase (COX) pathways via a G_i-dependent mechanism [25].

CaSR activation is linked also to pro-proliferative stimuli in many cell systems. The regulation of pro-proliferative CaSR signalling involves activation of the mitogen-activated protein (MAP) kinases extracellular-signal-regulated kinases (ERK) and $p38^{MAPK}$ protein kinases [26,27]. High Ca^{2+}_o induces CaSR-mediated activation of ERK1/2 in bovine parathyroid and HEK-CaSR cells, and the calcimimetic NPS-R467 markedly enhances this effect [28,29]. L-amino acids have only a relatively small effect, possibly operating as a fine-tuning mechanism [30]. Classically, ERK1/2 is activated via Ras-dependent activation of MAP kinase kinase (MEK), and specific inhibition of MEK suppresses high Ca^{2+}_o-induced ERK1/2 activation in both bovine parathyroid and HEK-CaSR cells [28].

ERK1/2 appears to be a significant site of signal convergence downstream of CaSR activation, as pertussis toxin, the PLC inhibitor U73122 and PKC inhibitor GF109203X, have all been shown to inhibit ERK1/2 activation partially implying a role for $G\alpha_i$, as well as $G\alpha_{q/11}$ and PI-PLC [28,29]. CaSR-mediated activation of ERK1/2 via $G\alpha_i$ also requires dynamin/β-arrestin-independent receptor internalisation [29]. In addition, CaSR-mediated ERK phosphorylation can also occur through "triple-pass" signalling in which CaSR activation leads to the release of an epidermal growth factor (EGF)-like peptide by matrix metalloproteinases, which in turn evokes EGF receptor-mediated cell signalling [31].

2. CaSR in cancer: tumour suppressor or oncogene?

Intracellular Ca^{2+}-dependent signalling mechanisms are frequently remodelled or deregulated in cancer cells. The current understanding is that the CaSR can either prevent, or promote tumourigenesis depending on the type of cancer [32] (see also Table 1). The mechanisms behind its impact on carcinogenesis are multiple and not well understood.

The expression of the CaSR can be decreased or even absent, as it is in parathyroid and colorectal cancer. It has been shown recently that the CaSR is expressed in differentiated neuroblastic tumours, but it is silenced in unfavourable neuroblastomas [33]. In these tumours, dearth of CaSR expression results in loss of the growth suppressing effects of high levels of Ca^{2+}_o. Activation of the receptor inhibits proliferation of these cancer cells, suggesting a tumour suppressor function for CaSR. In contrast, increased expression is observed in highly metastatic primary breast and prostate cancer cells. Furthermore, in breast cancer cells CaSR activates preferentially $G\alpha_s$ proteins and not $G\alpha_i$, as in normal breast cells [34], resulting in increased production of parathyroid hormone-related peptide (PTHrP), which is considered a primary cause of hypercalcaemia of malignancy, and a contributor to metastatic processes involving bone. In these settings, the CaSR seems to have an oncogenic role (Table 1).

Table 1
The CaSR in cancer (adapted from Chakravarti et al. [182]).

Cancer type	Expression of the CaSR	Effect of CaSR
Parathyroid adenoma/carcinoma	Decreased	Inhibits proliferation
Colorectal cancer	Decreased	Inhibits proliferation Enhances differentiation
Neuroblastoma	Expressed in favourable tumours	
Gliomas		Enhances proliferation
Breast cancer	Increased expression in metastasising tumours	Facilitates metastasis to bone (through PTHrP)
Prostate cancer		Facilitates metastasis to bone (through PTHrP)
Leydig cancer cells		Enhances proliferation
Ovarian cancer		Enhances proliferation

CaSR signalling might play a role in mediating pro/anti-oncogenic signalling by regulation of cell migration. Indeed, CaSR-dependent ERK phosphorylation requires receptor binding to the cytoskeletal protein filamin A, while CaSR activation in breast cancer cells evokes rho-dependent actin filament formation through $G\alpha_{12/13}$ [35]. Since $G\alpha_{12/13}$ proteins have been implicated in many different processes, including cell migration [36], it has been hypothesised that CaSR-dependent signalling in breast and prostate cancer cells could favour the metastatic spread of tumours (a concept that is discussed further below) [37].

Another mechanism leading to altered signalling through the CaSR is loss of one or more signalling partners (e.g. $G\alpha_q$ in parathyroid tumours). In some organs, as the gut, the CaSR is activated by a plethora of different ligands, such as spermine, amino acids, etc. The impact of changes in CaSR ligand concentration in the extracellular milieu on the activation of specific signalling pathways (as it might be the case in the intestinal tract) also requires further investigation. There is no evidence as yet that mutations of the CaSR play a role in tumourigenesis.

Changes in CaSR signalling during tumourigenesis seem to involve very different pathways, depending on the type of cancer, the tissue/cells involved, consistent with the different roles the CaSR plays in normal physiology.

Pharmacological agonists and antagonists of the receptor might find a much broader therapeutic usage, depending on whether activation or inhibition of the receptor is required, but first we have to understand in more detail the mechanisms driving CaSR signalling in the different tissues.

2.1. The role of the CaSR in parathyroid tumourigenesis

Parathyroid adenoma, parathyroid hyperplasia and parathyroid cancer are the main disorders of the parathyroid glands. Parathyroid adenoma and cancer lead to primary hyperparathyroidism (pHPT). pHPT is a relatively common endocrine disorder, with 25–30 new cases per 100,000 people each year [38]. Parathyroid adenomas account for 80% of the pHPT cases, whereas parathyroid cancer is an extremely rare disease responsible for less than 1% of all pHPT cases [38]. Parathyroid adenomas are more frequent in 42- to 59-year-old women, while parathyroid carcinoma develops usually much later in life with equal frequency in both sexes. No dietary or lifestyle factors are associated with the risk to develop parathyroid cancer [39]. The CaSR mRNA and protein expression levels were found to be decreased or lost in parathyroid adenomas and carcinomas. Whether this is the effect or cause of the tumourigenesis is not yet clear.

2.1.1. Expression of the CaSR in parathyroid tumours

Several studies have demonstrated that the expression levels of CaSR mRNA and protein are decreased in parathyroid adenomas, as well as in hyperplastic parathyroid glands from patients with uremic secondary hyperparathyroidism, compared with normal glands [40–42]. In addition, the study of Haven et al. suggests a role for the CaSR in prevention of malignant parathyroid tumours as the CaSR expression is decreased or absent in parathyroid carcinomas, compared with parathyroid adenomas and hyperplastic glands [43].

The molecular mechanisms that drive the loss of CaSR expression, as well as the mechanisms underlying abnormal PTH secretion in response to Ca^{2+}_o, in parathyroid tumours are still not completely understood. One possible explanation for the loss of CaSR expression was the loss of CaSR allele(s) on chromosome 3q leading to decreased CaSR mRNA levels observed in pathological parathyroid glands, however, this has turned out not to be the case [42,44]. Furthermore, no mutations in the coding region of the CaSR have been identified in parathyroid adenomas, hyperplasia or carcinomas, suggesting that mutations are not involved either in loss of CaSR expression or abnormal PTH release in these tissues [44].

2.1.2. CaSR-mediated signalling in parathyroid tumours

Uncontrolled parathyroid cell proliferation is a common feature in pathological parathyroid glands. Physiological activation of the CaSR appears to be related with suppression of parathyroid proliferation [5,43,45]. Patients with inactivating CaSR mutations and mice homozygous for the CaSR knockout generally exhibit marked parathyroid hyperplasia, indicative of the inhibitory role of the CaSR in parathyroid cell proliferation [5]. Furthermore, Haven et al. showed that the Ki67 proliferation index is significantly higher in parathyroid carcinomas, where it is significantly correlated with down-regulation of CaSR expression, than in parathyroid adenomas and hyperplastic lesions [43].

The inhibitory role of the CaSR in parathyroid proliferation was demonstrated by using calcimimetics, allosteric activators of the CaSR. Recent work by Miller et al., in a rodent model of chronic kidney disease characterised by parathyroid hyperplasia and excessive PTH secretion, showed that treatment with the calcimimetic cinacalcet mediated regression of parathyroid hyperplasia and PTH decrease, which could be reversed by discontinuation of the treatment. These data further support the need of an active CaSR for inhibition of parathyroid proliferation [46]. Interestingly, calcimimetics not only activate the CaSR but seem to be able to increase its expression as well. Studies in subtotally nephrectomised rats showed an increase in CaSR mRNA and protein levels after administration of the calcimimetic NPS-R568. In addition administration of the calcimimetic AMG 641 increased CaSR transcription and was able to prevent decrease of CaSR protein expression [47,48].

Impaired signalling through the CaSR is considered to be a major factor in promoting parathyroid hyperplasia and abnormal Ca^{2+}_o sensing. As some parathyroid adenomas show a reduced sensitivity to Ca^{2+}_o even when CaSR expression levels are normal, loss of CaSR expression might not be the only factor involved in the abnormal PTH secretion in parathyroid adenomas [40,49]. A number of possible candidates have been proposed that could interact with the CaSR and modulate its signalling, such as cyclin D1, regulator of G protein signalling 5 (RGS5), caveolin-1, and $G\alpha_q$ protein.

Cyclin D1 belongs to the cyclin protein family and is involved in regulation and progression of the cell cycle. It is overexpressed in 20–40% of parathyroid adenomas and in a subset of the tumours

cyclin D1 becomes controlled by the 5′-regulatory region of PTH due to a chromosomal rearrangement and, as a result, it is overexpressed [50,51]. Furthermore, overexpression of cyclin D1 may also be attributed to a misregulation of the wingless (Wnt) / β-catenin signalling pathway, leading to accumulation of non-phosphorylated β-catenin in the cytoplasm and nucleus in a PTH-producing parathyroid tumour cell line [52]. Whether in the parathyroid cyclin D1 expression is modulated by CaSR-dependent regulation of the Wnt pathway, as seen in colonocytes, is currently not known.

It has been suggested that cyclin D1 can interact with the CaSR and support development of parathyroid tumours by increasing parathyroid proliferation [53]. The role of cyclin D1 in parathyroid tumourigenesis is supported by the study of Imanishi et al. [50], who reported that a transgenic mouse model of hyperparathyroidism, that mimics the overexpression of cyclin D1 in parathyroid adenomas, presented with decreased CaSR expression levels, increased parathyroid cell proliferation and a right-shift in $Ca^{2+}{}_o$-dependent PTH response [50,54]. Furthermore, Corbetta et al. [53] showed that in parathyroid adenomas the CaSR inhibits cyclin D1 expression in the presence of growth factors, such as basic fibroblast growth factor (bFGF) and EGF, preventing the oncogenic actions of cyclin D1 in these tumours. Interestingly, the CaSR was unable to inhibit cyclin D1 activation in the absence of bFGF and EGF, supporting the existence of an interaction between the CaSR and these growth factors [53].

Koh et al. have recently shown that RGS5 is up-regulated in parathyroid tumours when compared with normal parathyroid glands [49]. RGS5 is part of the R4 subtype of the RGS proteins that inhibit signal transduction through regulation of heterotrimeric G proteins. Class C GPCRs, including the CaSR, are regulated by RGS proteins. In HEK-CaSR cells, transiently expressing RGS5, ERK1/2 phosphorylation was inhibited, indicating that RGS5 is able to inhibit CaSR signal transduction in this heterologous expression system. Furthermore, $RGS5^{-/-}$ knockout mice displayed abnormally low plasma PTH levels with normal Ca^{2+} serum levels and normal responsiveness to $Ca^{2+}{}_o$. Therefore, it has been suggested that the RGS5 is a negative regulator of CaSR activity in parathyroid cells. It seems that RGS5 may compete with the CaSR in binding to $G\alpha_i$ and $G\alpha_q$ proteins preventing CaSR activation and thus maintaining the sensitivity of the receptor to deviations from normal $Ca^{2+}{}_o$ levels. Furthermore, overexpression of RGS5 in parathyroid adenomas inhibited normal CaSR signalling and contributed to the abnormal Ca^{2+} sensing observed in parathyroid tumours. However, the normal Ca^{2+} serum levels present in the $RGS5^{-/-}$ mice indicate that the absence of RGS5 may not create a complete opposition to CaSR activity as it is seen in genetic conditions such as autosomal dominant hypocalcemia (ADH) and $CaSR^{-/-}$ mice [49].

In many cell types, e.g. bovine parathyroid cells, the CaSR is found in caveolae, a plasma membrane organelle. Caveolin-1 is a major component of caveolae and is thought to inhibit signal transduction and proliferation. Approximately 62% of parathyroid adenomas express caveolin-1, and these tumours appear to have a better PTH response to $Ca^{2+}{}_o$ compared with those where caveolin-1 expression is lower or lost [55]. Studies by Kifor et al. [55] have shown in freshly isolated bovine parathyroid cells that activated ERK 1/2 colocalises with caveolin-1 at the plasma membrane, whereas in bovine parathyroid cells cultured for 10 days they observed translocation of activated ERK1/2 and caveolin-1 to the nucleus and cytosol, in parallel with decreased expression of caveolin-1. ERK1/2 activation was increased both at low and high $Ca^{2+}{}_o$ in bovine cells cultured for 10 days, which is in accordance with the possible role of caveolin-1 as a negative regulator in the MAPK cascade. Similarly, in parathyroid adenomas, where caveolin-1 expression is decreased or lost, ERK1/2 was localised in the cytosol and nucleus, and a reduced ability for high $Ca^{2+}{}_o$-mediated suppression of PTH secretion was observed. In the majority of the adenomas ERK1/2 was activated independently of $Ca^{2+}{}_o$ [55]. Thus, contrary to bovine parathyroid glands, ERK1/2 signalling pathway appears to be lost in parathyroid adenomas.

Corbetta et al. have reported that $G\alpha_q$ protein levels were lower in pathological parathyroid glands than in normal glands [40]. These results are supported by another study in which increasing $Ca^{2+}{}_o$ concentrations and the CaSR agonist, gadolinium (Gd^{3+}), failed to activate ERK1/2 in parathyroid adenomas [56]. Consequently, in parathyroid adenomas low expression of caveolin-1 and $G\alpha_q$ protein levels could possibly cause altered PTH release in response to $Ca^{2+}{}_o$ [40,55].

In conclusion, parathyroid tumours are characterised by decreased CaSR expression levels, increased cell proliferation and abnormal PTH secretion. Although the exact mechanisms that lead to these events are still unknown, a number of studies have suggested that the CaSR plays a role in these processes, including CaSR-mediated modulation of cyclin D1 expression and abnormal CaSR signalling due to RGS5 overexpression in these tumours. In addition, CaSR-induced activation of ERK1/2 signalling does not seem to be active in parathyroid tumours. Further studies are needed to fully understand the mechanisms responsible for the development of parathyroid tumours and the possible CaSR signalling mechanisms in this process.

2.2. The role of CaSR in colorectal tumourigenesis

The CaSR seems to play an important protective role in colorectal cancer. Colorectal cancer is the fourth most frequent form of cancer in men and the third in women worldwide [57,58]. Diet plays a major role in colorectal tumourigenesis and epidemiological studies show an inverse correlation between calcium intake and the risk of tumour development [59]. Calcium exerts its chemopreventive features through a plethora of mechanisms, such as binding toxic secondary bile acids and/or ionised fatty acids and neutralising them in form of insoluble calcium soaps [60,61], or by activating several downstream signalling cascades such as stimulating cell differentiation, inducing apoptosis and inhibiting proliferation [62–64]. There is evidence that some of these molecular mechanisms are mediated, at least in part, by the CaSR [65–68].

2.2.1. Expression of the CaSR in the colon

In 1991, Whitfield [69] hypothesised that the $Ca^{2+}{}_o$ concentration in the lumen of colonic crypts increases from sub-physiological levels at the base reaching physiological or even higher levels at the top. Cell proliferation is driven by the combination of several factors in the lower two-thirds of the crypt while proliferation is stopped and differentiation is triggered when the cells reach a critical level in the $Ca^{2+}{}_o$ concentration. In a later review, Whitfield hypothesised that it is the CaSR that senses the changes in $Ca^{2+}{}_o$ levels and acts as a molecular switch that turns off proliferation and turns on differentiation in colonocytes [70,71].

Furthermore, besides calcium, there are numerous other CaSR ligands (like polyamines, amino acids) in the gastrointestinal tract, ingested through the diet or produced by local bacterial microflora that might alter the responsiveness of the receptor to $Ca^{2+}{}_o$. These ligands activate a wide spectrum of signalling pathways in a cell specific manner.

Although the CaSR is present along the entire length of the digestive tract [72], the exact localisation and expression pattern in the colon is not clear. Published data are discordant. Chattopadhyay and colleagues [10] have shown that the CaSR is expressed in different regions of the rat colon, including the basal region of the crypt, the serosa, sub mucosa and also in the nerve endings around the myentric plexus. Sheinin et al. [73] found that the CaSR was present only in the entero-endocrine cells of the human colonic crypt. Later, Cheng et al. [8] showed cytoplasmic and membrane expression of the CaSR protein in rat and human colonic epithelial cells both at the top and base of the crypt and in the enteric nervous system [74]. Chakrabarty and his colleagues detected perinuclear CaSR expression in the columnar epithelial cells of the colonic crypt, and showed an increase in CaSR expression along the crypt axis, from the basal

region to the top of the crypt [75,76]. Contrary to that, Ahearn et al. found the highest expression of the CaSR at the base of the crypt, decreasing towards the apex [77].

A clear consensus regarding the expression of the CaSR protein in the colon is needed to validate Whitfield's hypothesis. Similarly, the existence of a $Ca^{2+}{}_o$ gradient seems to be a hypothesis based on $Ca^{2+}{}_i$ concentrations in colonocytes lining the crypt [8,78]. The existence of a gradient in the crypt lumen also needs to be verified.

Contrary to the inconsistent data of protein expression pattern in the normal colon, there is a consensus that the CaSR expression is down-regulated during colorectal tumourigenesis [73,75,76,79] just as in parathyroid tumours. The underlying mechanisms leading to this loss of expression are not completely understood.

Epigenetic alterations such as DNA methylation and histone modifications, which are frequent events in tumours [80], might cause silencing of the *CaSR* gene, as suggested by Hizaki et al. [79]. In normal cells DNA methylation assures regulation of proper gene expression and generally affects cytosines within CpG islands [81], regions of the DNA with clusters of cytosines followed by guanine [82]. The CaSR has two promoters and two 5′-untranslated exons (exon 1A and exon 1B) yielding alternative transcripts, but encoding the same protein [83,84]. The upstream promoter contains a TATA and a CAAT box and only few sporadic CpGs, whereas the downstream promoter contains a large CpG island that could be susceptible to methylation [83,84].

Interestingly, it seems that exon 1A might have more impact in regulating the expression level of the CaSR then exon 1B. In parathyroid adenomas the expression of exon 1A was much lower than in the normal gland [83]. Similarly, in colorectal tumours exon 1A mRNA expression correlated inversely with tumour grade [85] while expression of exon 1B did not change significantly.

Several transcription factor binding sites like vitamin D response elements (VDRE), and NFκB, Stat1/3, Sp1/3 binding sites are located in both CaSR promoters [84,86,87]. The two VDREs in the CaSR promoter suggest a role for vitamin D in regulating CaSR expression. Indeed, injection of the active vitamin D metabolite, 1,25-dihydroxyvitamin D_3, in rats resulted in an enhanced CaSR expression in the parathyroid glands, thyroid and kidneys [84]. Similar results were observed also in the human colon cancer cell line CBS [75]. Whether lower levels of the vitamin D receptor in colorectal tumours could be one cause of decreased CaSR expression in these tumours [88], needs to be proven.

2.2.2. CaSR-mediated signalling in colon cancer

The CaSR is linked to an intricate network of calcium signalling pathways that control normal and cancer cell growth, depending on cell-specific coupling to appropriate G proteins. High $Ca^{2+}{}_o$ levels reduce the rate of proliferation in numerous colon cancer cell lines [65,89]. The signalling cascade seems to begin with inhibition of phospholipase A_2 [85] leading to reduced levels of arachidonic acid, precursor of the proliferative prostaglandins (e.g. prostaglandin E2). Activation of the CaSR sustains differentiation in several colon cancer cell lines [66,89,90]. This has been suggested to be dependent on PKC-regulated c-myc down-regulation [65,91] and mediated via the MAP kinase pathway [89].

Chakrabarty et al. have shown that in several colon cancer cell lines $Ca^{2+}{}_o$ increases expression of the tumour suppressor E-cadherin [76]. As an intercellular adhesion protein the major role of E-cadherin is maintenance of intact cell–cell contacts for which E-cadherin complexes with ß-catenin, reducing its availability to translocate into the nucleus. In the nucleus the role of ß-catenin is to activate, together with TCF4, the so-called canonical Wnt pathway (for rev. see [92,93]).

The Wingless (Wnt) pathway is crucial for the maintenance of both foetal and adult intestinal crypt architecture and is one of the main pathways regulated by $Ca^{2+}{}_o$ in the colon [93–97]. The adenomatous polyposis coli (APC) protein, a major component of the Wnt pathway, is altered in more than 80% of colorectal tumours, leading to accumulation of ß-catenin in the nucleus [94,98]. Extracellular calcium inhibits the canonical Wnt pathway by preventing nuclear translocation of ß-catenin, reducing β-catenin-TCF4 complex formation, downregulating c-myc and cyclin D1 expression and thus inhibiting proliferation in colon cancer cell lines, an effect mediated at least in part by the CaSR [76] (Fig. 1). Rey et al., using an intestine specific $CaSR^{-/-}$ mouse model, have recently shown that colonic crypts lacking the CaSR have increased proliferation rate and higher nuclear β-catenin levels, and that absence of the CaSR allows phosphorylation of β-catenin at Ser-552/675, which promotes its nuclear translocation [67].

Activation of CaSR by increased $Ca^{2+}{}_o$ mediates Wnt5a secretion from colonic myofibroblasts. $Ca^{2+}{}_o$ also enhances Wnt5a secretion in HT-29 colon cancer cells expressing truncated APC, leading to inhibition of the defective Wnt signalling in these cells [97,99]. In colon cancer cells CaSR activation led to upregulation of the receptor tyrosine kinase-like orphan receptor 2 (Ror2) protein which acts as a receptor for Wnt5a [97]. Taken together, it is proposed that the CaSR-mediated overexpression of Ror2 leads to the Wnt5a-mediated inhibition of β-catenin signalling in these cells [97,99,100] (see also Fig. 1). Although Wnt5a can be considered a tumour suppressor, there is also evidence for its involvement in metastasis, as seen in melanoma cells [92].

Furthermore, interactions between CaSR-mediated stromal Wnt5a and epithelial Ror2 in colonic epithelial cells seem to increase caudal homeobox type 2 (CDX2) production suggesting that activated CaSR stimulated epithelial differentiation through Wnt5a-mediated non-canonical Wnt signalling [97]. The homeobox gene CDX2 stimulates expression of sucrase-isomaltase, liver-intestine cadherin and mucin2 in intestinal epithelial cells, regulating cell growth and differentiation [97,101]. A number of studies illustrate that the CDX2 expression is clearly reduced in colon cancer [97,102,103], probably as a result of loss of CaSR expression (see Fig. 1).

The anti-proliferative effect of $Ca^{2+}{}_o$ seems to be lost in transformed cells during colon carcinogenesis [104,105]. Therefore, it is of paramount importance to find molecular markers that would identify whether a subject would benefit from calcium supplementation or whether high calcium intake would inhibit only the growth of normal cells while supporting proliferation of malignant cells. The CaSR could be this marker, however this still needs scientific proof.

Our knowledge regarding CaSR signalling is still very basic. The exact, step-by-step signalling in colon cancer cells, including other signalling systems involved, is still unravelled. Considering the diversity of ligands present in the colon and the phenomenon of biased signalling as depicted in Fig. 3, we need better models and systems to understand the role of the CaSR in normal intestinal physiology and in the process of colon tumourigenesis.

2.3. The role of the CaSR in breast and prostate tumourigenesis

Calcium and the CaSR are considered to be involved in both breast and prostate cancer development as well as in the formation of bone metastasis. Breast and prostate cancer are the most frequent malignancies, with one in every 8 women and one in every 6 men born in the United States today to be diagnosed with breast or prostate cancer respectively [106]. Nutritional factors, amongst others dietary calcium, are considered to modulate the risk of breast and prostate cancer. The anti-tumour effects of calcium involve regulation of cell proliferation, differentiation, and apoptosis, partially mediated through interaction with the vitamin D system (reviewed in [107]). However, in contrast to colorectal cancer [108], there is no conclusive evidence to date for the cancer preventive effects of dietary and/or supplemental calcium intake in breast [109–115] and prostate cancer patients [116]. The underlying mechanisms of whether and how calcium influences the risk of breast or prostate cancer are not yet elucidated.

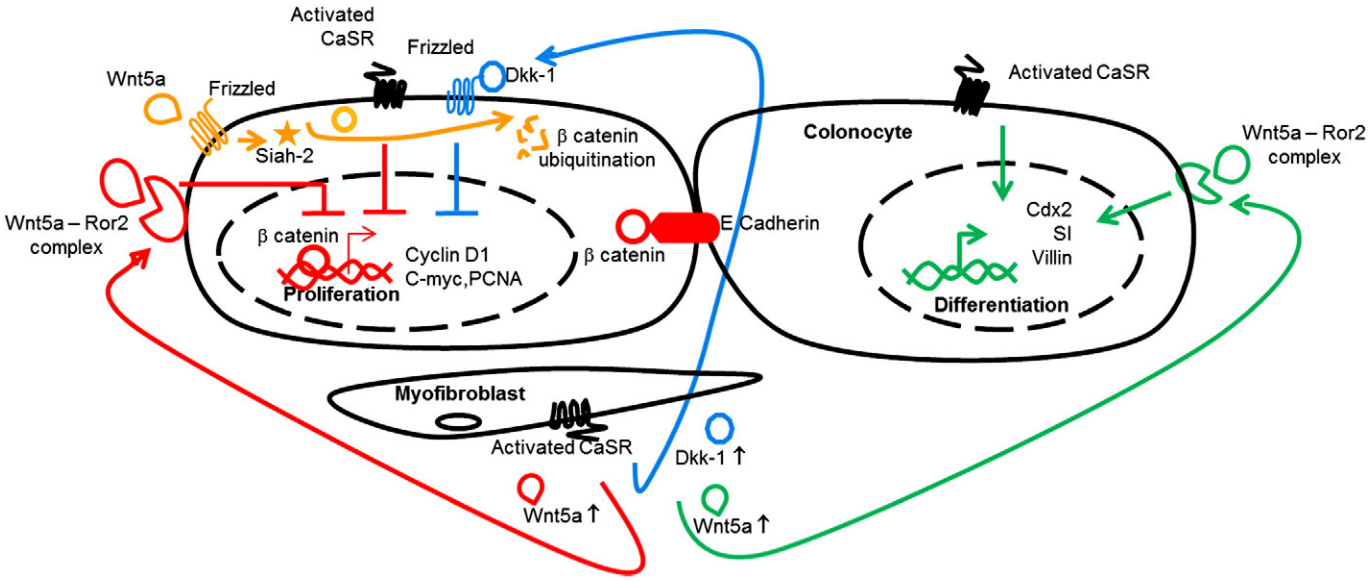

Fig. 1. Regulation of growth control mediated by calcium sensing receptor in colonocytes. The CaSR can be activated by several ligands in the extracellular mileu of the colonocytes which regulate proliferation and differentiation in these cells. There is cross talk between myofibroblasts and colonocytes. Upon receptor activation, Wnt5a is secreted by the myofibroblasts. Wnt5a then (i) increases the expression of Ror2, a receptor for Wnt5a. Upon Wnt5a-Ror2 binding, differentiation is promoted by signalling cascades leading to regulation of genes like Cdx2, sucrase isomaltase (SI) and villin (green) (ii) The Wnt5a-Ror2 binding can further inhibit nuclear translocation of β-catenin (red) or leads to degradation of β-catenin by upregulation of Siah-2, a ubiquitination ligase (orange) regulating the transcription of genes like cyclin D1, C-myc, PCNA and/or replication licensing genes, thus inhibiting proliferation. (iii) A third mechanism of growth control is by activation of myofibroblast-CaSR which increases secretion of Dickkopf-related protein 1 (Dkk-1) which then inhibits downstream signalling of its receptor, frizzled, inhibiting Wnt signalling(blue) [178–180].

2.3.1. Expression of the CaSR in the breast and prostate

The CaSR is expressed in the ductal epithelial cells of normal breast tissue [117] where it is involved in regulating calcium concentrations in the breast milk during lactation via modulation of PTHrP secretion [118]. Moreover, both ductal and lobular carcinomas express the CaSR. Interestingly, its highest expression was observed in breast cancer patients with bone metastasis, making the authors hypothesise that CaSR-positive tumours are more likely to metastasise to the skeleton [119]. In accordance with this hypothesis, expression of the CaSR was higher in breast cancer cells with relatively increased bone metastatic potential (MDA-MB-231) compared with cell lines showing lower tendency to metastasise to bone (MCF-7, T47D) [120]. Besides breast cancer cells, CaSR mRNA and protein was detected also in the highly bone metastatic prostate cancer cell lines PC-3 and C4-2B as well as in the non-metastatic LNCaP cells, albeit at a lower level [121,122]. So far, no reports on expression of the CaSR in normal prostate tissue or prostate cancer patients are available.

Currently, the mechanisms causing altered expression of the CaSR in breast cancer are not known. A constitutive activation of Nuclear Factor-kappaB (NFκB) has been observed in breast cancer [123,124] and might contribute to increased transcription of the CaSR gene, which has functional kappa b elements [86]. CaSR promoters are also known to have vitamin D responsive elements, which allow regulation of CaSR gene expression by 1,25-dihydroxyvitamin D_3, as observed in parathyroid glands [84]. In breast malignancies, however, the availability of the active vitamin D metabolite might be reduced as a result of an altered expression of the activating and degrading vitamin D enzymes (reviewed in [125]), which would lead to a decrease in the CaSR expression. Moreover, transactivation of the EGFR by the CaSR has been reported [126], however, it remains speculative whether there is a reciprocal transactivation and whether this has an impact on the expression level of CaSR, as it was observed for the oestrogen-sensitive class C G-protein coupled receptor, GPR30 [127]. Whether epigenetics, gene amplification or alterations in CaSR trafficking [128] modulate the expression of the CaSR in breast cancer remains to be investigated.

2.3.2. CaSR-mediated signalling in breast and prostate cancer

CaSR stimulation by high $Ca^{2+}{}_o$ levels promoted proliferation of MCF-7, PC-3 and C4-2B breast and prostate cancer cells known to metastasise to the skeleton. Whereas in MCF-7 breast cancer cells, $Ca^{2+}{}_o$-induced cell proliferation appears to be linked to ERK1/2 phosphorylation, which in turn stimulates expression of the Transient Receptor Potential Canonical 1 (TRPC1) cation channel and subsequent calcium entry [126,129], in PC-3 cells, Cyclin D1 is associated with the $Ca^{2+}{}_o$-induced proliferative effect [122]. In contrast, $Ca^{2+}{}_o$ did not affect proliferation of non-bone-metastatic LNCaP prostate cancer cells [122]. The effects of $Ca^{2+}{}_o$ on proliferation in these cells are likely mediated by the CaSR, as its knockdown by shRNA or siRNA decreased $Ca^{2+}{}_o$-induced cell proliferation in PC-3 and MCF-7 cells *in vitro*. It has been suggested that physiological concentrations of $Ca^{2+}{}_o$ (1.4 mM) would reduce breast cancer cell proliferation compared with low concentrations of $Ca^{2+}{}_o$ (between 0.175 and 0.2 mM), proposing the CaSR as a tumour suppressor in MCF-7 and MDA-MB-435 breast cancer cells, similar to BRCA1 [130]. These findings might be due to i) a dose-dependent biphasic response of the CaSR to $Ca^{2+}{}_o$ and/or ii) a switch in the G-proteins, as shown for CaSR-dependent regulation of PTHrP secretion in MCF-7 and Comma-D cells [34]. Furthermore, proliferation under higher concentrations of $Ca^{2+}{}_o$ was not tested in absence of vitamins, peptones and amino acids, since some of these might have been responsible for "ligand biased signalling" of the CaSR, as described later in the present review (Section 3.2).

2.3.3. The CaSR in bone metastasis

In advanced breast and prostate cancer, the CaSR seems to play a key role in the development of bone metastasis. For both types of cancer the preferential site of metastasis is the skeleton. Approximately 70% of patients with advanced breast cancer develop bone metastases with a predominantly osteolytic phenotype, while prostate cancer mostly forms osteoblastic bone metastases [131]. For both types of metastases, it is believed that breast or prostate cancer cells and bone cells communicate with each other, thereby triggering a vicious circle that results in progressive bone destruction and/or tumour growth as depicted in Fig. 2.

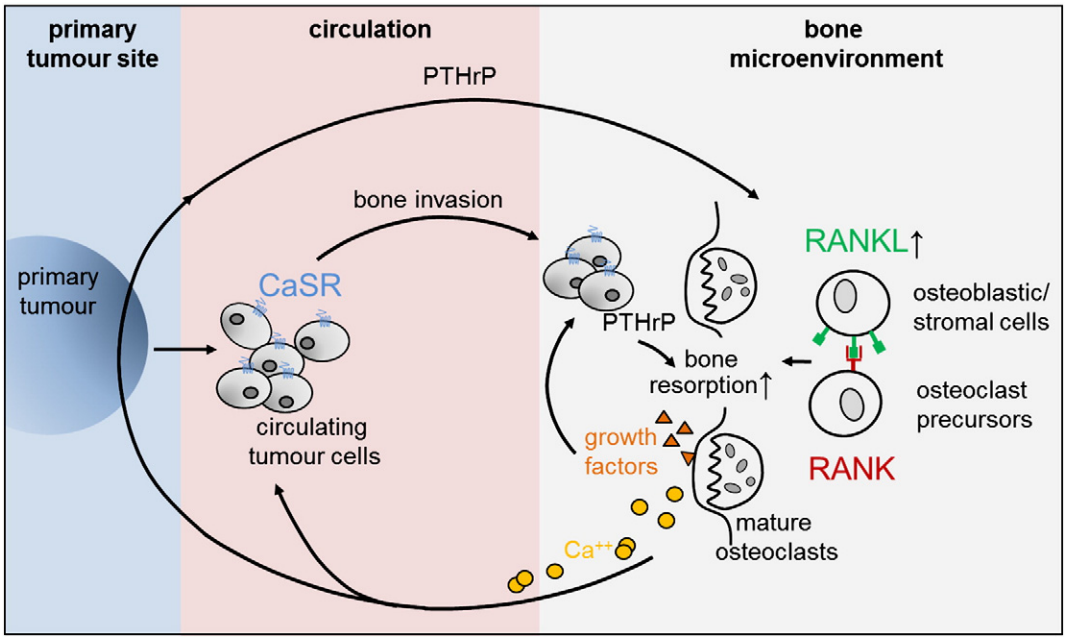

Fig. 2. The vicious circle of bone metastasis in breast and prostate cancer. Breast and prostate tumours produce parathyroid hormone related peptide (PTHrP) which up-regulates receptor activator for NFκB ligand (RANKL) on osteoblastic and stromal cells. Upon binding of RANKL to its receptor on osteoclast precursor cells, i.e. Receptor activator for nuclear factor κB (RANK), maturation and differentiation of osteoclast precursors to activated osteoclasts is initiated and bone resorption stimulated. During bone resorption, large amounts of calcium (Ca^{2+}) as well as diverse growth factors are released. Calcium promotes further PTHrP production in the tumour, thereby supporting the vicious circle. Acting via the calcium sensing receptor (CaSR), Ca^{2+} is considered to act as a chemoattractant factor and to facilitate tumour cell migration into the bone. Growth factors enable tumour cell survival and growth in the bone microenvironment and in consequence the manifestation of bone metastases which in turn increases the rate of bone turnover, thus feeding the vicious circle.

Cancer cells are capable of producing factors that stimulate bone resorption. In this regard, PTHrP is considered one of the major factors that promote bone turnover via up-regulation of Receptor Activator of NFκB Ligand (RANKL) in osteoblasts. Binding of RANKL to its receptor RANK (Receptor Activator of NFκB), which is expressed by cells of the osteoclastic lineage, increases the development, activity, and survival of mature osteoclasts in both physiological and pathological circumstances [132,133]. PTHrP is expressed in both primary breast tumours and, to a higher extent, in bone metastases of breast tumours [134]. It is therefore suggested to act both in an endocrine and paracrine manner. Endocrine secretion of PTHrP by the cancer cells at the primary tumour site may result in so-called hypercalcemia of malignancy, which occurs in up to 30% of cancer patients and is attributable to excessive bone resorption (reviewed in [135]). On the other hand, PTHrP is likely produced by metastatic cancer cells locally in the bone, where it stimulates bone resorption in a paracrine manner thereby allowing tumour expansion. In normal breast epithelial cells, PTHrP secretion is subject to a negative feedback regulation by calcium [136]. However, this negative feedback regulation is lost in both malignant breast (MCF-7, MDA-MB-231) and prostate cells (LNCaP and PC-3), where activation of the CaSR stimulates PTHrP secretion [137,138]. The effect of $Ca^{2+}{}_o$ on PTHrP secretion is potentiated by transforming growth factor-beta (TGF-β) [121,137]. Recently, Mamillapalli et al. demonstrated that a shift from $G\alpha_i$ to $G\alpha_s$ binding was responsible for the CaSR-dependent stimulation of PTHrP secretion in Comma-D and MCF-7 cells, instead of the inhibitory effect observed in normal mammary epithelial cells [34].

Circulating cancer cells are believed to sense calcium from bone through the CaSR by a mechanism which is not yet clearly understood. The activation of the CaSR might promote the retention and colonisation of these cells in the bone. This hypothesis is strongly supported by the findings of Liao and colleagues, who used an intra-cardiac injection model and demonstrated that the CaSR is essential for prostate cancer cells to develop bone metastases in mice. They provided evidence that *in vitro*, $Ca^{2+}{}_o$ stimulates prostate cancer cell proliferation and enhances the attachment of these cells via the CaSR and Akt signalling [122], probably promoting their metastatic properties. Moreover, the fact that highly-bone metastatic MDA-MB-231 breast cancer cells showed a stronger CaSR-dependent migratory response to $Ca^{2+}{}_o$ compared with cells with a lower metastatic potential (MCF-7, T47D) [120], strongly supports the role of the CaSR in migration of these cancer cells. Thus, calcium should be considered as a bone-derived chemoattractant factor similar to RANKL [139] and stromal cell-derived factor-1 (SDF-1) [140,141]. As previously shown by Kang and colleagues, metastatic breast cancer cells seem to be a subpopulation of primary tumour cells which, through genetic alteration, acquire features allowing proliferation, invasion, neovascularisation and finally metastatic capabilities. They even obtain the ability to influence bone remodelling to their advantage [142]. An adaptation of breast cancer cells seems to be essential for them to survive in such a hostile environment and it can be speculated that only a few highly adapted metastatic cells are needed to produce bone lesions. Up-regulation of the CaSR in breast cancer could be part of this adaptation. To address this issue, studies on the expression of CaSR in bone metastatic specimens are urgently needed. Clearly, our understanding of how the CaSR is involved in the development of bone metastasis is very limited and warrants further investigation. Future studies might also focus on the effect of CaSR activation on Wnt signalling, which has been implicated in bone metastasis [143].

3. The downstream signalling network of CaSR

The CaSR is a complex receptor with a plethora of ligands, participating in numerous different intracellular signalling pathways. Signal processing occurs already at the receptor level [144,145]. The CaSR has several orthosteric agonists and its activity is modulated by a large variety of allosteric modulators [146]. The binding of those extracellular compounds to the CaSR induces intracellular signalling via three different G proteins. The molecular mechanisms endowing the CaSR with the possibility to activate preferentially one specific G protein upon binding a particular ligand, without activating others are not completely

understood as yet. Ligand binding at the receptor level is modulated by intracellular phosphorylation of the CaSR at T888. Many of the dynamic and spatial aspects of CaSR signalling are undoubtedly underappreciated. For a number of other signalling systems dynamic and spatial aspects have turned out to be of key importance for understanding the signalling process [147,148]. Real-time dynamic measurement of the signalling activity of the receptor, using for instance fluorescence resonance energy transfer (FRET) [149,150], would ensure a better understanding of the dynamic competition of the G proteins for binding to the receptor and, perhaps, indicate how the receptor achieves ligand-biased signalling via modulation of its affinity for the target G proteins. In addition, to such short-term dynamic investigations of receptor activity, phosphoproteomics [151,152] or reverse phase protein arrays [153] could be used at a much slower time scale to elucidate how signalling, induced by the CaSR, propagates through the signalling network and controls various cellular processes, such as cell motility and gene expression. Such studies can go hand in hand with computational modelling efforts to study how signalling dynamics emerges from the kinetics of protein-protein interactions [154].

3.1. Mathematical modelling of the CaSR

One approach to tackle the mechanisms underlying ligand-biased signalling is to study the CaSR using biochemical models of cooperative proteins. Multi-protein complexes, such as the CaSR can display cooperativity, allostery and conformational transitions, central attributes of GPCRs [155]. Cooperativity and conformational changes are biochemical mechanisms that confer sensitisation towards signals; in the limit of strong cooperativity the saturation of a receptor with a signal is described by a sigmoidal Hill-type equation. Allostery is the phenomenon that one signal can influence the GPCR affinity for another signal. All these phenomena likely play a role in CaSR-mediated signalling [156,157]. Such models can facilitate interpretation of experimental data, obtained for instance with FRET measurements of G-protein activation, and support molecular understanding of the altered behaviour of wild-type vs. mutated cooperative proteins [23,144,158–160]. Several studies on GPCRs concluded that the Monod–Wyman–Changeux (MWC) model of cooperative proteins applies to membrane receptors, e.g. GPCRs [155,161–163]. The cooperativity between subunits of the CaSR suggests that the MWC model would be suitable to describe the CaSR.

3.2. Ligand biased signalling

The CaSR exhibits “ligand biased signalling” or “biased agonism”, a phenomenon common to GPCRs [164], indicating that distinct ligands or modulators bind preferentially to a specific state of the GPCR and stabilise it. Thereby, ligands induce a bias in signalling towards a specific pathway at the expense of others. By investigating the influence of several modulators on CaSR-associated signalling pathways, it has been shown that the allosteric modulators cinacalcet, NPS-R568 and the inhibitor NPS-2143 induce a preference towards intracellular Ca^{2+} mobilisation rather than the phosphorylation of ERK1/2 as a response of the activated receptor (Fig. 3) [145]. The evidence for biased signalling of the CaSR through different orthosteric agonists such as divalent cations, polyamines and aminoglycosides, is increasing. For example, Thomsen et al. showed that barium (Ba^{2+}) biases towards $G\alpha_{i/o}$ signalling in comparison to its potency to activate $G\alpha_{q/11}$ signalling. Further, the authors describe a general trend of polyamines and aminoglycosides to influence CaSR signalling towards ERK1/2 phosphorylation compared to $G\alpha_{q/11}$ signalling [165]. In addition, the same authors demonstrated a biasing effect of strontium on the potency of calcitonin secretion in comparison to calcium stimulation in cells endogenously expressing the CaSR [166]. Not only small molecule ligands, but also mutations of the CaSR gene can induce biasing effects on the signal transduction [144], further indicating that the stabilisation of altered receptor conformations is of key importance in the phenomenon of ligand biased signalling. An extreme example of biased signalling by the CaSR has been found in human oesophageal epithelial cells by Mulder et al. where, the major basic protein (MBP) and its mimetic polyarginine induced fibroblast growth factor 9 (FGF9) secretion in a CaSR-dependent manner while calcium alone had no effect [167]. The MWC model allows for a molecular explanation of CaSR ligand-biased signalling.

A changed pattern of information flow through a signalling network is one of the defining features of cancer; as an outcome the balance between apoptosis, differentiation and proliferation is disturbed. Alterations in ligand-biased signalling because of mutations in GPCRs could be one of the associated mechanisms. In addition, ligand biased signalling could be used in chemotherapy to enhance the therapeutic effect of drugs, enabling lower dosage and thus reducing side-effects. Indeed, calcium can enhance sensitivity of colon cancer cell lines to cytotoxic drugs [168]. Development of biased CaSR ligands that are more selective for specific signalling pathways could widen the field of utilisation of calcimimetics and calcilytics [145]. These examples illustrate the importance of quantitative approaches to GPCRs. Moreover, mathematical models of proteins can be a useful tool for drug design together with protein structural modelling and the analysis of mutated receptors.

4. The CaSR as a potential cancer drug target

The role of CaSR agonists and antagonists for tumour prevention or progression needs deeper understanding of the CaSR-mediated signalling mechanisms. However, at present data on the potential benefit of cinacalcet, the only FDA-approved calcimimetic, for cancer treatment are missing, except for parathyroid neoplasia.

As described above, treatment with cinacalcet was shown to reverse parathyroid gland hyperplasia in a rodent model of chronic kidney disease [46]. Furthermore, cinacalcet was effective in a murine model of primary hyperparathyroidism, which exhibited reduced CaSR expression in parathyroid glands [169]. These improvements may be, in part, due to calcimimetics promoting CaSR expression, as these disorders are characterised by decreased CaSR expression. In nephrectomised rats, for example, the calcimimetic NPS R-568 reversed reductions in CaSR mRNA and protein expression caused by a high-phosphorous diet [47], while the calcimimetic AMG 641 up-regulated CaSR and vitamin D receptor mRNA expression in uremic rats [48].

In addition, the CaSR seems to have a role in chemotherapy. Recent studies have shown that CaSR signalling regulates the expression of thymidylate synthase and survivin and intensifies the effect of 5-fluorouracil, one of the drugs of choice in colon cancer chemotherapy [170,171]. Furthermore, in breast cancer, knocking down the tumour suppressor gene BRAC1 leads to a downregulation of CaSR expression and, consequently, to upregulation of survivin which reduced sensitivity to paclitaxel, a mitotic inhibitor used in chemotherapy [172].

Targeting the CaSR could therefore be important, not only for finding new therapeutic avenues to prevent/delay malignant transformation, but also to improve the efficiency of current treatments. However, there are a number of important considerations that must be taken into account:

1) The CaSR is widely expressed in many different cell types and tissues throughout the body, therefore it may be important to develop drugs and delivery forms that only reach the CaSR in a tissue-specific way. This is an important consideration, as off-target effects of potential CaSR-based therapies could be detrimental to patient health. Currently, the class of drugs used for CaSR-based therapy, the phenylalkyamines, are themselves partially selective for the parathyroid CaSR, over the CaSR expressed in the thyroid C cells [173]. Therefore, selective targeting of the CaSR in other organs, such as the breast, colon and prostate, is important.

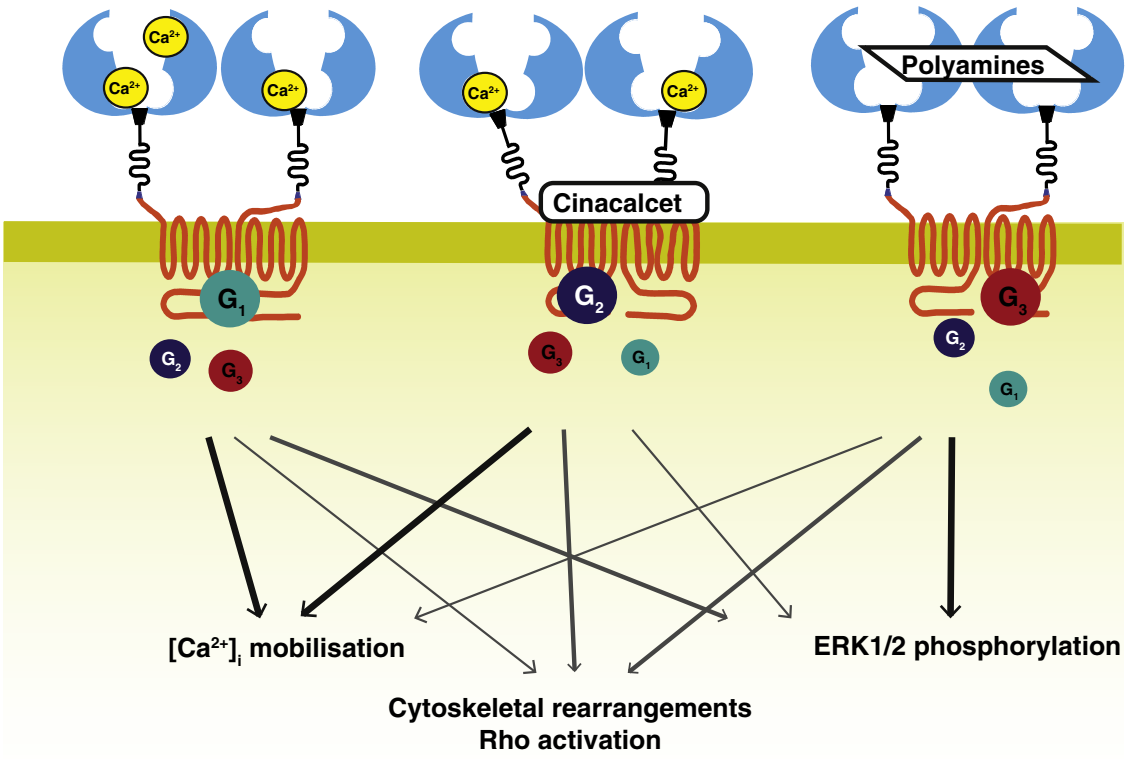

Fig. 3. Physiological ligand biased signalling of the calcium sensing receptor (CaSR). The receptor conformation is dependent on the amount and type of ligands bound. Different effector molecules bind preferentially to the different receptor states. This results in differential activation of the downstream signalling pathways. Arrow thickness correlates with the direction of the bias signaling. Adapted from [181].

2) While $Ca^{2+}{}_o$ has long been considered the main physiological agonist of the CaSR, it is now becoming apparent that a number of different molecules — including polyamines [174], L-amino acids [175,176] and γ-glutamyl peptides [177] — are in fact primary ligands in some physiological settings. These changes in primary ligands are important, as the different ligands lead to preferential stimulation of diverse signalling pathways, through 'ligand-biased signalling'. Consequently, identification and targeting of different CaSR ligands for specific tissues may also be an important part of the drug development process.
3) Finally, the CaSR is a pleiotropic GPCR and readily couples to at least three functionally diverse groups of heterotrimeric G proteins. Furthermore, as mentioned previously, the CaSR is also reported to switch G protein coupling from $G\alpha_{i/0}$ to $G\alpha_s$ in certain pathophysiological situations, including breast cancer [34]. Therefore, potential novel therapies will most likely need to target specific G protein usage.

These above considerations underline the multitude and complexity of CaSR-mediated signalling and highlight the potential difficulties in developing novel chemotherapeutics targeting the CaSR. A greater understanding of these biological networks and the molecular interactions that take place, aided by a systems biology approach, may be essential in helping to address these issues.

5. Conclusions

Epidemiological and experimental evidence suggests that the CaSR might play a role in tumour progression, however unequivocal evidence for a direct link between the CaSR and tumourigenesis is still lacking. CaSR seems to play a yin and yang role in cancer/cell physiology because it transduces both anti- and pro-proliferative signals, and inhibits or facilitates cell migration. The methodology of systems biology is an excellent tool to help understand these complex signalling networks. Using the ligand-biased signalling properties of the CaSR could be instrumental in designing specific therapeutics in different pathophysiological conditions.

Acknowledgments

M.L.B., F.B., R.M., D.R. and E.K. are funded by the Marie Curie Initial Training Network "Multifaceted CaSR", FP7-264663.

References

[1] E.M. Brown, R.J. MacLeod, Extracellular calcium sensing and extracellular calcium signaling, Physiol. Rev. 81 (2001) 239–297.
[2] A.D. Conigrave, E.M. Brown, Taste receptors in the gastrointestinal tract. II. L-amino acid sensing by calcium-sensing receptors: implications for GI physiology, Am. J. Physiol. Gastrointest. Liver Physiol. 291 (2006) G753–G761.
[3] E.M. Brown, G. Gamba, D. Riccardi, M. Lombardi, R. Butters, O. Kifor, A. Sun, M.A. Hediger, J. Lytton, S.C. Hebert, Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid, Nature 366 (1993) 575–580.
[4] N. Wettschureck, E. Lee, S.K. Libutti, S. Offermanns, P.G. Robey, A.M. Spiegel, Parathyroid-specific double knockout of Gq and G11 alpha-subunits leads to a phenotype resembling germline knockout of the extracellular Ca2+-sensing receptor, Mol. Endocrinol. 21 (2007) 274–280.
[5] C. Ho, D.A. Conner, M.R. Pollak, D.J. Ladd, O. Kifor, H.B. Warren, E.M. Brown, J.G. Seidman, C.E. Seidman, A mouse model of human familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism, Nat. Genet. 11 (1995) 389–394.
[6] S.J. Marx, R.D. Lasker, E.M. Brown, L.A. Fitzpatrick, N.B. Sweezey, R.B. Goldbloom, D.A. Gillis, D.E. Cole, Secretory dysfunction in parathyroid cells from a neonate with severe primary hyperparathyroidism, J. Clin. Endocrinol. Metab. 62 (1986) 445–449.
[7] G.N. Hendy, V. Guarnieri, L. Canaff, Calcium-sensing receptor and associated diseases, Prog. Mol. Biol. Transl. Sci. 89 (2009) 31–95.
[8] S.X. Cheng, M. Okuda, A.E. Hall, J.P. Geibel, S.C. Hebert, Expression of calcium-sensing receptor in rat colonic epithelium: evidence for modulation of fluid secretion, Am. J. Physiol. Gastrointest. Liver Physiol. 283 (2002) G240–G250.

[9] J. Geibel, K. Sritharan, R. Geibel, P. Geibel, J.S. Persing, A. Seeger, T.K. Roepke, M. Deichstetter, C. Prinz, S.X. Cheng, D. Martin, S.C. Hebert, Calcium-sensing receptor abrogates secretagogue-induced increases in intestinal net fluid secretion by enhancing cyclic nucleotide destruction, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 9390–9397.
[10] N. Chattopadhyay, I. Cheng, K. Rogers, D. Riccardi, A. Hall, R. Diaz, S.C. Hebert, D.I. Soybel, E.M. Brown, Identification and localization of extracellular Ca(2+)-sensing receptor in rat intestine, Am. J. Physiol. 274 (1998) G122–G130.
[11] O. Rey, S.H. Young, R. Jacamo, M.P. Moyer, E. Rozengurt, Extracellular calcium sensing receptor stimulation in human colonic epithelial cells induces intracellular calcium oscillations and proliferation inhibition, J. Cell. Physiol. 225 (2010) 73–83.
[12] G.E. Breitwieser, Calcium sensing receptors and calcium oscillations: calcium as a first messenger, Curr. Top. Dev. Biol. 73 (2006) 85–114.
[13] H. Miki, P.B. Maercklein, L.A. Fitzpatrick, Spontaneous oscillations of intracellular calcium in single bovine parathyroid cells may be associated with the inhibition of parathyroid hormone secretion, Endocrinology 136 (1995) 2954–2959.
[14] P. Ridefelt, E. Bjorklund, G. Akerstrom, M.J. Olsson, J. Rastad, E. Gylfe, Ca(2+)-induced Ca2+ oscillations in parathyroid cells, Biochem. Biophys. Res. Commun. 215 (1995) 903–909.
[15] G.E. Breitwieser, L. Gama, Calcium-sensing receptor activation induces intracellular calcium oscillations, Am. J. Physiol. Cell Physiol. 280 (2001) C1412–C1421.
[16] D. Szekely, S.C. Brennan, H.C. Mun, A.D. Conigrave, P.W. Kuchel, Effectors of the frequency of calcium oscillations in HEK-293 cells: wavelet analysis and a computer model, Eur. Biophys. J. 39 (2009) 149–165.
[17] S.H. Young, E. Rozengurt, Amino acids and Ca2+ stimulate different patterns of Ca2+ oscillations through the Ca2+-sensing receptor, Am. J. Physiol. Cell Physiol. 282 (2002) C1414–C1422.
[18] S.H. Young, S.V. Wu, E. Rozengurt, Ca2+-stimulated Ca2+ oscillations produced by the Ca2+-sensing receptor require negative feedback by protein kinase C, J. Biol. Chem. 277 (2002) 46871–46876.
[19] S.L. Davies, A. Ozawa, W.D. McCormick, M.M. Dvorak, D.T. Ward, Protein kinase C-mediated phosphorylation of the calcium-sensing receptor is stimulated by receptor activation and attenuated by calyculin-sensitive phosphatase activity, J. Biol. Chem. 282 (2007) 15048–15056.
[20] W.D. McCormick, R. Atkinson-Dell, K.L. Campion, H.C. Mun, A.D. Conigrave, D.T. Ward, Increased receptor stimulation elicits differential calcium-sensing receptor (T888) dephosphorylation, J. Biol. Chem. 285 (2010) 14170–14177.
[21] M. Bai, S. Trivedi, C.R. Lane, Y. Yang, S.J. Quinn, E.M. Brown, Protein kinase C phosphorylation of threonine at position 888 in Ca2+o-sensing receptor (CaR) inhibits coupling to Ca2+ store release, J. Biol. Chem. 273 (1998) 21267–21275.
[22] Y.F. Jiang, Z. Zhang, O. Kifor, C.R. Lane, S.J. Quinn, M. Bai, Protein kinase C (PKC) phosphorylation of the Ca2+ o-sensing receptor (CaR) modulates functional interaction of G proteins with the CaR cytoplasmic tail, J. Biol. Chem. 277 (2002) 50543–50549.
[23] S. Lazarus, C.J. Pretorius, F. Khafagi, K.L. Campion, S.C. Brennan, A.D. Conigrave, E.M. Brown, D.T. Ward, A novel mutation of the primary protein kinase C phosphorylation site in the calcium-sensing receptor causes autosomal dominant hypocalcemia, Eur. J. Endocrinol. 164 (2011) 429–435.
[24] M. Freichel, A. Zink-Lorenz, A. Holloschi, M. Hafner, V. Flockerzi, F. Raue, Expression of a calcium-sensing receptor in a human medullary thyroid carcinoma cell line and its contribution to calcitonin secretion, Endocrinology 137 (1996) 3842–3848.
[25] N.R. Ferreri, S. Hao, P.L. Pedraza, B. Escalante, C.P. Vio, Eicosanoids and tumor necrosis factor-alpha in the kidney, Prostaglandins Other Lipid Mediat. 98 (2012) 101–106.
[26] S.A. Hobson, S.E. McNeil, F. Lee, K.D. Rodland, Signal transduction mechanisms linking increased extracellular calcium to proliferation in ovarian surface epithelial cells, Exp. Cell Res. 258 (2000) 1–11.
[27] M.E. Handlogten, C. Huang, N. Shiraishi, H. Awata, R.T. Miller, The Ca2+-sensing receptor activates cytosolic phospholipase A2 via a Gqalpha-dependent ERK-independent pathway, J. Biol. Chem. 276 (2001) 13941–13948.
[28] O. Kifor, R.J. MacLeod, R. Diaz, M. Bai, T. Yamaguchi, T. Yao, I. Kifor, E.M. Brown, Regulation of MAP kinase by calcium-sensing receptor in bovine parathyroid and CaR-transfected HEK293 cells, Am. J. Physiol. Renal Physiol. 280 (2001) F291–F302.
[29] D.M. Holstein, K.A. Berg, L.M. Leeb-Lundberg, M.S. Olson, C. Saunders, Calcium-sensing receptor-mediated ERK1/2 activation requires Galphai2 coupling and dynamin-independent receptor internalization, J. Biol. Chem. 279 (2004) 10060–10069.
[30] H.J. Lee, H.C. Mun, N.C. Lewis, M.F. Crouch, E.L. Culverston, R.S. Mason, A.D. Conigrave, Allosteric activation of the extracellular Ca2+-sensing receptor by L-amino acids enhances ERK1/2 phosphorylation, Biochem. J. 404 (2007) 141–149.
[31] R.J. MacLeod, S. Yano, N. Chattopadhyay, E.M. Brown, Extracellular calcium-sensing receptor transactivates the epidermal growth factor receptor by a triple-membrane-spanning signaling mechanism, Biochem. Biophys. Res. Commun. 320 (2004) 455–460.
[32] Z. Saidak, R. Mentaverri, E.M. Brown, The role of the calcium-sensing receptor in the development and progression of cancer, Endocr. Rev. 30 (2009) 178–195.
[33] C. Casala, E. Gil-Guinon, J.L. Ordonez, S. Miguel-Queralt, E. Rodriguez, P. Galvan, C. Lavarino, F. Munell, E. de Alava, J. Mora, C. de Torres, The calcium-sensing receptor is silenced by genetic and epigenetic mechanisms in unfavorable neuroblastomas and its reactivation induces ERK1/2-dependent apoptosis, Carcinogenesis (2012).
[34] R. Mamillapalli, J. VanHouten, W. Zawalich, J. Wysolmerski, Switching of G-protein usage by the calcium-sensing receptor reverses its effect on parathyroid hormone-related protein secretion in normal versus malignant breast cells, J. Biol. Chem. 283 (2008) 24435–24447.
[35] C. Huang, L.M. Hydo, S. Liu, R.T. Miller, Activation of choline kinase by extracellular Ca2+ is Ca(2+)-sensing receptor, Galpha12 and Rho-dependent in breast cancer cells, Cell. Signal. 21 (2009) 1894–1900.
[36] T. Worzfeld, N. Wettschureck, S. Offermanns, G(12)/G(13)-mediated signalling in mammalian physiology and disease, Trends Pharmacol. Sci. 29 (2008) 582–589.
[37] D.T. Ward, D. Riccardi, New concepts in calcium-sensing receptor pharmacology and signalling, Br. J. Pharmacol. 165 (2012) 35–48.
[38] J.P. Bilezikian, S.J. Silverberg, Clinical spectrum of primary hyperparathyroidism, Rev. Endocr. Metab. Disord. 1 (2000) 237–245.
[39] C.H. Wei, A. Harari, Parathyroid carcinoma: update and guidelines for management, Curr. Treat. Options Oncol. 13 (2012) 11–23.
[40] S. Corbetta, G. Mantovani, A. Lania, S. Borgato, L. Vicentini, E. Beretta, G. Faglia, A.M. Di Blasio, A. Spada, Calcium-sensing receptor expression and signalling in human parathyroid adenomas and primary hyperplasia, Clin. Endocrinol. (Oxf) 52 (2000) 339–348.
[41] J. Gogusev, P. Duchambon, B. Hory, M. Giovannini, Y. Goureau, E. Sarfati, T.B. Drueke, Depressed expression of calcium receptor in parathyroid gland tissue of patients with hyperparathyroidism, Kidney Int. 51 (1997) 328–336.
[42] F. Farnebo, U. Enberg, L. Grimelius, M. Backdahl, M. Schalling, C. Larsson, L.O. Farnebo, Tumor-specific decreased expression of calcium sensing receptor messenger ribonucleic acid in sporadic primary hyperparathyroidism, J. Clin. Endocrinol. Metab. 82 (1997) 3481–3486.
[43] C.J. Haven, M. van Puijenbroek, M. Karperien, G.J. Fleuren, H. Morreau, Differential expression of the calcium sensing receptor and combined loss of chromosomes 1q and 11q in parathyroid carcinoma, J. Pathol. 202 (2004) 86–94.
[44] F. Cetani, A. Pinchera, E. Pardi, L. Cianferotti, E. Vignali, A. Picone, P. Miccoli, P. Viacava, C. Marcocci, No evidence for mutations in the calcium-sensing receptor gene in sporadic parathyroid adenomas, J. Bone Miner. Res. 14 (1999) 878–882.
[45] S. Yano, T. Sugimoto, T. Tsukamoto, K. Chihara, A. Kobayashi, S. Kitazawa, S. Maeda, R. Kitazawa, Association of decreased calcium-sensing receptor expression with proliferation of parathyroid cells in secondary hyperparathyroidism, Kidney Int. 58 (2000) 1980–1986.
[46] G. Miller, J. Davis, E. Shatzen, M. Colloton, D. Martin, C.M. Henley, Cinacalcet HCl prevents development of parathyroid gland hyperplasia and reverses established parathyroid gland hyperplasia in a rodent model of CKD, Nephrol. Dial. Transplant. 27 (2012) 2198–2205.
[47] M. Mizobuchi, I. Hatamura, H. Ogata, F. Saji, S. Uda, K. Shiizaki, T. Sakaguchi, S. Negi, E. Kinugasa, S. Koshikawa, T. Akizawa, Calcimimetic compound upregulates decreased calcium-sensing receptor expression level in parathyroid glands of rats with chronic renal insufficiency, J. Am. Soc. Nephrol. 15 (2004) 2579–2587.
[48] F.J. Mendoza, I. Lopez, R. Canalejo, Y. Almaden, D. Martin, E. Aguilera-Tejero, M. Rodriguez, Direct upregulation of parathyroid calcium-sensing receptor and vitamin D receptor by calcimimetics in uremic rats, Am. J. Physiol. Renal Physiol. 296 (2009) F605–F613.
[49] J. Koh, M. Dar, B.R. Untch, D. Dixit, Y. Shi, Z. Yang, M.A. Adam, H. Dressman, X. Wang, D. Gesty-Palmer, J.R. Marks, R. Spurney, K.M. Druey, J.A. Olson Jr., Regulator of G protein signaling 5 is highly expressed in parathyroid tumors and inhibits signaling by the calcium-sensing receptor, Mol. Endocrinol. 25 (2011) 867–876.
[50] Y. Imanishi, Y. Hosokawa, K. Yoshimoto, E. Schipani, S. Mallya, A. Papanikolaou, O. Kifor, T. Tokura, M. Sablosky, F. Ledgard, G. Gronowicz, T.C. Wang, E.V. Schmidt, C. Hall, E.M. Brown, R. Bronson, A. Arnold, Primary hyperparathyroidism caused by parathyroid-targeted overexpression of cyclin D1 in transgenic mice, J. Clin. Invest. 107 (2001) 1093–1102.
[51] S.M. Mallya, A. Arnold, Cyclin D1 in parathyroid disease, Front. Biosci. 5 (2000) D367–D371.
[52] P. Bjorklund, G. Akerstrom, G. Westin, Activated beta-catenin in the novel human parathyroid tumor cell line sHPT-1, Biochem. Biophys. Res. Commun. 352 (2007) 532–536.
[53] S. Corbetta, C. Eller-Vainicher, L. Vicentini, A. Lania, G. Mantovani, P. Beck-Peccoz, A. Spada, Modulation of cyclin D1 expression in human tumoral parathyroid cells: effects of growth factors and calcium sensing receptor activation, Cancer Lett. 255 (2007) 34–41.
[54] S.M. Mallya, J.J. Gallagher, Y.K. Wild, O. Kifor, J. Costa-Guda, K. Saucier, E.M. Brown, A. Arnold, Abnormal parathyroid cell proliferation precedes biochemical abnormalities in a mouse model of primary hyperparathyroidism, Mol. Endocrinol. 19 (2005) 2603–2609.
[55] O. Kifor, I. Kifor, F.D. Moore Jr., R.R. Butters Jr., T. Cantor, P. Gao, E.M. Brown, Decreased expression of caveolin-1 and altered regulation of mitogen-activated protein kinase in cultured bovine parathyroid cells and human parathyroid adenomas, J. Clin. Endocrinol. Metab. 88 (2003) 4455–4464.
[56] S. Corbetta, A. Lania, M. Filopanti, L. Vicentini, E. Ballare, A. Spada, Mitogen-activated protein kinase cascade in human normal and tumoral parathyroid cells, J. Clin. Endocrinol. Metab. 87 (2002) 2201–2205.
[57] D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, Global cancer statistics, 2002, CA Cancer J. Clin. 55 (2005) 74–108.
[58] M.M. Center, A. Jemal, R.A. Smith, E. Ward, Worldwide variations in colorectal cancer, CA Cancer J. Clin. 59 (2009) 366–378.
[59] C. Garland, R.B. Shekelle, E. Barrett-Connor, M.H. Criqui, A.H. Rossof, O. Paul, Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men, Lancet 1 (1985) 307–309.
[60] R. Van der Meer, H.T. De Vries, Differential binding of glycine- and taurine-conjugated bile acids to insoluble calcium phosphate, Biochem. J. 229 (1985) 265–268.

[61] H.L. Newmark, M.J. Wargovich, W.R. Bruce, Colon cancer and dietary fat, phosphate, and calcium: a hypothesis, J. Natl. Cancer Inst. 72 (1984) 1323–1325.
[62] V. Fedirko, R.M. Bostick, W.D. Flanders, Q. Long, E. Sidelnikov, A. Shaukat, C.R. Daniel, R.E. Rutherford, J.J. Woodard, Effects of vitamin d and calcium on proliferation and differentiation in normal colon mucosa: a randomized clinical trial, Cancer Epidemiol. Biomarkers Prev. 18 (2009) 2933–2941.
[63] V. Fedirko, R.M. Bostick, W.D. Flanders, Q. Long, A. Shaukat, R.E. Rutherford, C.R. Daniel, V. Cohen, C. Dash, Effects of vitamin D and calcium supplementation on markers of apoptosis in normal colon mucosa: a randomized, double-blind, placebo-controlled clinical trial, Cancer Prev. Res. (Phila.) 2 (2009) 213–223.
[64] M. Lipkin, H. Newmark, Effect of added dietary calcium on colonic epithelial-cell proliferation in subjects at high risk for familial colonic cancer, N. Engl. J. Med. 313 (1985) 1381–1384.
[65] E. Kallay, O. Kifor, N. Chattopadhyay, E.M. Brown, M.G. Bischof, M. Peterlik, H.S. Cross, Calcium-dependent c-myc proto-oncogene expression and proliferation of Caco-2 cells: a role for a luminal extracellular calcium-sensing receptor, Biochem. Biophys. Res. Commun. 232 (1997) 80–83.
[66] E. Kallay, E. Bajna, F. Wrba, S. Kriwanek, M. Peterlik, H.S. Cross, Dietary calcium and growth modulation of human colon cancer cells: role of the extracellular calcium-sensing receptor, Cancer Detect. Prev. 24 (2000) 127–136.
[67] O. Rey, W. Chang, D. Bikle, N. Rozengurt, S.H. Young, E. Rozengurt, Negative cross-talk between calcium-sensing receptor and beta-catenin signaling systems in colonic epithelium, J. Biol. Chem. 287 (2012) 1158–1167.
[68] T.U. Ahearn, M.L. McCullough, W.D. Flanders, Q. Long, E. Sidelnikov, V. Fedirko, C.R. Daniel, R.E. Rutherford, A. Shaukat, R.M. Bostick, A randomized clinical trial of the effects of supplemental calcium and vitamin D3 on markers of their metabolism in normal mucosa of colorectal adenoma patients, Cancer Res. 71 (2011) 413–423.
[69] J. Whitfield, Calcium switches, cell cycles, differentiation and, death, CRC Press, 1991.
[70] J.F. Whitfield, The calcium-sensing receptor—a driver of colon cell differentiation, Curr. Pharm. Biotechnol. 10 (2009) 311–316.
[71] J.F. Whitfield, Calcium, calcium-sensing receptor and colon cancer, Cancer Lett. 275 (2009) 9–16.
[72] L. Gama, L.M. Baxendale-Cox, G.E. Breitwieser, Ca2+-sensing receptors in intestinal epithelium, Am. J. Physiol. 273 (1997) C1168–C1175.
[73] Y. Sheinin, E. Kallay, F. Wrba, S. Kriwanek, M. Peterlik, H.S. Cross, Immunocytochemical localization of the extracellular calcium-sensing receptor in normal and malignant human large intestinal mucosa, J. Histochem. Cytochem. 48 (2000) 595–602.
[74] S.X. Cheng, Calcium-sensing receptor inhibits secretagogue-induced electrolyte secretion by intestine via the enteric nervous system, Am. J. Physiol. Gastrointest. Liver Physiol. 303 (2012) G60–G70.
[75] S. Chakrabarty, H. Wang, L. Canaff, G.N. Hendy, H. Appelman, J. Varani, Calcium sensing receptor in human colon carcinoma: interaction with Ca(2+) and 1,25-dihydroxyvitamin D(3), Cancer Res. 65 (2005) 493–498.
[76] S. Chakrabarty, V. Radjendirane, H. Appelman, J. Varani, Extracellular calcium and calcium sensing receptor function in human colon carcinomas: promotion of E-cadherin expression and suppression of beta-catenin/TCF activation, Cancer Res. 63 (2003) 67–71.
[77] T.U. Ahearn, M.L. McCullough, W.D. Flanders, Q. Long, E. Sidelnikov, V. Fedirko, C.R. Daniel, R.E. Rutherford, A. Shaukat, R.M. Bostick, A randomized clinical trial of the effects of supplemental calcium and vitamin D3 on markers of their metabolism in normal mucosa of colorectal adenoma patients, Cancer Res. 71 (2011) 413–423.
[78] B.M. Brenner, N. Russell, S. Albrecht, R.J. Davies, The effect of dietary vitamin D3 on the intracellular calcium gradient in mammalian colonic crypts, Cancer Lett. 127 (1998) 43–53.
[79] K. Hizaki, H. Yamamoto, H. Taniguchi, Y. Adachi, M. Nakazawa, T. Tanuma, N. Kato, Y. Sukawa, J.V. Sanchez, H. Suzuki, S. Sasaki, K. Imai, Y. Shinomura, Epigenetic inactivation of calcium-sensing receptor in colorectal carcinogenesis, Mod. Pathol. 24 (2011) 876–884.
[80] P.A. Jones, S.B. Baylin, The fundamental role of epigenetic events in cancer, Nat. Rev. Genet. 3 (2002) 415–428.
[81] M. Kulis, M. Esteller, DNA methylation and cancer, Adv. Genet. 70 (2010) 27–56.
[82] Y. Wang, F.C. Leung, An evaluation of new criteria for CpG islands in the human genome as gene markers, Bioinformatics 20 (2004) 1170–1177.
[83] N. Chikatsu, S. Fukumoto, Y. Takeuchi, M. Suzawa, T. Obara, T. Matsumoto, T. Fujita, Cloning and characterization of two promoters for the human calcium-sensing receptor (CaSR) and changes of CaSR expression in parathyroid adenomas, J. Biol. Chem. 275 (2000) 7553–7557.
[84] L. Canaff, G.N. Hendy, Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D, J. Biol. Chem. 277 (2002) 30337–30350.
[85] E. Kallay, E. Bonner, F. Wrba, R.V. Thakker, M. Peterlik, H.S. Cross, Molecular and functional characterization of the extracellular calcium-sensing receptor in human colon cancer cells, Oncol. Res. 13 (2003) 551–559.
[86] L. Canaff, G.N. Hendy, Calcium-sensing receptor gene transcription is up-regulated by the proinflammatory cytokine, interleukin-1beta. Role of the NF-kappaB PATHWAY and kappaB elements, J. Biol. Chem. 280 (2005) 14177–14188.
[87] L. Canaff, X. Zhou, G.N. Hendy, The proinflammatory cytokine, interleukin-6, up-regulates calcium-sensing receptor gene transcription via Stat1/3 and Sp1/3, J. Biol. Chem. 283 (2008) 13586–13600.
[88] H.C. Horváth, P. Lakatos, J.P. Kósa, K. Bácsi, K. Borka, G. Bises, T. Nittke, P.A. Hershberger, G. Speer, E. Kállay, The candidate oncogene CYP24A1: a potential biomarker for colorectal tumorigenesis, J. Histochem. Cytochem. 58 (2010) 277–285.
[89] N. Bhagavathula, E.A. Kelley, M. Reddy, K.C. Nerusu, C. Leonard, K. Fay, S. Chakrabarty, J. Varani, Upregulation of calcium-sensing receptor and mitogen-activated protein kinase signalling in the regulation of growth and differentiation in colon carcinoma, Br. J. Cancer 93 (2005) 1364–1371.
[90] N. Bhagavathula, A.W. Hanosh, K.C. Nerusu, H. Appelman, S. Chakrabarty, J. Varani, Regulation of E-cadherin and beta-catenin by Ca2+ in colon carcinoma is dependent on calcium-sensing receptor expression and function, Int. J. Cancer 121 (2007) 1455–1462.
[91] O. Rey, S.H. Young, R. Jacamo, M.P. Moyer, E. Rozengurt, Extracellular calcium sensing receptor stimulation in human colonic epithelial cells induces intracellular calcium oscillations and proliferation inhibition, J. Cell. Physiol. 225 (2010) 73–83.
[92] R.T. Moon, A.D. Kohn, G.V. De Ferrari, A. Kaykas, WNT and beta-catenin signalling: diseases and therapies, Nat. Rev. Genet. 5 (2004) 691–701.
[93] H. Clevers, R. Nusse, Wnt/beta-catenin signaling and disease, Cell 149 (2012) 1192–1205.
[94] A.W. Burgess, M.C. Faux, M.J. Layton, R.G. Ramsay, Wnt signaling and colon tumorigenesis—a view from the periphery, Exp. Cell Res. 317 (2011) 2748–2758.
[95] F. Kuhnert, C.R. Davis, H.T. Wang, P. Chu, M. Lee, J. Yuan, R. Nusse, C.J. Kuo, Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 266–271.
[96] A.R. Clarke, Wnt signalling in the mouse intestine, Oncogene 25 (2006) 7512–7521.
[97] I.I. Pacheco, R.J. Macleod, CaSR stimulates secretion of Wnt5a from colonic myofibroblasts to stimulate CDX2 and sucrase-isomaltase using Ror2 on intestinal epithelia, Am. J. Physiol. Gastrointest. Liver Physiol. 295 (2008) G748–G759.
[98] R. Najdi, R.F. Holcombe, M.L. Waterman, Wnt signaling and colon carcinogenesis: beyond APC, J. Carcinog. 10 (2011) 5.
[99] R.J. MacLeod, M. Hayes, I. Pacheco, Wnt5a secretion stimulated by the extracellular calcium-sensing receptor inhibits defective Wnt signaling in colon cancer cells, Am. J. Physiol. Gastrointest. Liver Physiol. 293 (2007) G403–G411.
[100] J.C. Kelly, P. Lungchukiet, R.J. Macleod, Extracellular calcium-sensing receptor inhibition of intestinal epithelialTNF signaling requires CaSR-mediated Wnt5a/Ror2 interaction, Front. Physiol. 2 (2011) 17.
[101] C.A. Moskaluk, H. Zhang, S.M. Powell, L.A. Cerilli, G.M. Hampton, H.F. Frierson, Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays, Mod. Pathol. 16 (2003) 913–919.
[102] G.V. Mallo, P. Soubeyran, J.C. Lissitzky, F. Andre, C. Farnarier, J. Marvaldi, J.C. Dagorn, J.L. Iovanna, Expression of the Cdx1 and Cdx2 homeotic genes leads to reduced malignancy in colon cancer-derived cells, J. Biol. Chem. 273 (1998) 14030–14036.
[103] I. Gross, I. Duluc, T. Benameur, A. Calon, E. Martin, T. Brabletz, M. Kedinger, C. Domon-Dell, J.N. Freund, The intestine-specific homeobox gene Cdx2 decreases mobility and antagonizes dissemination of colon cancer cells, Oncogene 27 (2008) 107–115.
[104] M. Buset, M. Lipkin, S. Winawer, S. Swaroop, E. Friedman, Inhibition of human colonic epithelial cell proliferation in vivo and in vitro by calcium, Cancer Res. 46 (1986) 5426–5430.
[105] A.C. Rogers, A.M. Hanly, D. Collins, A.W. Baird, D.C. Winter, Review article: loss of the calcium-sensing receptor in colonic epithelium is a key event in the pathogenesis of colon cancer, Clin. Colorectal Cancer 11 (2012) 24–30.
[106] In: N. Howlader, A.M. Noone, M. Krapcho, N. Neyman, R. Aminou, S.F. Altekruse, C.L. Kosary, J. Ruhl, Z. Tatalovich, H. Cho, A. Mariotto, M.P. Eisner, D.R. Lewis, H.S. Chen, E.J. Feuer, K.A. Cronin (Eds.), SEER Cancer Statistics Review, 1975–2009 (Vintage 2009 Populations), National Cancer Institute, 2012, (http://seer.cancer.gov/csr/1975_2009_pops1909/index.html accessed July 19, 2012).
[107] Y. Cui, T.E. Rohan, Vitamin D, calcium, and breast cancer risk: a review, Cancer Epidemiol. Biomarkers Prev. 15 (2006) 1427–1437.
[108] M. Huncharek, J. Muscat, B. Kupelnick, Colorectal cancer risk and dietary intake of calcium, vitamin D, and dairy products: a meta-analysis of 26,335 cases from 60 observational studies, Nutr. Cancer 61 (2009) 47–69.
[109] R.T. Chlebowski, K.C. Johnson, C. Kooperberg, M. Pettinger, J. Wactawski-Wende, T. Rohan, J. Rossouw, D. Lane, M.J. O'Sullivan, S. Yasmeen, R.A. Hiatt, J.M. Shikany, M. Vitolins, J. Khandekar, F.A. Hubbell, Calcium plus vitamin D supplementation and the risk of breast cancer, J. Natl. Cancer Inst. 100 (2008) 1581–1591.
[110] S.C. Larsson, L. Bergkvist, A. Wolk, Long-term dietary calcium intake and breast cancer risk in a prospective cohort of women, Am. J. Clin. Nutr. 89 (2009) 277–282.
[111] P.G. Moorman, P.D. Terry, Consumption of dairy products and the risk of breast cancer: a review of the literature, Am. J. Clin. Nutr. 80 (2004) 5–14.
[112] V. Bissonauth, B. Shatenstein, P. Ghadirian, Nutrition and breast cancer among sporadic cases and gene mutation carriers: an overview, Cancer Detect. Prev. 32 (2008) 52–64.
[113] P.W. Parodi, Dairy product consumption and the risk of breast cancer, J. Am. Coll. Nutr. 24 (2005) 556S–568S.
[114] W. Al Sarakbi, M. Salhab, K. Mokbel, Dairy products and breast cancer risk: a review of the literature, Int. J. Fertil. Womens Med. 50 (2005) 244–249.
[115] S. Abbas, J. Linseisen, J. Chang-Claude, Dietary vitamin D and calcium intake and premenopausal breast cancer risk in a German case-control study, Nutr. Cancer 59 (2007) 54–61.
[116] J.A. Baron, M. Beach, K. Wallace, M.V. Grau, R.S. Sandler, J.S. Mandel, D. Heber, E.R. Greenberg, Risk of prostate cancer in a randomized clinical trial of calcium supplementation, Cancer Epidemiol. Biomarkers Prev. 14 (2005) 586–589.

[117] I. Cheng, M.E. Klingensmith, N. Chattopadhyay, O. Kifor, R.R. Butters, D.I. Soybel, E.M. Brown, Identification and localization of the extracellular calcium-sensing receptor in human breast, J. Clin. Endocrinol. Metab. 83 (1998) 703–707.
[118] J.N. VanHouten, M.C. Neville, J.J. Wysolmerski, The calcium-sensing receptor regulates plasma membrane calcium adenosine triphosphatase isoform 2 activity in mammary epithelial cells: a mechanism for calcium-regulated calcium transport into milk, Endocrinology 148 (2007) 5943–5954.
[119] R. Mihai, J. Stevens, C. McKinney, N.B. Ibrahim, Expression of the calcium receptor in human breast cancer—a potential new marker predicting the risk of bone metastases, Eur. J. Surg. Oncol. 32 (2006) 511–515.
[120] Z. Saidak, C. Boudot, R. Abdoune, L. Petit, M. Brazier, R. Mentaverri, S. Kamel, Extracellular calcium promotes the migration of breast cancer cells through the activation of the calcium sensing receptor, Exp. Cell Res. 315 (2009) 2072–2080.
[121] J.L. Sanders, N. Chattopadhyay, O. Kifor, T. Yamaguchi, E.M. Brown, Ca(2+)-sensing receptor expression and PTHrP secretion in PC-3 human prostate cancer cells, Am. J. Physiol. Endocrinol. Metab. 281 (2001) E1267–E1274.
[122] J. Liao, A. Schneider, N.S. Datta, L.K. McCauley, Extracellular calcium as a candidate mediator of prostate cancer skeletal metastasis, Cancer Res. 66 (2006) 9065–9073.
[123] D.K. Biswas, Q. Shi, S. Baily, I. Strickland, S. Ghosh, A.B. Pardee, J.D. Iglehart, NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 10137–10142.
[124] L. Connelly, W. Barham, H.M. Onishko, T. Sherrill, L.A. Chodosh, T.S. Blackwell, F.E. Yull, Inhibition of NF-kappa B activity in mammary epithelium increases tumor latency and decreases tumor burden, Oncogene 30 (2011) 1402–1412.
[125] J. Höbaus, U. Thiem, D.M. Hummel, E. Kallay, Role of calcium, Vitamin D, and the extrarenal vitamin D hydroxylases during carcinogenesis, Anticancer Agents Med. Chem. 13 (2012) 20–35.
[126] Y. El Hiani, V. Lehen'kyi, H. Ouadid-Ahidouch, A. Ahidouch, Activation of the calcium-sensing receptor by high calcium induced breast cancer cell proliferation and TRPC1 cation channel over-expression potentially through EGFR pathways, Arch. Biochem. Biophys. 486 (2009) 58–63.
[127] A. Vivacqua, R. Lappano, P. De Marco, D. Sisci, S. Aquila, F. De Amicis, S.A. Fuqua, S. Ando, M. Maggiolini, G protein-coupled receptor 30 expression is up-regulated by EGF and TGF alpha in estrogen receptor alpha-positive cancer cells, Mol. Endocrinol. 23 (2009) 1815–1826.
[128] G.E. Breitwieser, Minireview: the intimate link between calcium sensing receptor trafficking and signaling: implications for disorders of calcium homeostasis, Mol. Endocrinol. 26 (2012) 1482–1495.
[129] Y. El Hiani, A. Ahidouch, V. Lehen'kyi, F. Hague, F. Gouilleux, R. Mentaverri, S. Kamel, K. Lassoued, G. Brule, H. Ouadid-Ahidouch, Extracellular signal-regulated kinases 1 and 2 and TRPC1 channels are required for calcium-sensing receptor-stimulated MCF-7 breast cancer cell proliferation, Cell. Physiol. Biochem. 23 (2009) 335–346.
[130] G. Liu, X. Hu, S. Chakrabarty, Calcium sensing receptor down-regulates malignant cell behavior and promotes chemosensitivity in human breast cancer cells, Cell Calcium 45 (2009) 216–225.
[131] G.D. Roodman, Mechanisms of bone metastasis, N. Engl. J. Med. 350 (2004) 1655–1664.
[132] R.J. Thomas, T.A. Guise, J.J. Yin, J. Elliott, N.J. Horwood, T.J. Martin, M.T. Gillespie, Breast cancer cells interact with osteoblasts to support osteoclast formation, Endocrinology 140 (1999) 4451–4458.
[133] J.M. Brown, E. Corey, Z.D. Lee, L.D. True, T.J. Yun, M. Tondravi, R.L. Vessella, Osteoprotegerin and rank ligand expression in prostate cancer, Urology 57 (2001) 611–616.
[134] G.J. Powell, J. Southby, J.A. Danks, R.G. Stillwell, J.A. Hayman, M.A. Henderson, R.C. Bennett, T.J. Martin, Localization of parathyroid hormone-related protein in breast cancer metastases: increased incidence in bone compared with other sites, Cancer Res. 51 (1991) 3059–3061.
[135] A.F. Stewart, Clinical practice. Hypercalcemia associated with cancer, N. Engl. J. Med. 352 (2005) 373–379.
[136] J.N. VanHouten, Calcium sensing by the mammary gland, J. Mammary Gland Biol. Neoplasia 10 (2005) 129–139.
[137] J.L. Sanders, N. Chattopadhyay, O. Kifor, T. Yamaguchi, R.R. Butters, E.M. Brown, Extracellular calcium-sensing receptor expression and its potential role in regulating parathyroid hormone-related peptide secretion in human breast cancer cell lines, Endocrinology 141 (2000) 4357–4364.
[138] S. Yano, R.J. Macleod, N. Chattopadhyay, J. Tfelt-Hansen, O. Kifor, R.R. Butters, E.M. Brown, Calcium-sensing receptor activation stimulates parathyroid hormone-related protein secretion in prostate cancer cells: role of epidermal growth factor receptor transactivation, Bone 35 (2004) 664–672.
[139] D.H. Jones, T. Nakashima, O.H. Sanchez, I. Kozieradzki, S.V. Komarova, I. Sarosi, S. Morony, E. Rubin, R. Sarao, C.V. Hojilla, V. Komnenovic, Y.Y. Kong, M. Schreiber, S.J. Dixon, S.M. Sims, R. Khokha, T. Wada, J.M. Penninger, Regulation of cancer cell migration and bone metastasis by RANKL, Nature 440 (2006) 692–696.
[140] M.C. Smith, K.E. Luker, J.R. Garbow, J.L. Prior, E. Jackson, D. Piwnica-Worms, G.D. Luker, CXCR4 regulates growth of both primary and metastatic breast cancer, Cancer Res. 64 (2004) 8604–8612.
[141] Y.X. Sun, A. Schneider, Y. Jung, J. Wang, J. Dai, K. Cook, N.I. Osman, A.J. Koh-Paige, H. Shim, K.J. Pienta, E.T. Keller, L.K. McCauley, R.S. Taichman, Skeletal localization and neutralization of the SDF-1(CXCL12)/CXCR4 axis blocks prostate cancer metastasis and growth in osseous sites in vivo, J. Bone Miner. Res. 20 (2005) 318–329.
[142] Y. Kang, P.M. Siegel, W. Shu, M. Drobnjak, S.M. Kakonen, C. Cordon-Cardo, T.A. Guise, J. Massague, A multigenic program mediating breast cancer metastasis to bone, Cancer Cell 3 (2003) 537–549.
[143] N. Sethi, Y. Kang, Dysregulation of developmental pathways in bone metastasis, Bone 48 (2011) 16–22.
[144] K. Leach, A. Wen, A.E. Davey, P.M. Sexton, A.D. Conigrave, A. Christopoulos, Identification of molecular phenotypes and biased signaling induced by naturally occurring mutations of the human calcium-sensing receptor, Endocrinology 153 (2012) 4304–4316.
[145] A.E. Davey, K. Leach, C. Valant, A.D. Conigrave, P.M. Sexton, A. Christopoulos, Positive and negative allosteric modulators promote biased signaling at the calcium-sensing receptor, Endocrinology 153 (2012) 1232–1241.
[146] A.A. Jensen, H. Brauner-Osborne, Allosteric modulation of the calcium-sensing receptor, Curr. Neuropharmacol. 5 (2007) 180–186.
[147] B.N. Kholodenko, J.F. Hancock, W. Kolch, Signalling ballet in space and time, Nat. Rev. Mol. Cell Biol. 11 (2010) 414–426.
[148] H.E. Grecco, M. Schmick, P.I. Bastiaens, Signaling from the living plasma membrane, Cell 144 (2011) 897–909.
[149] M.J. Adjobo-Hermans, J. Goedhart, W.L. van, S. Nijmeijer, E.M. Manders, S. Offermanns, T.W. Gadella Jr., Real-time visualization of heterotrimeric G protein Gq activation in living cells, BMC Biol. 9 (2011) 32.
[150] C. Janetopoulos, T. Jin, P. Devreotes, Receptor-mediated activation of heterotrimeric G-proteins in living cells, Science 291 (2001) 2408–2411.
[151] P.A. Grimsrud, D.L. Swaney, C.D. Wenger, N.A. Beauchene, J.J. Coon, Phosphoproteomics for the masses, ACS Chem. Biol. 5 (2010) 105–119.
[152] L. Rubbi, B. Titz, L. Brown, E. Galvan, E. Komisopoulou, S.S. Chen, T. Low, M. Tahmasian, B. Skaggs, M. Muschen, M. Pellegrini, T.G. Graeber, Global phosphoproteomics reveals crosstalk between Bcr-Abl and negative feedback mechanisms controlling Src signaling, Sci. Signal. 4 (2011) ra18.
[153] T.S. Gujral, R.L. Karp, A. Finski, M. Chan, P.E. Schwartz, G. Macbeath, P. Sorger, Profiling phospho-signaling networks in breast cancer using reverse-phase protein arrays, Oncogene (2012), [Sep. 3, Electronic publication ahead of print].
[154] B. Kholodenko, M.B. Yaffe, W. Kolch, Computational approaches for analyzing information flow in biological networks, Sci. Signal. 5 (2012) re1.
[155] J.P. Changeux, S.J. Edelstein, Allosteric mechanisms of signal transduction, Science 308 (2005) 1424–1428.
[156] F. De Luca, K. Ray, E.E. Mancilla, G.F. Fan, K.K. Winer, P. Gore, A.M. Spiegel, J. Baron, Sporadic hypoparathyroidism caused by de novo gain-of-function mutations of the Ca(2+)-sensing receptor, J. Clin. Endocrinol. Metab. 82 (1997) 2710–2715.
[157] M.R. Pollak, E.M. Brown, H.L. Estep, P.N. McLaine, O. Kifor, J. Park, S.C. Hebert, C.E. Seidman, J.G. Seidman, Autosomal dominant hypocalcaemia caused by a Ca(2+)-sensing receptor gene mutation, Nat. Genet. 8 (1994) 303–307.
[158] H.C. Mun, E.L. Culverston, A.H. Franks, C.A. Collyer, R.J. Clifton-Bligh, A.D. Conigrave, A double mutation in the extracellular Ca2+-sensing receptor's venus flytrap domain that selectively disables L-amino acid sensing, J. Biol. Chem. 280 (2005) 29067–29072.
[159] A. Terranegra, A. Ferraretto, E. Dogliotti, M. Scarpellini, S. Corbetta, A.M. Barbieri, A. Spada, T. Arcidiacono, F. Rainone, A. Aloia, D. Cusi, G. Vezzoli, L. Soldati, Calcimimetic R-568 effects on activity of R990G polymorphism of calcium-sensing receptor, J. Mol. Endocrinol. 45 (2010) 245–256.
[160] K. Ray, B.C. Hauschild, P.J. Steinbach, P.K. Goldsmith, O. Hauache, A.M. Spiegel, Identification of the cysteine residues in the amino-terminal extracellular domain of the human Ca(2+) receptor critical for dimerization. Implications for function of monomeric Ca(2+) receptor, J. Biol. Chem. 274 (1999) 27642–27650.
[161] L.T. May, K. Leach, P.M. Sexton, A. Christopoulos, Allosteric modulation of G protein-coupled receptors, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 1–51.
[162] J.P. Changeux, S. Edelstein, Conformational selection or induced fit? 50 years of debate resolved, F1000 Biol. Rep. 3 (2011) 19.
[163] A. Shenker, G protein-coupled receptor structure and function: the impact of disease-causing mutations, Baillieres Clin. Endocrinol. Metab. 9 (1995) 427–451.
[164] T. Kenakin, Functional selectivity and biased receptor signaling, J. Pharmacol. Exp. Ther. 336 (2011) 296–302.
[165] A.R. Thomsen, M. Hvidtfeldt, H. Brauner-Osborne, Biased agonism of the calcium-sensing receptor, Cell Calcium 51 (2012) 107–116.
[166] A.R. Thomsen, J. Worm, S.E. Jacobsen, M. Stahlhut, M. Latta, H. Brauner-Osborne, Strontium is a biased agonist of the calcium-sensing receptor in rat medullary thyroid carcinoma 6-23 cells, J. Pharmacol. Exp. Ther. 343 (2012) 638–649.
[167] D.J. Mulder, I. Pacheco, D.J. Hurlbut, N. Mak, G.T. Furuta, R.J. MacLeod, C.J. Justinich, FGF9-induced proliferative response to eosinophilic inflammation in oesophagitis, Gut 58 (2009) 166–173.
[168] G. Liu, X. Hu, J. Varani, S. Chakrabarty, Calcium and calcium sensing receptor modulates the expression of thymidylate synthase, NAD(P)H:quinone oxidoreductase 1 and survivin in human colon carcinoma cells: promotion of cytotoxic response to mitomycin C and fluorouracil, Mol. Carcinog. 48 (2009) 202–211.
[169] T. Kawata, Y. Imanishi, K. Kobayashi, T. Kenko, M. Wada, E. Ishimura, T. Miki, N. Nagano, M. Inaba, A. Arnold, Y. Nishizawa, Relationship between parathyroid calcium-sensing receptor expression and potency of the calcimimetic, cinacalcet, in suppressing parathyroid hormone secretion in an in vivo murine model of primary hyperparathyroidism, Eur. J. Endocrinol. 153 (2005) 587–594.
[170] G. Liu, X. Hu, S. Chakrabarty, Vitamin D mediates its action in human colon carcinoma cells in a calcium-sensing receptor-dependent manner: downregulates malignant cell behavior and the expression of thymidylate synthase and survivin and promotes cellular sensitivity to 5-FU, Int. J. Cancer 126 (2010) 631–639.

[171] G. Liu, X. Hu, J. Varani, S. Chakrabarty, Calcium and calcium sensing receptor modulates the expression of thymidylate synthase, NAD(P)H:quinone oxidoreductase 1 and survivin in human colon carcinoma cells: promotion of cytotoxic response to mitomycin C and fluorouracil, Mol. Carcinog. 48 (2009) 202–211.
[172] M. Promkan, G. Liu, P. Patmasiriwat, S. Chakrabarty, BRCA1 suppresses the expression of survivin and promotes sensitivity to paclitaxel through the calcium sensing receptor (CaSR) in human breast cancer cells, Cell Calcium 49 (2011) 79–88.
[173] J. Fox, S.H. Lowe, R.L. Conklin, B.A. Petty, E.F. Nemeth, Calcimimetic compound NPS R-568 stimulates calcitonin secretion but selectively targets parathyroid gland Ca(2+) receptor in rats, J. Pharmacol. Exp. Ther. 290 (1999) 480–486.
[174] S.X. Cheng, J.P. Geibel, S.C. Hebert, Extracellular polyamines regulate fluid secretion in rat colonic crypts via the extracellular calcium-sensing receptor, Gastroenterology 126 (2004) 148–158.
[175] A.P. Liou, Y. Sei, X. Zhao, J. Feng, X. Lu, C. Thomas, S. Pechhold, H.E. Raybould, S.A. Wank, The extracellular calcium-sensing receptor is required for cholecystokinin secretion in response to L-phenylalanine in acutely isolated intestinal I cells, Am. J. Physiol. Gastrointest. Liver Physiol. 300 (2011) G538–G546.
[176] Y. Wang, R. Chandra, L.A. Samsa, B. Gooch, B.E. Fee, J.M. Cook, S.R. Vigna, A.O. Grant, R.A. Liddle, Amino acids stimulate cholecystokinin release through the Ca2+-sensing receptor, Am. J. Physiol. Gastrointest. Liver Physiol. 300 (2011) G528–G537.
[177] T. Ohsu, Y. Amino, H. Nagasaki, T. Yamanaka, S. Takeshita, T. Hatanaka, Y. Maruyama, N. Miyamura, Y. Eto, Involvement of the calcium-sensing receptor in human taste perception, J. Biol. Chem. 285 (2010) 1016–1022.
[178] R. Cheung, J. Kelly, R.J. Macleod, Regulation of villin by wnt5a/ror2 signaling in human intestinal cells, Front. Physiol. 2 (2011) 58.
[179] R.J. MacLeod, M. Hayes, I. Pacheco, Wnt5a secretion stimulated by the extracellular calcium-sensing receptor inhibits defective Wnt signaling in colon cancer cells, Am. J. Physiol. Gastrointest. Liver Physiol. 293 (2007) G403–G411.
[180] R.J. Pacheco II, Macleod, CaSR stimulates secretion of Wnt5a from colonic myofibroblasts to stimulate CDX2 and sucrase-isomaltase using Ror2 on intestinal epithelia, Am. J. Physiol. Gastrointest. Liver Physiol. 295 (2008) G748–G759.
[181] W. Chang, D. Shoback, Extracellular Ca2+-sensing receptors—an overview, Cell Calcium 35 (2004) 183–196.
[182] B. Chakravarti, S.K. Dwivedi, A. Mithal, N. Chattopadhyay, Calcium-sensing receptor in cancer: good cop or bad cop? Endocrine 35 (2009) 271–284.