Biochimica et Biophysica Acta 1863 (2016) 1398–1407

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

The calcium-sensing receptor and the hallmarks of cancer☆

Samawansha Tennakoon [a,1], Abhishek Aggarwal [a,b,1], Enikö Kállay [a,*]

[a] Department of Pathophysiology and Allergy Research, Medical University of Vienna, Austria
[b] Department of Pediatrics/Endocrinology, Stanford University School of Medicine, Stanford, CA, USA

ARTICLE INFO

Article history:
Received 9 October 2015
Received in revised form 17 November 2015
Accepted 18 November 2015
Available online 30 November 2015

Keywords:
Cancer
Calcium
G protein-coupled receptors
Biased signalling
Oncogene
Tumour suppressor

ABSTRACT

The calcium-sensing receptor (CaSR) plays a pivotal role in systemic calcium metabolism by regulating parathyroid hormone secretion and urinary calcium excretion. The CaSR is ubiquitously expressed, implying a wide range of functions regulated by this receptor. Abnormal CaSR function affects the development of both calciotropic disorders such as hyperparathyroidism, and non-calciotropic disorders such as cardiovascular disease and cancer, which are the leading causes of mortality worldwide.

The CaSR is able to bind a plethora of ligands; it interacts with multiple G protein subtypes, and regulates highly divergent downstream signalling pathways, depending on the cellular context. The CaSR is a key regulator for such diverse processes as hormone secretion, gene expression, inflammation, proliferation, differentiation, and apoptosis. Due to this pleiotropy, the CaSR is able to regulate cell fate and is implicated in the development of many types of benign or malignant tumours of the breast, prostate, parathyroid, and colon. In cancer, the CaSR appears to have paradoxical roles, and depending on the tissue involved, it is able to prevent or promote tumour growth. In tissues like the parathyroid or colon, the CaSR inhibits proliferation and induces terminal differentiation of the cells. Therefore, loss of the receptor, as seen in colorectal or parathyroid tumours, confers malignant potential, suggestive of a tumour suppressor role. In contrast, in prostate and breast tumours the expression of the CaSR is increased and it seems that it favours metastasis to the bone, acting as an oncogene.

Deciphering the molecular mechanism driving the CaSR in the different tissues could lead to development of new allosteric drug compounds that selectively target the CaSR and have therapeutic potential for cancer. This article is part of a Special Issue entitled: Calcium and Cell Fate . Guest Editors: Jacques Haiech, Claus Heizmann, Joachim Krebs, Thierry Capiod and Olivier Mignen.

1. Introduction

The extracellular calcium-sensing receptor (CaSR) is a ubiquitously expressed class C G-protein coupled receptor (GPCR), the master regulator of calcium homeostasis [1,2]. It is highly expressed in the parathyroid and thyroid glands and in the kidneys [2]. In foetal tissues, the CaSR is abundant in the peripheral nervous system, heart and the lungs [3], suggestive of a role in the development of these organs. The CaSR was identified as the molecular sensor of free ionised serum calcium (Ca_o^{2+}) [4]. Ca^{2+} plays the role of first messenger for the CaSR and links changes in extracellular Ca^{2+} concentration with intracellular signalling networks critical for many physiological and pathological processes [1]. Besides the systemic regulation of Ca_o^{2+} homeostasis, the CaSR controls numerous other processes, such as axon and dendrite development in the brain, regulates insulin secretion, blood pressure and myogenic tone, bone remodelling, intestinal water absorption and pH regulation, synthesis of enteroendocrine hormones, and transport of calcium into milk. On cellular level, it regulates gene expression, cell proliferation, differentiation, and cell death. The CaSR responds to numerous signalling molecules, such as other cations, metabolites,

Abbreviations: 1,25-D_3, 1,25-Dihydroxy vitamin D_3; ACF, Aberrant crypt foci; AC, Adenylate cyclase; ADH, Autosomal dominant hypocalcaemia; CaSR, Calcium-sensing receptor; cAMP, Cyclic adenosine 3′,5′-monophosphate; Ca_i^{2+}, Intracellular free ionised calcium; Ca_o^{2+}, Extracellular free ionised calcium; CRC, Colorectal cancer; ECD, Extracellular domain; EGFR, Epithelial growth factor receptor; EMT, Epithelial-to-mesenchymal transition; ER, Endoplasmic reticulum; FHH, Familial hypocalciuric hypercalcaemia; G proteins, Guanine nucleotide-binding proteins; GI, Gastro intestinal; GPCR, G-protein coupled receptor; HCO_3^-, Bicarbonate ion; HHM, Humoral hypercalcaemia of malignancy; mGluRs, Metabotropic glutamate receptors; miRNA, MicroRNA; NSHPT, Neonatal severe hyperparathyroidism; OR, Odds ratio; PKC, Protein kinase C; PLC, Phospholipase C; PLA, Phospholipase A; PLD, Phospholipase D; PTH, Parathyroid hormone; PTHrP, Parathyroid hormone-related peptide; RANKL, Receptor activator of nuclear factor κ b ligand; RCC, Renal cell carcinoma; SNP, Single nucleotide polymorphism; TRPC1, Transient receptor potential canonical 1; TM, Transmembrane domain; VDR, Vitamin D receptor; VDRE, Vitamin D response element.

☆ This article is part of a Special Issue entitled: Calcium and Cell Fate . Guest Editors: Jacques Haiech, Claus Heizmann, Joachim Krebs, Thierry Capiod and Olivier Mignen.

* Corresponding author at: Department of Pathophysiology and Allergy Research, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria.
E-mail address: enikoe.kallay@meduniwien.ac.at (E. Kállay).

[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.bbamcr.2015.11.017

nutrients, and activates a plethora of signalling pathways. It is a multifaceted receptor with multiple ligands and pleiotropic effects [1].

In the present review we explore the multiple signalling pathways regulated by this multimodal chemosensor in cancer, to understand how the pleiotropy of the CaSR reflects on its contradictory roles in tumour development.

1.1. The structure of the CaSR

The *CASR* gene is located on the long arm of chromosome 3 (3q13.3-21) and consists of seven exons. It is under the control of two promoters (the upstream P1 containing a TATA box and a CAAT box, and the downstream P2, which is GC rich) that result in alternative transcripts of exon 1 (exon 1A and exon 1B) [5]. Expression of exon 1A is lower in parathyroid adenomas compared with the normal parathyroid tissue [6]. In colorectal tumours, exon 1A mRNA levels inversely correlate with tumour grade [7]. Therefore, it appears that exon 1A has more impact on CaSR expression than exon 1B. The presence of the two promoter regions suggests tissue-specific *CASR* promoter regulation and alternatively spliced mRNA transcripts [6,8]. Both promoters contain vitamin D response elements (VDREs) enabling 1,25-dihydroxy vitamin D_3 (1,25-D_3), the active form of vitamin D, to induce CaSR expression [5].

The CaSR, a large, 1078 amino acid long protein, consists of four major domains: a large extracellular N terminal domain (ECD), a cysteine-rich domain linking the ECD to the first transmembrane helix, the seven-transmembrane (TM) domain, and an intracellular C-terminal domain [9,10]. The large extracellular domain, characteristic of several GPCRs, is organised as a Venus-flytrap structure. This motif contains the majority of ligand binding sites of the CaSR, others are found in the transmembrane domain. Because the crystal structure of the CaSR is still not known, most of the predictions on the regions involved in ligand binding are performed using the X-ray structure of the metabotropic glutamate receptors (mGluRs) [11] which belong to the same GPCR family. Naturally occurring mutations are most common in the ECD [12]. The N-terminus of this domain contains a signal peptide cleavage site. The conserved cysteine rich domain is important for receptor dimerization, cell surface expression, and signalling [13]. The 216 amino acid long intracellular tail is crucial for CaSR signalling and for surface expression [14].

During its biosynthesis, the signal peptide targets the CaSR to the endoplasmic reticulum (ER) where it is dimerized and then glycosylated in the Golgi before reaching the cell surface [15,16]. Although the CaSR is usually present as a homodimer at the cell surface, the CaSR can form heterodimeric complexes with other GPCRs like the glutamate receptors or the γ-aminobutyric acid-B receptor 1 and can also bind to other proteins like filamins, dorfin, and β arrestins [17,18]. Dimer formation is important, but not sufficient to release the CaSR from the ER pool.

In contrast to most GPCRs, binding to its ligands does not lead to desensitisation of the CaSR [15]. Grant et al. have demonstrated that although endocytosis remains active, CaSR signalling drives biosynthesis of the receptor, release of the new receptor molecules from the ER pool, and trafficking and insertion to the plasma membrane, a phenomenon known as the agonist-driven insertional signalling [19].

1.2. The role of the calcium-sensing receptor in physiology

1.2.1. Role of the CaSR in calciotropic tissues

The primary function of the CaSR is maintenance of systemic calcium homeostasis, by maintaining the balance between absorption of Ca^{2+} in the gastro intestinal (GI) tract, excretion of Ca^{2+} by the kidneys, and the release of Ca^{2+} from the bone. The CaSR in the parathyroid senses minute changes in serum calcium levels (1.1–1.3 mM) and regulates parathyroid hormone (PTH) synthesis and secretion. When serum Ca^{2+} levels are high, the receptor is activated, inhibiting PTH synthesis and secretion. When serum Ca^{2+} concentration is low, CaSR is inactive, and PTH is secreted into the serum. This enhances Ca^{2+} uptake from the intestine, Ca^{2+} release from the bone and reduces urinary Ca^{2+} secretion until serum Ca^{2+} concentration is restored [1]. Renal CaSR controls calcium and phosphate homeostasis, ion transport, the release of renin, and maintains urinary acidification and concentration [16]. In the bone, the CaSR is involved in bone cell metabolism, osteogenesis and in linking bone formation to resorption during bone remodelling [20], although the role of the CaSR in skeletal development is still controversial [21,22]. CaSR mediated reduction in calcitonin secretion in response to low extracellular calcium is also considered important in maintenance of systemic calcium homeostasis. Calcitonin inhibits bone resorption while increasing urinary Ca^{2+} excretion [23].

1.2.2. Role of the CaSR in non-calciotropic tissues

Although the central role of the CaSR is regulation of calcium homeostasis, the CaSR is expressed in non-calciotropic tissues as well. In these tissues, the CaSR regulates a multitude of cellular processes [1]. In the central nervous system, the CaSR is involved in regulation of neuronal cell growth as well as maturation and function of oligodendroglial cells. In nerve endings, the CaSR regulates synaptic functions like plasticity and neurotransmission [24]. In the epidermis, the CaSR regulates cell–cell adhesion and differentiation [25]. In the breast, the CaSR is involved in lactation and enables Ca^{2+} transport into milk [26]. In the pancreas, the CaSR mediates cellular adhesion, cell-to-cell communication and insulin secretion [27]. In the cardiovascular system, the CaSR regulates blood vessel tone and blood pressure [28].

The CaSR is also expressed along the entire GI tract including the taste buds [29], oesophagus [30], stomach [31], and the small and large intestine [32]. In the gut the CaSR is considered to serve as a nutrient sensor [33]. The CaSR stimulates the H^+-K^+-ATPase, thus regulating secretion of gastrin from G cells of the stomach [34]. It also stimulates secretion of cholecystokinin from enteroendocrine cells [35,36], and secretion of bone morphogenetic protein 2 [37] and Wnt5a from colonic myofibroblasts [38]. In the intestine, the CaSR is involved in regulating fluid transport and intestinal ion transport. Bicarbonate (HCO_3^-) secretion in the colon is fine-tuned by the CaSR: in physiological settings it stimulates chloride- and short fatty acid-dependent secretion of HCO_3^-, while in experimental conditions resulting in HCO_3^- loss, that also occurs in diarrhoea and cholera, the CaSR inhibits cAMP-dependent HCO_3^- secretion [39].

1.3. CaSR-mediated signalling

The CaSR is a promiscuous receptor that recognises many different ligands. Upon ligand binding, the conformation of the CaSR changes, leading to binding and activating of associated guanine nucleotide-binding proteins (G proteins) and initiating a complex, G protein-mediated downstream signalling. Depending on the cell and tissue type the CaSR activates a whole network of specific signalling cascades, tightly regulating not only calcium homeostasis but also several pivotal cell functions such as proliferation, differentiation, and apoptosis [40,41]. Moreover the quantity of the CaSR in the cell affects its own signalling as shown recently by Brennan et al. [42].

1.3.1. CaSR ligands

CaSR is activated by a plethora of ligands (Table 1). Type-I or orthosteric ligands directly activate the receptor whereas, type-II ligands are allosteric modulators which sensitise the receptor to type-I ligands [11].

Ca^{2+} is the main orthosteric physiological ligand of the CaSR. Five putative Ca^{2+} binding sites were identified in the ECD [43]. Site 1 is located in the hinge region between the two lobes of the Venus-flytrap region. The sites 1, 2, and 4 are considered non-continuous while the sites 3 and 5 are continuous binding sites. The binding of Ca^{2+} to the different binding sites impacts differently the binding of subsequent Ca^{2+} ions and produces highly cooperative intracellular Ca^{2+} responses.

Table 1
CaSR ligands and physiological conditions which affect CaSR activity.

Type I ligands	**Cations**
	Ca^{2+}, Mg^{2+}, Be^{2+}, Ba^{2+}, Sr^{2+}, La^{3+}, Gd^{3+}
	Polyamines
	Spermine, spermidine, putrescine
	Polypeptides
	Polyarginine, polylysine, protamine, γ-glutamyl peptides, amyloid-β peptide
	Aminoglycoside antibiotics
	Neomycin, tobramycin, gentamicin, kanamycin
Type II ligands	**Pharmacological agents**
	Positive modulators (calcimimetics): Cinacalcet, NPS R-467, NPS R-568, AMG 641
	Negative modulators (calcilytics): NPS 2143, Calhex 231, compound 3, Ronacaleret
	Amino acids
	L-Trp, L-Phe, L-His, L-Ala, L-Glu, L-Leu, L-Arg
Physiological conditions	Ionic strength, pH

Moreover the interaction among the five different Ca^{2+}-binding sites depends on the concentration of the extracellular Ca^{2+} [44].

A number of other cations can also activate the CaSR (Table 1). Experiments with chimeric receptors suggest that the different cations bind to different regions in the extracellular domain of the CaSR [11]. In addition to the cations, amino acids, such as large aromatic L-amino acids, bind to the ECD and act as allosteric modulators. Their binding site is different from the Ca^{2+} binding pockets [45]. Endogenous polyamines, even aminoglycoside antibiotics activate the receptor, broadening the range of CaSR ligands [1,11,44]. Ligand binding to the CaSR modulates subsequent ligand-receptor interaction, leading either to homotropic cooperativity if the ligands are identical, or to heterotropic cooperativity, if they are different (i.e. Ca^{2+} and an aromatic amino acid) [44].

Physiological conditions such as ionic strength and pH also affect CaSR activity. Ionic strength reduces, whereas pH levels higher than the physiological levels (7–7.8) increase the sensitivity of the receptor towards the agonists [46,47].

In addition to these biological ligands, pharmacological modulators: calcimimetics and calcilytics were developed to target the CaSR (Table 1). These not only modulate the activity of the receptor, but act also as pharmaco-chaperones and influence CaSR expression as well. Calcimimetics increase the number of CaSR molecules on the cell surface, whereas calcilytics either downregulate [48] or upregulate CaSR expression [49] in a cell type-dependent manner. Experiments performed by Huang and Breitwieser using proteasomal inhibitors suggested that NPS 2143-dependent downregulation of the CaSR expression was due to increased sensitivity to degradation [48].

Ca^{2+} and calcimimetics trigger the phosphorylation of Thr888 in the C terminal domain of the CaSR. The CaSR antagonist NPS 2143 prevented this phosphorylation and decreased CaSR activity [50]. Davies et al. demonstrated that de-phosphorylation of Thr888 inhibited CaSR signalling [50], identifying this process as a negative feedback loop in the regulation of CaSR function.

1.3.2. G protein activation and downstream signalling

The calcium-sensing receptor regulates diverse downstream signalling pathways by binding different G protein subunits. Kinetics of G protein activation depend on a number of factors including receptor type, ligands, intracellular protein content, expression of G proteins and their binding affinity and deactivation rate of the receptor [51]. To date, four classes of Gα subunits ($G_{i/o}$, G_s, $G_{q/11}$ and $G_{12/13}$) have been shown to be involved in CaSR-mediated signalling, activating complex downstream pathways (Fig. 1) [52].

Coupling of the CaSR to $G\alpha_{q/11}$ leads to phospholipase C (PLC) activation, IP_3-mediated intracellular Ca^{2+} (Ca_i^{2+}) release and protein kinase C (PKC) activation (Fig. 1) [1,52,53]. CaSR-dependent upregulation of PLC/IP_3-mediated Ca_i^{2+} levels have been observed in liver cells [54], parathyroid cells [4,55], lung epithelial cells [56], and kidney cells [57]. CaSR-mediated activation of phospholipase (PL) C, A, and D is dependent on PKC stimulation [1,58,59]. The CaSR itself can be phosphorylated by PKC on its PKC-phosphorylation sites (Thr888, Ser895, Ser 915) [14].

In parathyroid cells, coupling of the CaSR to $G_{i/o}$, leads to inhibition of adenylate cyclase (AC) reducing the level of 3′,5′-cyclic adenosine monophosphate (cAMP) and inhibition of CaSR-mediated PTH secretion [1,58]. Upregulation of AC activity triggers PKA activation via cAMP generation. PKA signalling, Ca^{2+}/CAM/calcineurin and MAP/ERK pathway are the main downstream events regulated by cAMP [60,61]. This in turn regulates a wide range of downstream proteins amplifying and diversifying the initial signal. CaSR-mediated $G_{i/o}$ activation has been reported in the cells of the medullary thick ascending limb [62], in human embryonic kidney cells transfected with CaSR [63], and in

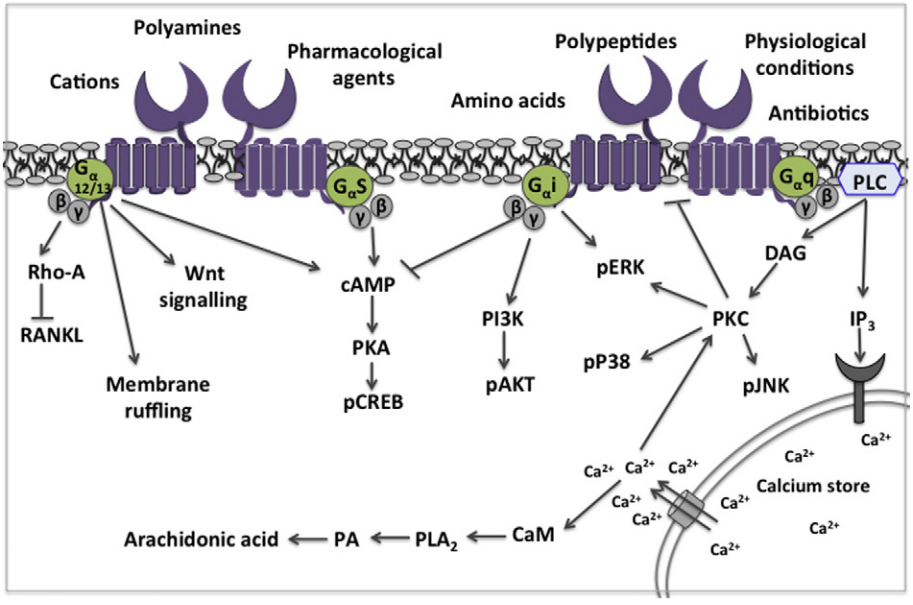

Fig. 1. G protein activation and downstream signalling of the active CaSR. The spectrum of CaSR ligands includes endogenous cations, polyamines, amino acids, pharmacological agents, and physiological conditions. Intracellular Ca^{2+} signal, cAMP synthesis, and protein phosphorylation are some of the key signalling events regulated by the CaSR.

normal breast cells, while in breast cancer cells cAMP levels were upregulated [64]. Mamillapalli et al. demonstrated that CaSR is coupled to $G_{i/o}$ in normal breast cells whereas it binds to G_s, in breast cancer cells [64]. In addition to breast cancer cells, CaSR-mediated synthesis of cAMP via activation of G_s has been observed in pituitary cells [65], hamster ovary cells [66], and in colon cancer cells [7].

$G_{12/13}$-mediated CaSR signalling primarily influences cell shape and cell migration by activation of Rho GTPases [67] and modulation of processes involved in maintaining cell architecture such as β-catenin release from E-cadherin [52,68]. Understanding the crosstalk within and between cellular signalling networks is crucial not only for identifying molecular basis of pathological conditions but also for the development of novel therapeutic agents.

1.3.3. Biased signalling of the CaSR

The CaSR functions as a multimodal chemosensor and a key transducer of signals from the extracellular milieu to the intracellular environment. It integrates a variety of extracellular metabolic stimuli (e.g. polyvalent cations, amino acids, pH, and ionic strength), which preferentially activate distinct intracellular signalling cascades in a process known as "ligand-directed targeting of receptor stimulus" or "stimulus bias" [52,69].

CaSR signalling depends on the type of the cell, expression of G protein isoforms, enzymes, and adapter proteins that control the assembly of signalling scaffolds, and mutations of the receptor [52,68,69]. The phenomenon of ligand-dependent bias is used as a new strategy to develop GPCR-targeting drugs, including CaSR-targeting candidates, with very specific on-target effects and no, or minimal side-effects [69,70]. Due to the diversity and versatility of the CaSR-mediated signalling, the CaSR has very different roles in different tissues and pathologies.

1.4. CaSR mutations and their clinical implications

Aberrant CaSR expression and activity is linked to several inherited disorders of calcium homeostasis. Numerous *CASR* mutations, both activating and inactivating, have been identified in humans (http://www.casrdb.mcgill.ca/). The best-characterised disorders caused by inactivating mutations are disorders of calcium metabolism including familial hypocalciuric hypercalcaemia (FHH) and neonatal severe hyperparathyroidism (NSHPT). FHH is an autosomal dominant disorder associated with increased serum Ca^{2+}, inappropriately normal PTH levels and decreased urinary Ca^{2+} excretion as a result of loss of function mutations of the CaSR on a single allele [58,71,72]. NSHPT, caused by homozygous inactivating mutations is characterised by life threatening hypercalcaemia and under-mineralized bones [58,72]. Gain of function mutations of the *CASR* result in autosomal dominant hypocalcaemia (ADH) and type-V Bartter syndrome. ADH patients have mild hypocalcaemia, low PTH levels, seizures during childhood, while the type-V Bartter syndrome is characterised by chronic metabolic alkalosis, hypocalcaemia and increased plasma renin activity [58,73].

2. The calcium-sensing receptor in cancer

Outside its central role in calcium homeostasis, the CaSR mediates cell fate-regulating processes including hormone secretion, control of proliferation, differentiation, apoptosis, and chemotaxis [1]. The ability of the CaSR to regulate cell fate predicts that it will have substantial impact in cancer development. Interestingly, the CaSR functions in a yin-yang fashion acting both as an oncogene and a tumour suppressor gene, depending on the site of cancer (Fig. 2).

Several groups have tested whether common genetic variants of the *CASR* influence the risk, incidence, recurrence, or lethality of cancers. In a large cohort, nested in the prospective Health Professionals Follow-up study, three genetic variants of the *CASR* were linked with lethal prostate cancer. Two single nucleotide polymorphisms (SNPs) were nominally

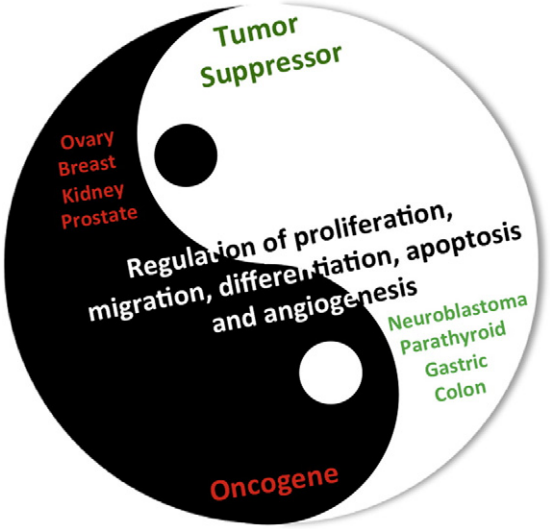

Fig. 2. The yin-yang role of the CaSR in cancer: In cancer the CaSR regulates proliferation, differentiation, cell death, angiogenesis, and migration. It functions either as a tumour suppressor as in colon, parathyroid, gastric cancer, and neuroblastomas (shown in green) or as an oncogene in breast, prostate, ovary, and kidney cancer (shown in red).

associated with odds ratios (OR) of 0.65 (rs6438705; rs13083990), the third (rs2270916) was linked to an OR of 1.55, while 3 others (rs1801725, rs1042636, and rs1801726) showed no associations [74]. A recent study however, found no association between 65 *CASR* SNPs and the recurrence or aggressiveness of prostate cancer [75].

The G allele of the CaSR rs17251221 polymorphism seems to protect against ovarian cancer [76].

Three nonsynonymous SNPs in the coding region of the intracellular *CASR* tail (Q1011E, A986S, R990G) have been suggested to be linked to colorectal cancer susceptibility [77]. The *CASR* SNP rs1801726 (Q1011E) was significantly associated with reduced risk for rectal cancer [78]. Another study identified four polymorphisms significantly associated with increased colorectal cancer risk, but only in patients that received low calcium diet [79].

In a cohort of neuroblastic tumours three functionally relevant CaSR polymorphisms (rs1801725, rs1042636 and rs1801726) were analysed as a block, the tri-locus haplotype TAC, that suggests a potentially less active CaSR, was associated with an increased risk of death in the whole cohort including the neuroblastoma patients [80]. Apart from the SNPs, there is no real evidence until now, that would link CASR mutations to cancer development. It appears that it is the expression level of the receptor that changes during cancer development. It is either increased, as in cancers where the CaSR acts as an oncogene, or decreased, as in cancers where it functions as tumour suppressor [68].

2.1. The CaSR: an oncogene

In several cancers, such as prostate, testicular, ovarian, and breast cancer the CaSR seems to act as an oncogene, often by promoting proliferation and inhibiting apoptosis. However, the mechanisms are not yet completely understood.

2.1.1. Humoral hypercalcaemia of malignancy

The oncogenic role of the CaSR is often linked to its involvement in regulating the synthesis of parathyroid hormone-related peptide (PTHrP) [81]. PTHrP is a peptide growth factor that binds the same receptor as PTH. It is a stimulator of osteoclastic bone resorption that activates osteoclasts by increasing the expression of receptor activator of nuclear factor κ B ligand (RANKL), a member of the tumour necrosis factor cytokine family [81]. PTHrP contributes to the pathogenesis of cancers by stimulating osteolytic bone destruction and releasing bone-

derived growth factors. Humoral hypercalcemia of malignancy (HHM) is a common paraneoplastic syndrome and a frequent complication of breast, prostate, lung, kidney cancer and of multiple myeloma. HHM is often caused by PTHrP produced by tumour cells [82,83] and is frequently seen in patients with tumours that metastasize to the bone.

One of the first evidences that the CaSR might be involved in the pathophysiology of HHM was based on studies in the Rice H-500 rat Leydig tumour cell line [84]. These cells cause PTHrP-dependent hypercalcaemia in rats bearing Leydig tumour fragment xenografts. Treatment of H-500 cells with Ca^{2+} resulted in a concentration-dependent increase of PTHrP release [84], an effect that could be inhibited by the transfection of these cells with a dominant negative *CASR* mutant (bearing the R185Q mutation) [85].

In H-500 cells, the CaSR stimulated proliferation and conferred resistance to apoptosis [86], although this effect seemed to be direct, by activation of the PI_3-kinase/Akt pathway, and not mediated by PTHrP [87]. In the H-500 cells, activation of the CaSR increased the expression of the pituitary tumour-transforming gene [88], an oncogene that affects proliferation and angiogenesis, two hallmarks of cancer (Fig. 3).

2.1.2. The role of the CaSR in favouring metastasis to the bone

The bone niche has unique characteristics that provide homing signals to several cancers such as breast, prostate, lung and kidney cancer. These cancers develop bone metastases with an incidence of 65–40% [89]. Multiple growth factors and cytokines in combination with physical properties provide favourable chemotactic and growth-promoting conditions [90]. High extracellular Ca^{2+} is one of the most important inorganic factors involved in this process [91]. A recent study showed that in the highly invasive MDA-MB-231 breast cancer cell line activation of the CaSR stimulated the secretion of several pro-angiogenic and chemotactic cytokines and growth factors [92]. The CaSR seems to facilitate formation and growth of skeletal metastases of prostate, breast, and kidney cancer cells [81].

Prostate cancer metastasizes almost exclusively to bone. The CaSR is higher expressed in the bone metastases than in the primary prostate tumour, although no differences were seen in primary prostate tumours with or without concurrent metastases [93]. High Ca_o^{2+} increased proliferation of bone-colonising prostate cancer cell lines (PC-3, C4-2B) but had no effect on the LNCaP prostate cancer cells derived from a lymph node metastasis. The proliferative effect of Ca_o^{2+} was mediated by the CaSR, at least in part, because knocking down CaSR expression inhibited proliferation and metastatic potential of PC-3 cells [91].

About 30–40% of renal cell carcinomas (RCC) metastasize to the bone. A recent study found that expression of CaSR was highest in the primary renal cell tumours of patients with bone metastases, compared with tumours from patients with lung metastases or no metastases. Moreover, Ca_o^{2+} treatment induced proliferation and migration solely in RCC cells obtained from patients with bone metastases, an effect that was inhibited by the CaSR inhibitor NPS 2143 [94].

Understanding the role of the CaSR in development of bone metastases could provide new application for CaSR targeting drugs. Antagonising the tumour cell CaSR with calcilytics that prevent its activation in conjunction with antiresorptive agents, such as bisphosphonates, might result in a novel therapeutic approach to control both osteolysis and tumour cell proliferation.

2.1.3. Mitogenic pathways stimulated by the CaSR

Among early responses to mitogenic GPCR agonists is the activation of protein phosphorylation cascades, including Akt/mTOR/p70S6K, Raf/MEK/ERK, and PKC/PKD [95]. The tumour promoting effects of the CaSR seem to involve the same or similar pathways. Thus, stimulation of the CaSR by Ca_o^{2+} induced proliferation in both normal and malignant ovarian epithelial cells by activation of the ERK1/2 pathway [96,97].

The signalling pathways underlying the mitogenic effects of the CaSR in prostate cancer are still not completely understood. Although most of the prostate cancer cells cause osteoblastic and not osteolytic metastases, PTHrP seems to be involved in the CaSR-mediated stimulation of metastasis. PTHrP levels were higher in prostate tumours compared with normal prostate epithelial cells. Moreover, PTHrP increased proliferation of the bone metastasizing PC-3 cells but not that of LNCaP cells [98]. Activation of the CaSR in prostate cancer cells stimulated PTHrP secretion probably by transactivation of the epithelial growth factor receptor (EGFR) and activation of ERK1/2 [99]. Knockdown of PTHrP in PC-3 cells inhibited epithelial-to-mesenchymal transition (EMT) and tumour progression when injected orthotopically in mice [100]. There is some evidence suggesting a novel linkage between PTHrP and the Wnt pathway. PTHrP inhibits the expression of Dickkopf-1, a canonical Wnt inhibitor, leading to activation of the Wnt pathway in the bone metastases of prostate cancer [101]. Whether the CaSR is involved in this mechanism, needs to be proven. A further pathway involved in the CaSR-mediated proliferation is activation of Akt [91], while another seems to involve Rho signalling [102].

2.1.4. The role of the CaSR in breast cancer

The CaSR is expressed both in the normal and cancerous mammary gland. In normal mammary epithelial cells activation of the CaSR during lactation inhibits PTHrP synthesis and secretion. In contrast, in breast cancer cells the CaSR stimulates PTHrP secretion in a cAMP-dependent way [103]. This contradictory function is possible due to the ability of the CaSR to bind and activate different G proteins, in this case, to switch from activation of the inhibitory $G\alpha_i$ and suppression of cAMP levels in the normal mammary cells, to activation of $G\alpha_s$ and stimulation of cAMP levels in breast cancer cells [64].

It seems that the role of the CaSR in breast cancer is equivocal [103]. There is evidence both for pro- and anti-tumourigenic effects. Mihai et al. [104] found positive correlation between CaSR expression in primary breast tumours and the development of osteolytic bone metastases and showed that the CaSR expression was higher in bone metastases than in primary breast tumours.

Recently Li et al. observed that lower expression of CaSR significantly associated with poor overall survival, cause-specific survival, and distant metastasis-free survival of breast cancer patients [105]. Additionally, in a large prospective cohort, postmenopausal women with a dietary calcium intake of >1250 mg/d had reduced risk to develop breast cancer [106].

Several studies suggested that the CaSR promoted a more aggressive behaviour of breast tumours. Treatment with high calcium concentration (> 15 mM) reduced oestrogen receptor expression but increased its transcriptional activity in the breast cancer cell line MCF-7 transfected with a vector containing oestrogen response element and a luciferase reporter gene. Activation of the CaSR by the calcimimetic NPS R-467 mimicked the effect of Ca^{2+} on oestrogen receptor expression and activity [107]. In MCF-7 cells activation of the CaSR with increasing Ca_o^{2+} levels stimulated proliferation and transient receptor potential canonical 1 (TRPC1) channel expression in a PLC-, PKC-, and ERK-dependent manner [108,109]. The effect of the CaSR involved transactivation of the EGFR [109]. Such "triple-pass" signalling through the CaSR is common for several cell types [103]. The CaSR promoted breast cancer cell migration, however only in cells that are capable to form bone metastases (e.g. MDA-MB-231 and MCF-7). In BT474 cells, that have no bone-metastatic potential, Ca_o^{2+} had no effect on migration, although the CaSR levels were similar as in the metastatic cells [110].

The group of Chakrabarty [111] however, observed that treatment with Ca_o^{2+} reduced malignant behaviour and induced the sensitivity of MCF-7 and MDA-MB-435 cells to the chemotherapeutic drug paclitaxel, by inhibiting the expression of survivin. Moreover, the surviving paclitaxel-resistant cells expressed no CaSR [111]. This group also suggested that some of the tumour suppressive functions of BRCA1 are CaSR-dependent [112].

The oncogenic potential of the CaSR is demonstrated by stimulation of proliferative and angiogenic signals and promotion of metastasis (Fig. 3). The contradictory findings with respect to the role of the CaSR

in breast cancer point to the limitations of cell line-based *in vitro* studies and show the need to study the effect of the receptor in a more natural environment, either in organoids or *in vivo* in animal models.

2.2. The CaSR, a tumour suppressor

Increased intake of calcium reduces the risk of several cancers. There is some evidence that the CaSR is one of the central mediators of the anti-tumourigenic effects of calcium. However, expression of the CaSR is reduced or even lost in several malignancies, including parathyroid cancer, colorectal cancer and neuroblastomas. In these tumours, loss of receptor function leads to loss of the protective effects of calcium because the decrease in CaSR levels affects both key characteristics of the Ca_i^{2+} response at the single-cell level and the proportion of cells responding to Ca_o^{2+} [42]. The mechanisms leading to loss of expression are different in the different tumours. In neuroblastomas both genetic and epigenetic mechanisms contribute to silencing of the CaSR; in colorectal tumours the epigenetic mechanisms are primarily responsible for CaSR loss. However, in parathyroid tumours, neither of these mechanisms seem to be responsible for loss of CaSR expression.

2.2.1. The role of the CaSR in parathyroid cancer

In the parathyroid, expression of the CaSR progressively decreases in hyperplastic glands, adenomas, and carcinomas compared with normal parathyroid tissue [113]. An inactivating mutation of the *CASR* in a mouse model led to parathyroid hyperplasia [114] suggesting a causative relation between loss of CaSR function and loss of growth control. In another study using mice with chronic kidney disease, the authors demonstrated that treatment with Cinacalcet, a positive allosteric modulator of the CaSR, was able to control parathyroid hyperplasia, which was reversed upon discontinuation of the treatment [115].

Although it is clear that loss of CaSR function leads to an increase in PTH secretion, the molecular mechanisms governing parathyroid tumourigenesis is not yet clear. Neither loss of *CASR* alleles [116], nor epigenetic silencing [117,118] are responsible for the loss of CaSR expression in parathyroid tumours. Furthermore, no mutations in the *CASR* gene were associated with parathyroid hyperplasia [119]. Therefore, further mechanistic studies are needed to understand the causes of CaSR loss and to establish the role of the CaSR in parathyroid tumours. Recently, Fabbri and colleagues have generated a continuous rat parathyroid cell line [120], which would aid in understanding the role of the CaSR in the parathyroid.

2.2.2. The role of the CaSR in neuroblastomas

In neuroblastoma, a malignancy of the sympathetic nervous system, expression of the CaSR is lost in unfavourable tumours compared with differentiated tumours, which still express the receptor [121]. Loss of one chromosome 3 (monosomy), which harbours the *CASR* gene, as well as DNA hypermethylation of the *CASR* promoter region contribute to silencing of the receptor in this rare form of cancer. It seems that the CaSR is needed for ERK1/2-mediated induction of apoptosis in neuroblastoma cells [122]. A follow up study associated polymorphisms of the *CASR* with poor clinical outcome [80,122].

2.2.3. The role of the CaSR in colorectal cancer

The inverse correlation between calcium intake and risk of CRC has been known for decades [123], although the mechanisms driving the protective effect of calcium were not clear. In normal colonic epithelia calcium inhibits proliferation and induces differentiation. After identification of the CaSR on intestinal cells [32,124] it was suggested that the anti-proliferative effects of calcium were mediated, at least in part, by the CaSR [32,125,126]. The CaSR is expressed in normal colonic mucosa and in early adenomas; its expression is already decreased in advanced adenomas, and lost in late stage undifferentiated tumours [126,127,128]. This might be the reason why during colorectal carcinogenesis calcium is ineffective or even tumour promoting [129].

Compelling evidence from two independent studies demonstrated that promoter hypermethylation and histone deacetylation lead to silencing of the receptor in colorectal tumours [127,130]. We have recently demonstrated that a further cause of silencing the CaSR in colorectal tumours was increased expression of the microRNAs (miRNA) miR-135b and miR-146b [131]. MiRNAs are small non-coding RNAs which, in addition to DNA hypermethylation and histone deacetylation, play an important role in epigenetic regulation of gene expression [132,133]. Singh et al. showed that miR-21, miR-145 and miR135a also regulate CaSR expression in colon cancer cells [134,135]. It is likely that deregulation of miRNA expression occurs also in parathyroid tumours, and could be one of the mechanisms behind loss of expression in those tumours.

Colon cancer cells that lack the CaSR are less differentiated, grow faster, and in general have a more malignant phenotype [49,134,135]. Dearth of the *CASR* (both, global and intestine-specific) increased proliferation [49,136] and led to formation of pre-neoplastic lesions called aberrant crypt foci (ACFs) in the colon of mice [137]. Furthermore, the intestine of the CaSR-KO mice expresses increased levels of inflammation markers and is more susceptible to chemically-induced colitis compared with the intestine of wild type mice [137,138]. Activating the CaSR (by treatment with the calcimimetic NPS R-568) or increasing its expression resensitised colon cancer cells to the chemoprotective functions of calcium, inhibiting cell growth, and inducing differentiation and apoptosis [49]. These studies unanimously demonstrated that the CaSR plays a central role in mediating anti-tumourigenic effects of calcium in the colon.

Colorectal cancer is a disease of defective Wnt/β-catenin signalling. *In vitro*, *in vivo* and *ex situ* studies have linked the loss of CaSR expression or function to enhanced Wnt/β-catenin signalling in the colon. In colonic cells that lack the CaSR nuclear β-catenin expression increased [136,139] and the Wnt signalling was shifted from the non-canonical to the canonical Wnt/β-catenin pathway [38,137,140]. Moreover, the cells had a more mesenchymal and cancer stem cell-like phenotype, [134,135,139]. Increasing CaSR expression or activity prevented epithelial-to-mesenchymal transition (EMT) and reduced expression of cancer stem cell markers [49,139,141]. These studies demonstrate that the tumour suppressive characteristics of the CaSR in the colon are due to the ability of the CaSR to inhibit several hallmarks of cancer (Fig. 3). It inhibits sustained proliferation by regulating the canonical and non-canonical Wnt pathway, restricts invasion and metastasis by preventing EMT and acquisition of stem cell-like phenotype.

2.2.4. The role of the CaSR in other cancers

In gastric tissue the CaSR is expressed in gastric epithelial glands and ganglions, and is downregulated in gastric tumours [142]. A recent study found a negative correlation between CaSR expression and gastric cancer invasion suggesting that the CaSR might act as a tumour suppressor [142].

In the pancreas, the CaSR is highly expressed in the endocrine islets. The pancreatic ducts, exocrine acinar cells, intrapancreatic nerves and the blood vessels also express the CaSR, albeit at lower levels [143,144]. While protein expression of the receptor was found in both normal and neoplastic pancreatic tissue [143,144], mRNA expression was downregulated in pancreatic ductal adenocarcinomas compared with the normal, adjacent tissue from the same patient [143]. Given the heterogeneous nature of the pancreatic tissue and the differences in the CaSR expression in different regions, it is still unclear whether the expression of the receptor is indeed deregulated in pancreatic cancer.

Deregulation of CaSR expression and function contributes to progression and aggressiveness of several tumours due to its ability to regulate several hallmarks of cancer (Fig. 3). Finding means or drugs that modulate CaSR expression and activity in tumours could present the CaSR as a provocative target for anticancer drug design.

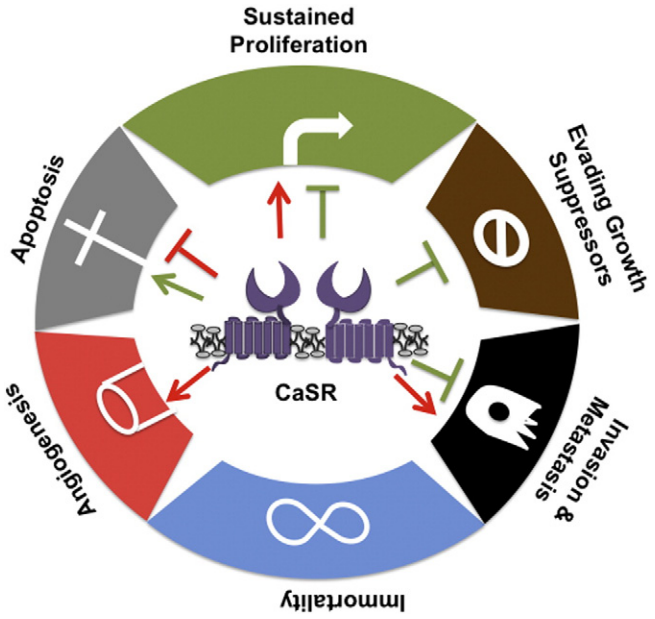

Fig. 3. The CaSR within the hallmarks of cancer as defined by Hanahan and Weinberg. Arrows depict the oncogenic role (in red), and tumour suppressor role (in green). (Adapted from Hanahan and Weinberg [145]).

2.3. Therapeutic implications for the CaSR in cancer

Due to increased life expectancy, cancer in the elderly has become an increasing problem in the Western world. Several studies have focused on establishing risk factors to facilitate the development of effective and well-tolerated treatments. Targeting the CaSR holds significant therapeutic potential depending on whether its expression is lost, as seen in colon and parathyroid cancers, increased as in breast and prostate cancers, or whether its signalling is altered. CaSR-targeting drugs could be used to prevent carcinogenesis or to improve the efficiency of current chemotherapeutics.

Although both positive and negative modulators of the CaSR are already in development, currently only the positive allosteric modulator, Cinacalcet, is approved for use in humans. It is the first FDA-approved allosteric GPCR modulator and is used to treat secondary hyperparathyroidism caused by chronic kidney disease, and hypercalcaemia in patients with inoperable parathyroid carcinoma [146]. However, it had no effect on cancer progression, and reduced only the hypercalcaemic symptoms [147]. Moreover, treatment with Cinacalcet has limits due to its side effects and tolerability.

Given the implication of the CaSR in many physiological and pathophysiological processes, allosteric CaSR modulators could be used in other disorders where the CaSR is involved [148]. Based on preclinical and clinical studies, several other CaSR-based therapeutics seem to emerge for non-parathyroid disorders.

Since the CaSR modulates key events in cancer, it presents itself as a potential therapeutic target. However, caution will be needed when using CaSR modulators, because of the different roles of the CaSR in the different malignancies. In order to prevent side effects in other tissues than the target, tissue-specific delivery should be considered.

Identification of ligands or development of drugs with high selectivity for the CaSR and specificity for the tissue of interest are needed. These ligands could be used to exploit the characteristic of the CaSR to signal in a ligand-biased manner; to activate preferentially one pathway upon binding a specific ligand, without activating others.

Another strategy could be to find natural substances that affect CaSR expression. We have shown previously that high vitamin D diet increased CaSR expression in the colon [141]. Whether dietary vitamin D would affect the expression level of the CaSR also in tumours, needs to be tested.

3. Conclusion

There is evidence for the involvement of the CaSR in the development of different tumours. The sheer diversity of the signalling mechanisms affected by the CaSR makes the identification of its precise role in the different malignancies a challenging task. However, only a deep understanding of the CaSR-mediated signalling mechanisms in the different normal and malignant tissues will provide sufficient support for the development and use of novel calcimimetics and calcilytics for treatment of cancer.

Financial support

ST and AA were Marie Curie Early Stage Researchers funded by the EU Marie Curie ITN grant Multifaceted CaSR (FP7-264663) to EK and by the Vienna Science and Technology Fund (WWTF) LS12-047 to EK.

Conflict of interest

None.

Transparency document

The Transparency document associated with this article can be found, in online version.

References

[1] E.M. Brown, R.J. MacLeod, Extracellular calcium sensing and extracellular calcium signaling, Physiol. Rev. 81 (2001) 239–297.
[2] D.T. Ward, D. Riccardi, New concepts in calcium-sensing receptor pharmacology and signalling, Br. J. Pharmacol. 165 (2012) 35–48.
[3] A. Diez-Fraile, T. Lammens, Y. Benoit, K.G. D'Herde, The calcium-sensing receptor as a regulator of cellular fate in normal and pathological conditions, Curr. Mol. Med. 13 (2013) 282–295.
[4] 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.
[5] 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-dihydroxy vitamin D, J. Biol. Chem. 277 (2002) 30337–30350.
[6] 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.
[7] 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.
[8] G.N. Hendy, L. Canaff, D.E. Cole, The CASR gene: alternative splicing and transcriptional control, and calcium-sensing receptor (CaSR) protein: structure and ligand binding sites, Best Pract. Res. Clin. Endocrinol. Metab. 27 (2013) 285–301.
[9] J.E. Garrett, I.V. Capuano, L.G. Hammerland, B.C. Hung, E.M. Brown, S.C. Hebert, E.F. Nemeth, F. Fuller, Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs, J. Biol. Chem. 270 (1995) 12919–12925.
[10] C. Zhang, C.L. Miller, E.M. Brown, J.J. Yang, The calcium sensing receptor: from calcium sensing to signaling, Sci. China. Life Sci. 58 (2015) 14–27.
[11] A.L. Magno, B.K. Ward, T. Ratajczak, The calcium-sensing receptor: a molecular perspective, Endocr. Rev. 32 (2011) 3–30.
[12] J. Hu, A.M. Spiegel, Naturally occurring mutations of the extracellular Ca^{2+}-sensing receptor: implications for its structure and function, Trends Endocrinol. Metab. 14 (2003) 282–288.
[13] K. Ray, K.A. Adipietro, C. Chen, J.K. Northup, Elucidation of the role of peptide linker in calcium-sensing receptor activation process, J. Biol. Chem. 282 (2007) 5310–5317.
[14] 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 Ca_o^{2+}-sensing receptor (CaR) inhibits coupling to Ca^{2+} store release, J. Biol. Chem. 273 (1998) 21267–21275.
[15] G.E. Breitwieser, The calcium sensing receptor life cycle: trafficking, cell surface expression, and degradation, Best Pract. Res. Clin. Endocrinol. Metab. 27 (2013) 303–313.
[16] D. Riccardi, E.M. Brown, Physiology and pathophysiology of the calcium-sensing receptor in the kidney, Am. J. Physiol. Renal Physiol. 298 (2010) F485–F499.
[17] M. Bai, S. Trivedi, E.M. Brown, Dimerization of the extracellular calcium-sensing receptor (CaR) on the cell surface of CaR-transfected HEK293 cells, J. Biol. Chem. 273 (1998) 23605–23610.
[18] L. Gama, S.G. Wilt, G.E. Breitwieser, Heterodimerization of calcium sensing receptors with metabotropic glutamate receptors in neurons, J. Biol. Chem. 276 (2001) 39053–39059.

[19] M.P. Grant, A. Stepanchick, A. Cavanaugh, G.E. Breitwieser, Agonist-driven maturation and plasma membrane insertion of calcium-sensing receptors dynamically control signal amplitude, Sci. Signal. 4 (2011), ra78.
[20] R. Caudarella, F. Vescini, A. Buffa, E. Rizzoli, L. Ceccoli, C.M. Francucci, Role of calcium-sensing receptor in bone biology, J. Endocrinol. Investig. 34 (2011) 13–17.
[21] W. Chang, C. Tu, T.H. Chen, D. Bikle, D. Shoback, The extracellular calcium-sensing receptor (CaSR) is a critical modulator of skeletal development, Sci. Signal. 1 (2008), ra1.
[22] Q. Tu, M. Pi, G. Karsenty, L. Simpson, S. Liu, L.D. Quarles, Rescue of the skeletal phenotype in CasR-deficient mice by transfer onto the Gcm2 null background, J. Clin. Invest. 111 (2003) 1029–1037.
[23] N.J. Fudge, C.S. Kovacs, Physiological studies in heterozygous calcium sensing receptor (CaSR) gene-ablated mice confirm that the CaSR regulates calcitonin release in vivo, BMC Physiol. 4 (2004) 5.
[24] M. Ruat, E. Traiffort, Roles of the calcium sensing receptor in the central nervous system, Best Pract. Res. Clin. Endocrinol. Metab. 27 (2013) 429–442.
[25] C.L. Tu, D.D. Bikle, Role of the calcium-sensing receptor in calcium regulation of epidermal differentiation and function, Best Pract. Res. Clin. Endocrinol. Metab. 27 (2013) 415–427.
[26] R. Mamillapalli, J. VanHouten, P. Dann, D. Bikle, W. Chang, E. Brown, J. Wysolmerski, Mammary-specific ablation of the calcium-sensing receptor during lactation alters maternal calcium metabolism, milk calcium transport, and neonatal calcium accrual, Endocrinology 154 (2013) 3031–3042.
[27] P.E. Squires, P.M. Jones, M.Y. Younis, C.E. Hills, The calcium-sensing receptor and β-cell function, Vitam. Horm. 95 (2014) 249–267.
[28] M. Schepelmann, P.L. Yarova, I. Lopez-Fernandez, T.S. Davies, S.C. Brennan, P.J. Edwards, A. Aggarwal, J. Graca, K. Rietdorf, V.V. Matchkov, R.A. Fenton, W. Chang, M. Krssak, A. Stewart, K.J. Broadley, D.T. Ward, S.A. Price, D.H. Edwards, P.J. Kemp, D. Riccardi, The vascular Ca^{2+}-sensing receptor regulates blood vessel tone and blood pressure, Am. J. Physiol. Cell Physiol. (2015) (ajpcell.00248.02015).
[29] M.F. Bystrova, R.A. Romanov, O.A. Rogachevskaja, G.D. Churbanov, S.S. Kolesnikov, Functional expression of the extracellular-Ca^{2+}-sensing receptor in mouse taste cells, J. Cell Sci. 123 (2010) 972–982.
[30] C.J. Justinich, N. Mak, I. Pacheco, D. Mulder, R.W. Wells, M.G. Blennerhassett, R.J. MacLeod, The extracellular calcium-sensing receptor (CaSR) on human esophagus and evidence of expression of the CaSR on the esophageal epithelial cell line (HET-1A), Am. J. Physiol. Gastrointest. Liver Physiol. 294 (2008) G120–G129.
[31] I. Cheng, I. Qureshi, N. Chattopadhyay, A. Qureshi, R.R. Butters, A.E. Hall, R.R. Cima, K.V. Rogers, S.C. Hebert, J.P. Geibel, E.M. Brown, D.I. Soybel, Expression of an extracellular calcium-sensing receptor in rat stomach, Gastroenterology 116 (1999) 118–126.
[32] 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.
[33] J.P. Geibel, S.C. Hebert, The functions and roles of the extracellular Ca^{2+}-sensing receptor along the gastrointestinal tract, Annu. Rev. Physiol. 71 (2009) 205–217.
[34] M.M. Dufner, P. Kirchhoff, C. Remy, P. Hafner, M.K. Muller, S.X. Cheng, L.Q. Tang, S.C. Hebert, J.P. Geibel, C.A. Wagner, The calcium-sensing receptor acts as a modulator of gastric acid secretion in freshly isolated human gastric glands, Am. J. Physiol. Gastrointest. Liver Physiol. 289 (2005) G1084–G1090.
[35] 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.
[36] 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 Ca^{2+}-sensing receptor, Am. J. Physiol. Gastrointest. Liver Physiol. 300 (2011) G528–G537.
[37] D. Peiris, I. Pacheco, C. Spencer, R.J. MacLeod, The extracellular calcium-sensing receptor reciprocally regulates the secretion of BMP-2 and the BMP antagonist Noggin in colonic myofibroblasts, Am. J. Physiol. Gastrointest. Liver Physiol. 292 (2007) G753–G766.
[38] 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.
[39] L. Tang, M. Peng, L. Liu, W. Chang, H.J. Binder, S.X. Cheng, Calcium-sensing receptor stimulates $Cl(-)$- and SCFA-dependent but inhibits cAMP-dependent $HCO_3(-)$ secretion in colon, Am. J. Physiol. Gastrointest. Liver Physiol. 308 (2015) G874–G883.
[40] E.K. Rowinsky, Signal events: cell signal transduction and its inhibition in cancer, Oncologist 8 (Suppl. 3) (2003) 5–17.
[41] J.M. Irish, N. Kotecha, G.P. Nolan, Mapping normal and cancer cell signalling networks: towards single-cell proteomics, Nat. Rev. Cancer 6 (2006) 146–155.
[42] S.C. Brennan, H.C. Mun, K. Leach, P.W. Kuchel, A. Christopoulos, A.D. Conigrave, Receptor expression modulates calcium-sensing receptor mediated intracellular Ca^{2+} mobilization, Endocrinology 156 (2015) 1330–1342.
[43] Y. Huang, Y. Zhou, A. Castiblanco, W. Yang, E.M. Brown, J.J. Yang, Multiple $Ca(2+)$-binding sites in the extracellular domain of the $Ca(2+)$-sensing receptor corresponding to cooperative $Ca(2+)$ response, Biochemistry 48 (2009) 388–398.
[44] J. Hu, A.M. Spiegel, Structure and function of the human calcium-sensing receptor: insights from natural and engineered mutations and allosteric modulators, J. Cell. Mol. Med. 11 (2007) 908–922.
[45] 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 Ca^{2+}-sensing receptor's venus flytrap domain that selectively disables L-amino acid sensing, J. Biol. Chem. 280 (2005) 29067–29072.
[46] S.J. Quinn, M. Bai, E.M. Brown, pH sensing by the calcium-sensing receptor, J. Biol. Chem. 279 (2004) 37241–37249.
[47] S.J. Quinn, O. Kifor, S. Trivedi, R. Diaz, P. Vassilev, E. Brown, Sodium and ionic strength sensing by the calcium receptor, J. Biol. Chem. 273 (1998) 19579–19586.
[48] Y. Huang, G.E. Breitwieser, Rescue of calcium-sensing receptor mutants by allosteric modulators reveals a conformational checkpoint in receptor biogenesis, J. Biol. Chem. 282 (2007) 9517–9525.
[49] A. Aggarwal, M. Prinz-Wohlgenannt, S. Tennakoon, J. Höbaus, C. Boudot, R. Mentaverri, E.M. Brown, S. Baumgartner-Parzer, E. Kállay, The calcium-sensing receptor: a promising target for prevention of colorectal cancer, Biochim. Biophys. Acta 1853 (2015) 2158–2167.
[50] 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.
[51] W.M. Oldham, H.E. Hamm, Heterotrimeric G protein activation by G-protein-coupled receptors, Nat. Rev. Mol. Cell Biol. 9 (2008) 60–71.
[52] A.D. Conigrave, D.T. Ward, Calcium-sensing receptor (CaSR): pharmacological properties and signaling pathways, Best Pract. Res. Clin. Endocrinol. Metab. 27 (2013) 315–331.
[53] 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 Ca^{2+}-sensing receptor, Mol. Endocrinol. 21 (2007) 274–280.
[54] W. Xing, G. Li, Y. Xi, J. Guo, H. Li, H. Li, W. Zhang, L. Zhang, L. Wu, R. Wang, C. Xu, The functional expression of calcium-sensing receptors in BRL cells and related signal transduction pathway responsible for intracellular calcium elevation, Mol. Cell. Biochem. 343 (2010) 13–19.
[55] 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.
[56] J. Cortijo, J. Milara, M. Mata, E. Donet, N. Gavara, S.E. Peel, I.P. Hall, E.J. Morcillo, Nickel induces intracellular calcium mobilization and pathophysiological responses in human cultured airway epithelial cells, Chem. Biol. Interact. 183 (2010) 25–33.
[57] M.E. Handlogten, C. Huang, N. Shiraishi, H. Awata, R.T. Miller, The Ca^{2+}-sensing receptor activates cytosolic phospholipase A2 via a Gqalpha-dependent ERK-independent pathway, J. Biol. Chem. 276 (2001) 13941–13948.
[58] R.V. Thakker, Diseases associated with the extracellular calcium-sensing receptor, Cell Calcium 35 (2004) 275–282.
[59] O. Kifor, R. Diaz, R. Butters, E.M. Brown, The Ca^{2+}-sensing receptor (CaR) activates phospholipases C, A2, and D in bovine parathyroid and CaR-transfected, human embryonic kidney (HEK293) cells, J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res. 12 (1997) 715–725.
[60] P. Sassone-Corsi, The cyclic AMP pathway, Cold Spring Harb. Perspect. Biol. 4 (2012).
[61] J.D. Graves, E.G. Krebs, Protein phosphorylation and signal transduction, Pharmacol. Ther. 82 (1999) 111–121.
[62] H.I. Abdullah, P.L. Pedraza, J.C. McGiff, N.R. Ferreri, CaR activation increases TNF production by mTAL cells via a Gi-dependent mechanism, Am. J. Physiol. Renal Physiol. 294 (2008) F345–F354.
[63] A. Gerbino, W.C. Ruder, S. Curci, T. Pozzan, M. Zaccolo, A.M. Hofer, Termination of cAMP signals by Ca^{2+} and G(alpha)i via extracellular Ca^{2+} sensors: a link to intracellular Ca^{2+} oscillations, J. Cell Biol. 171 (2005) 303–312.
[64] 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.
[65] R. Mamillapalli, J. Wysolmerski, The calcium-sensing receptor couples to Galpha(s) and regulates PTHrP and ACTH secretion in pituitary cells, J. Endocrinol. 204 (2010) 287–297.
[66] T. Miyashita, Y. Kubo, Extracellular Ca^{2+} sensitivity of mGluR1alpha induces an increase in the basal cAMP level by direct coupling with Gs protein in transfected CHO cells, Recept. Channels 7 (2000) 77–91.
[67] A.L. Magno, E. Ingley, S.J. Brown, A.D. Conigrave, T. Ratajczak, B.K. Ward, Testin, a novel binding partner of the calcium-sensing receptor, enhances receptor-mediated Rho-kinase signalling, Biochem. Biophys. Res. Commun. 412 (2011) 584–589.
[68] S.C. Brennan, U. Thiem, S. Roth, A. Aggarwal, I. Fetahu, S. Tennakoon, A.R. Gomes, M.L. Brandi, F. Bruggeman, R. Mentaverri, D. Riccardi, E. Kallay, Calcium sensing receptor signalling in physiology and cancer, Biochim. Biophys. Acta 1833 (2013) 1732–1744.
[69] K. Leach, A.D. Conigrave, P.M. Sexton, A. Christopoulos, Towards tissue-specific pharmacology: insights from the calcium-sensing receptor as a paradigm for GPCR (patho)physiological bias, Trends Pharmacol. Sci. 36 (2015) 215–225.
[70] A.R. Thomsen, S. Smajilovic, H. Brauner-Osborne, Novel strategies in drug discovery of the calcium-sensing receptor based on biased signaling, Curr. Drug Targets 13 (2012) 1324–1335.
[71] E.M. Brown, Mutations in the calcium-sensing receptor and their clinical implications, Horm. Res. 48 (1997) 199–208.
[72] M. Mrgan, S. Nielsen, K. Brixen, Familial hypocalciuric hypercalcemia and calcium sensing receptor, Acta Clin. Croat. 53 (2014) 220–225.
[73] G.N. Hendy, L. D'Souza-Li, B. Yang, L. Canaff, D.E. Cole, Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia, Hum. Mutat. 16 (2000) 281–296.
[74] I.M. Shui, L.A. Mucci, K.M. Wilson, P. Kraft, K.L. Penney, M.J. Stampfer, E. Giovannucci, Common genetic variation of the calcium-sensing receptor and lethal prostate cancer risk, Cancer Epidemiol. Biomark. Prev. 22 (2013) 118–126.

[75] M. Binder, I.M. Shui, K.M. Wilson, K.L. Penney, L.A. Mucci, A.S. Kibel, Calcium intake, polymorphisms of the calcium-sensing receptor, and recurrent/aggressive prostate cancer, Cancer Causes Control (2015).
[76] S. Yan, C. Yuan, Q. Yang, X. Li, N. Yang, X. Liu, R. Dong, X. Zhang, Z. Yuan, N. Zhang, B. Kong, A genetic polymorphism (rs17251221) in the calcium-sensing receptor is associated with ovarian cancer susceptibility, Oncol. Rep. 34 (2015) 2151–2155.
[77] U. Peters, N. Chatterjee, M. Yeager, S.J. Chanock, R.E. Schoen, K.A. McGlynn, T.R. Church, J.L. Weissfeld, A. Schatzkin, R.B. Hayes, Association of genetic variants in the calcium-sensing receptor with risk of colorectal adenoma, Cancer Epidemiol. Biomark. Prev. 13 (2004) 2181–2186.
[78] E.T. Jacobs, M.E. Martinez, P.T. Campbell, D.V. Conti, D. Duggan, J.C. Figueiredo, R.W. Haile, E.C. LeRoy, J.N. Poynter, P.A. Thompson, J.A. Baron, Genetic variation in the retinoid X receptor and calcium-sensing receptor and risk of colorectal cancer in the Colon Cancer Family Registry, Carcinogenesis 31 (2010) 1412–1416.
[79] K.Z. Kim, A. Shin, J. Kim, J.W. Park, S.C. Park, H.S. Choi, H.J. Chang, D.Y. Kim, J.H. Oh, Association between CASR polymorphisms, calcium intake, and colorectal cancer risk, PLoS One 8 (2013), e59628.
[80] L. Masvidal, R. Iniesta, C. Casala, P. Galvan, E. Rodriguez, C. Lavarino, J. Mora, C. de Torres, Polymorphisms in the calcium-sensing receptor gene are associated with clinical outcome of neuroblastoma, PLoS One 8 (2013), e59762.
[81] B. Chakravarti, S.K. Dwivedi, A. Mithal, N. Chattopadhyay, Calcium-sensing receptor in cancer: good cop or bad cop? Endocrine 35 (2009) 271–284.
[82] G.A. Clines, Mechanisms and treatment of hypercalcemia of malignancy, Curr. Opin. Endocrinol. Diabetes Obes. 18 (2011) 339–346.
[83] J.J. Wysolmerski, Parathyroid hormone-related protein: an update, J. Clin. Endocrinol. Metab. 97 (2012) 2947–2956.
[84] J.L. Sanders, N. Chattopadhyay, O. Kifor, T. Yamaguchi, E.M. Brown, Extracellular calcium-sensing receptor (CaR) expression and its potential role in parathyroid hormone-related peptide (PTHrP) secretion in the H-500 rat Leydig cell model of humoral hypercalcemia of malignancy, Biochem. Biophys. Res. Commun. 269 (2000) 427–432.
[85] J. Tfelt-Hansen, R.J. MacLeod, N. Chattopadhyay, S. Yano, S. Quinn, X. Ren, E.F. Terwilliger, P. Schwarz, E.M. Brown, Calcium-sensing receptor stimulates PTHrP release by pathways dependent on PKC, p38 MAPK, JNK, and ERK1/2 in H-500 cells, Am. J. Physiol. Endocrinol. Metab. 285 (2003) E329–E337.
[86] N. Chattopadhyay, Effects of calcium-sensing receptor on the secretion of parathyroid hormone-related peptide and its impact on humoral hypercalcemia of malignancy, Am. J. Physiol. Endocrinol. Metab. 290 (2006) E761–E770.
[87] J. Tfelt-Hansen, N. Chattopadhyay, S. Yano, D. Kanuparthi, P. Rooney, P. Schwarz, E.M. Brown, Calcium-sensing receptor induces proliferation through p38 mitogen-activated protein kinase and phosphatidylinositol 3-kinase but not extracellularly regulated kinase in a model of humoral hypercalcemia of malignancy, Endocrinology 145 (2004) 1211–1217.
[88] J. Tfelt-Hansen, P. Schwarz, E.F. Terwilliger, E.M. Brown, N. Chattopadhyay, Calcium-sensing receptor induces messenger ribonucleic acid of human securin, pituitary tumor transforming gene, in rat testicular cancer, Endocrinology 144 (2003) 5188–5193.
[89] G.R. Mundy, Metastasis to bone: causes, consequences and therapeutic opportunities, Nat. Rev. Cancer 2 (2002) 584–593.
[90] T. Guise, Examining the metastatic niche: targeting the microenvironment, Semin. Oncol. 37 (Suppl. 2) (2010) S2–14.
[91] 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.
[92] M.A. Hernandez-Bedolla, J. Carretero-Ortega, M. Valadez-Sanchez, J. Vazquez-Prado, G. Reyes-Cruz, Chemotactic and proangiogenic role of calcium sensing receptor is linked to secretion of multiple cytokines and growth factors in breast cancer MDA-MB-231 cells, Biochim. Biophys. Acta 1853 (2015) 166–182.
[93] J. Feng, X. Xu, B. Li, E. Brown, A.B. Farris, S.Y. Sun, J.J. Yang, Prostate cancer metastatic to bone has higher expression of the calcium-sensing receptor (CaSR) than primary prostate cancer, Recept. Clin. Investig. 1 (2014).
[94] E. Joeckel, T. Haber, D. Prawitt, K. Junker, C. Hampel, J.W. Thuroff, F.C. Roos, W. Brenner, High calcium concentration in bones promotes bone metastasis in renal cell carcinomas expressing calcium-sensing receptor, Mol. Cancer 13 (2014) 42.
[95] E. Rozengurt, Mitogenic signaling pathways induced by G protein-coupled receptors, J. Cell. Physiol. 213 (2007) 589–602.
[96] L. McNeil, S. Hobson, V. Nipper, K.D. Rodland, Functional calcium-sensing receptor expression in ovarian surface epithelial cells, Am. J. Obstet. Gynecol. 178 (1998) 305–313.
[97] S.A. Hobson, J. Wright, F. Lee, S.E. McNeil, T. Bilderback, K.D. Rodland, Activation of the MAP kinase cascade by exogenous calcium-sensing receptor, Mol. Cell. Endocrinol. 200 (2003) 189–198.
[98] F. Asadi, M. Faraj, S. Malakouti, S.C. Kukreja, Effect of parathyroid hormone related protein, and dihydrotestosterone on proliferation and ornithine decarboxylase mRNA in human prostate cancer cell lines, Int. Urol. Nephrol. 33 (2001) 417–422.
[99] 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.
[100] W.M. Ongkeko, D. Burton, A. Kiang, E. Abhold, S.Z. Kuo, E. Rahimy, M. Yang, R.M. Hoffman, J. Wang-Rodriguez, L.J. Deftos, Parathyroid hormone related-protein promotes epithelial-to-mesenchymal transition in prostate cancer, PLoS One 9 (2014), e85803.
[101] H. Zhang, C. Yu, J. Dai, J.M. Keller, A. Hua, J.L. Sottnik, G. Shelley, C.L. Hall, S.I. Park, Z. Yao, J. Zhang, L.K. McCauley, E.T. Keller, Parathyroid hormone-related protein inhibits DKK1 expression through c-Jun-mediated inhibition of beta-catenin activation of the DKK1 promoter in prostate cancer, Oncogene 33 (2014) 2464–2477.
[102] C. Huang, S. Liu, R.T. Miller, Role of p115RhoGEF in the regulation of extracellular Ca(2+)-induced choline kinase activation and prostate cancer cell proliferation, Int. J. Cancer 128 (2011) 2833–2842.
[103] J.N. Vanhouten, J.J. Wysolmerski, The calcium-sensing receptor in the breast, Best Pract. Res. Clin. Endocrinol. Metab. 27 (2013) 403–414.
[104] 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.
[105] X. Li, L. Li, M.S. Moran, L. Jiang, X. Kong, H. Zhang, X. Zhang, B.G. Haffty, Q. Yang, Prognostic significance of calcium-sensing receptor in breast cancer, Tumour Biol. 35 (2014) 5709–5715.
[106] M.L. McCullough, C. Rodriguez, W.R. Diver, H.S. Feigelson, V.L. Stevens, M.J. Thun, E.E. Calle, Dairy, calcium, and vitamin D intake and postmenopausal breast cancer risk in the Cancer Prevention Study II Nutrition Cohort, Cancer Epidemiol. Biomark. Prev. 14 (2005) 2898–2904.
[107] F. Journe, J.C. Dumon, N. Kheddoumi, J. Fox, I. Laios, G. Leclercq, J.J. Body, Extracellular calcium downregulates estrogen receptor alpha and increases its transcriptional activity through calcium-sensing receptor in breast cancer cells, Bone 35 (2004) 479–488.
[108] 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.
[109] 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.
[110] 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.
[111] 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.
[112] 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.
[113] 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.
[114] 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.
[115] 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.
[116] 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.
[117] S. Varshney, S.K. Bhadada, N. Sachdeva, A.K. Arya, U.N. Saikia, A. Behera, S.D. Rao, Methylation status of the CpG islands in vitamin D and calcium-sensing receptor gene promoters does not explain the reduced gene expressions in parathyroid adenomas, J. Clin. Endocrinol. Metab. 98 (2013) E1631–E1635.
[118] L. Sulaiman, C.C. Juhlin, I.L. Nilsson, O. Fotouhi, C. Larsson, J. Hashemi, Global and gene-specific promoter methylation analysis in primary hyperparathyroidism, Epigenetics 8 (2013) 646–655.
[119] 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.
[120] S. Fabbri, S. Ciuffi, V. Nardone, A.R. Gomes, C. Mavilia, R. Zonefrati, G. Galli, E. Luzi, A. Tanini, M.L. Brandi, PTH-C1: a rat continuous cell line expressing the parathyroid phenotype, Endocrine 47 (2014) 90–99.
[121] C. de Torres, H. Beleta, R. Diaz, N. Toran, E. Rodriguez, C. Lavarino, I. Garcia, S. Acosta, M. Sunol, J. Mora, The calcium-sensing receptor and parathyroid hormone-related protein are expressed in differentiated, favorable neuroblastic tumors, Cancer 115 (2009) 2792–2803.
[122] 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 34 (2013) 268–276.
[123] 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.
[124] L. Gama, L.M. Baxendale-Cox, G.E. Breitwieser, Ca^{2+}-sensing receptors in intestinal epithelium, Am. J. Physiol. 273 (1997) C1168–C1175.
[125] 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.
[126] 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.

[127] I.S. Fetahu, J. Hobaus, A. Aggarwal, D.M. Hummel, S. Tennakoon, I. Mesteri, S. Baumgartner-Parzer, E. Kallay, Calcium-sensing receptor silencing in colorectal cancer is associated with promoter hypermethylation and loss of acetylation on histone 3, Int. J. Cancer 135 (2014) 2014–2023.
[128] 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.
[129] R.R. Buras, M. Shabahang, F. Davoodi, L.M. Schumaker, K.J. Cullen, S. Byers, R.J. Nauta, S.R. Evans, The effect of extracellular calcium on colonocytes: evidence for differential responsiveness based upon degree of cell differentiation, Cell Prolif. 28 (1995) 245–262.
[130] 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.
[131] I.S. Fetahu, S. Tennakoon, K.E. Lines, C. Groschel, A. Aggarwal, I. Mesteri, S. Baumgartner-Parzer, R.M. Mader, R.V. Thakker, E. Kallay, miR-135b- and miR-146b-dependent silencing of calcium-sensing receptor expression in colorectal tumors, Int. J. Cancer 138 (2016) 137–145.
[132] J.J. Ye, J. Cao, MicroRNAs in colorectal cancer as markers and targets: recent advances, World J. Gastroenterol. 20 (2014) 4288–4299.
[133] T. Kunej, I. Godnic, S. Horvat, M. Zorc, G.A. Calin, Cross talk between microRNA and coding cancer genes, Cancer J. 18 (2012) 223–231.
[134] N. Singh, S. Chakrabarty, Induction of CaSR expression circumvents the molecular features of malignant CaSR null colon cancer cells, Int. J. Cancer 133 (2013) 2307–2314.
[135] N. Singh, G. Liu, S. Chakrabarty, Isolation and characterization of calcium sensing receptor null cells: a highly malignant and drug resistant phenotype of colon cancer, Int. J. Cancer 132 (2013) 1996–2005.
[136] O. Rey, W. Chang, D. Bikle, N. Rozengurt, S.H. Young, E. Rozengurt, Negative cross-talk between calcium-sensing receptor and β-catenin signaling systems in colonic epithelium, J. Biol. Chem. 287 (2012) 1158–1167.
[137] R.J. MacLeod, Extracellular calcium-sensing receptor/PTH knockout mice colons have increased Wnt/β-catenin signaling, reduced non-canonical Wnt signaling, and increased susceptibility to azoxymethane-induced aberrant crypt foci, Lab. Investig. 93 (2013) 520–527.
[138] S.X. Cheng, Y.L. Lightfoot, T. Yang, M. Zadeh, L. Tang, B. Sahay, G.P. Wang, J.L. Owen, M. Mohamadzadeh, Epithelial CaSR deficiency alters intestinal integrity and promotes proinflammatory immune responses, FEBS Lett. (2014).
[139] A. Aggarwal, M. Prinz-Wohlgenannt, C. Groschel, S. Tennakoon, A. Meshcheryakova, W. Chang, E.M. Brown, D. Mechtcheriakova, E. Kallay, The calcium-sensing receptor suppresses epithelial-to-mesenchymal transition and stem cell-like phenotype in the colon, Mol. Cancer 14 (2015) 61.
[140] 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.
[141] A. Aggarwal, J. Höbaus, S. Tennakoon, M. Prinz-Wohlgenannt, J. Graça, S.A. Price, P. Heffeter, W. Berger, S. Baumgartner-Parzer, E. Kállay, Active vitamin D potentiates the anti-neoplastic effects of calcium in the colon: a cross talk through the calcium-sensing receptor, J. Steroid Biochem. Mol. Biol. (2015).
[142] C.H. Tae, K.N. Shim, H.I. Kim, Y.H. Joo, J.H. Lee, M.S. Cho, C.M. Moon, S.E. Kim, H.K. Jung, S.A. Jung, Significance of calcium-sensing receptor expression in gastric cancer, Scand. J. Gastroenterol. (2015) 1–6.
[143] D. Hummel, A. Aggarwal, K. Borka, E. Bajna, E. Kallay, H.C. Horvath, The vitamin D system is deregulated in pancreatic diseases, J. Steroid Biochem. Mol. Biol. (2014) 402–409 (144 Pt B).
[144] G.Z. Racz, A. Kittel, D. Riccardi, R.M. Case, A.C. Elliott, G. Varga, Extracellular calcium sensing receptor in human pancreatic cells, Gut 51 (2002) 705–711.
[145] D. Hanahan, R.A. Weinberg, The hallmarks of cancer, Cell 100 (2000) 57–70.
[146] E.M. Brown, Clinical utility of calcimimetics targeting the extracellular calcium-sensing receptor (CaSR), Biochem. Pharmacol. 80 (2010) 297–307.
[147] A. Mingione, C. Verdelli, A. Terranegra, L. Soldati, S. Corbetta, Molecular and Clinical Aspects of the Target Therapy with the Calcimimetic Cinacalcet in the Treatment of Parathyroid Tumors, Curr. Cancer Drug Targets 15 (2015) 563–574.
[148] E.F. Nemeth, D. Shoback, Calcimimetic and calcilytic drugs for treating bone and mineral-related disorders, Best Pract. Res. Clin. Endocrinol. Metab. 27 (2013) 373–384.