MINI-REVIEW

Calcium Sensing Receptor Modulation for Cancer Therapy

Puja Sarkar, Sudhir Kumar*

Abstract

　　The calcium sensing receptor (CaSR) is a member of the largest family of cell surface receptors, the G protein-coupled receptors involved in calcium homeostasis. The role of the CaSR in neoplasia appears to be homeostatic; loss of normal CaSR-induced response to extracellular calcium is observed in cancers of the colon and ovary, while increased release of PTHrP is observed in cancers of the breast, prostate and Leydig cells. Currently CaSR can be considered as a molecule that can either promote or prevent tumor growth depending on the type of cancer. Therefore, recognition of the multifaceted role of CaSR in gliomas and other malignant tumors in general is fundamental to elucidating the mechanisms of tumor progression and the development of novel therapeutic agents. Emphasis should be placed on development of drug-targeting methods to modulate CaSR activity in cancer cells.

Keywords: CaSR - GPCR - metastasis - PTHrP - homeostasis - cancer cells

Introduction

In all living cells, calcium is required to maintain the normal structure and function (Barrett et al., 1986). Calcium signaling is also important for cell adhesion and mortality (Capiod et al., 2007). Certainly all events are integral to normal cell function, and disruption in calcium signaling is a well established feature in the generation and progression of cancer (Al-Haddad et al., 1999). The calcium sensing receptors (CaSR) are responsive to the changes in the extracellular Ca^{2+} concentration (Saidak et al., 2009). The enhanced expression of CaSR expression and altered proliferation of prostate cancer cells in response to increased Ca^{2+} have also been described(George et al., 2011), in contrast colon and parathyroid cancers often present with reduced or absent CaSR expression, hence decrease in cell proliferation, suggesting a role for the CaSR as a tumor suppressor gene (Saidak et al., 2009). The CaSR are expressed in both normal as well as malignant cells (Saidak et al., 2009), increased expression have been reported in highly metastatic primary tumor contributing to the oncogenic properties of CaSR(Schneider et al., 2005). Thus, the CaSR may play an important role in the development of various benign and malignant tumors. An analysis of intracellular signaling pathways revealed a key role for CaSR-induced phospholipase C activation and the release of intracellular calcium (Tharmalingam et al., 2011). These results demonstrated for the first time that an ion sensing G protein coupled receptor functionally couples to the integrins, and in conjunction with intracellular calcium release, promotes cellular adhesion

and migration in tumor cells. Herein, we review the role of calcium sensing receptor (CaSR) in the development and metastasis of cancer and manipulation of the receptor for the treatment of cancers in the future.

Calcium Sensing Receptor

The calcium sensing receptor (CaSR) is widely expressed homodimeric G protein-coupled receptor (GPCR) that functions as multi-modal nutrient sensor to maintain systemic calcium homeostasis by sensing changes in extracellular Ca^{2+} concentration (Ward and Riccardi, 2012). Ca^{2+} plays an essential role in numerous physiological processes, including blood clotting (Chattopadhyay et al., 1996), neuromuscular excitability (Chattopadhyay et al., 1996), and maintenance of skeletal integrity (Chattopadhyay et al., 1996). It has been known for years that Ca^{2+} selective ion channels enable calcium ion to move across the cell membrane (Brown and MacLeod, 2001). Most of the hormone receptors are activated by nanomolar to picomolar concentration of their agonists (Rodland, 2004). In contrast, the CaSR receptor is sensitive to relatively small changes in Ca^{2+} concentration in the extracellular fluid (Rodland, 2004).

In 1993, a membrane–spanning Ca^{2+} sensing receptor, containing 1085 amino acids was cloned from bovine parathyroid, and it was shown to belong to the GPCR superfamily (Brown et al., 1993). The structure of CaSR , like of other GPCR superfamily, consists of seven transmembane helices, an extracellular N terminal and an intracellular C terminal (Brown et al., 1993). The CaSR

*IGNOU-I²IT Centre of Excellence for Advanced Education and Research, Pune, Maharashtra, India *For correspondence: sudhirk@ isquareit.ac.in*

belongs to the family 3 (or C) of the GPCR, which also includes the metabotropic glutamate receptor (mGluR1-8), γ-aminobutyric acid receptor subunits (GABAB1 and GABAB2), sweet and umami taste receptor (T1R1, T1R2, and T1R3), as well as pheromone and seven orphan receptor, and is characterized by a large extracellular N-terminal ligand-binding domain that possesses structural similarity to venus flytrap domain motif of bacterial periplasmic binding protein (Pin et al., 2003). There is evidence that the CaSR may exist as a dimer linked by disulfide bonds on the surface of certain cells, moreover the dimer is more sensitive to changes in calcium and other agonists than the monomeric form (Ray et al., 1999). When the CaR was first cloned from the bovine parathyroid, it was identified as the cation-sensing receptor that is responsible for the sensitivity of the parathyroid chief cells to changes in Ca^{2+} (Brown et al., 1993) and it was suggested to be the primary player in Ca^{2+} homeostasis (Breitwieser, 2012). However, it has been shown that the CaSR is also expressed by various cells involved in Ca^{2+} homeostasis, such as the brain (Ruat et al., 1995), lens epithelial cells (Chattopadhyay et al., 1997), pancreas (Squires et al., 2000) and the antral gastrin-secreting cells of the stomach (Ray et al., 1997).

CaSR and Hematopoietic Stem Cell Niche

Since 1978 when Shofield and colleagues proposed the hematopoietic stem cell (HSCs) reside in a highly specific niche that regulates the overall size of the pool and their development (Holmbeck et al., 2003). Adult hematopoietic stem cell resides within the bone marrow in close association with the endosteal surface of bone (Nilsson et al., 2001). The ability of hematopoietic stem cells (HSCs) to maintain an undifferentiated state and undergo self-renewal is partly regulated by external signals originating from the stem cell niche (House et al., 1997). One receptor expressed on HSCs that is known to be involved in HSC niche biology is the calcium-sensing receptor (Drueke, 2006). During mammalian hematopoiesis, HSCs shifts from fetal liver to the bone morrow shortly after the onset of bone mineralization (Cumano and Godin, 2007). HSCs obtained from the fetal liver of mice are deficient in CaSR has shown the crucial role of CaSR in HSC lodgment and engraftment in the bone.

Osteopontin, an acidic glycoprotein produced by osteoblasts is highly enriched at the endosteal surface and was also shown necessary for the HSC lodgment (Haylock and Nilsson, 2006; Nilsson et al., 2005). The endosteal niche has uniquely enriched calcium content relative to serum, CaSR expression has been demonstrated on HSCs, including the stem cell-enriched (lin⁻) Sca-1⁺ c-Kit⁺(LSK) population (Adams et al., 2006). Finally, in a series of elegant experiments using CaSR-/- mice, Adams et al demonstrated that it is the mineral content of the niche dictates HSC localization via the CaSR(Adams et al., 2006). These mechanisms by which the CaR dictates preferential localization of HSCs in the bone marrow endosteal region may provide additional insights for the fundamental interrelationship between the stem cell niche and stem cell fate (Theman and Collins, 2009).

CaSR and Cancer

The CaSR has recently been documented to be expressed in a variety of benign tumor and malignancies, often at expression levels that differ from those in their healthy counterparts like in breast cancer, prostate cancer, as well as in cancers originating from organs involved in Ca^{2+} homeostasis, including colorectal cancer and parathyroid adenomas. A recent examination of publicly available gene expression data identified a variety of types of GPCRs (Dores and Trejo, 2012), including protease activated receptor and receptors for various chemokines, adenosine 2B, neuropeptide, metabotropic glutamate, and CaSR that are overexpressed in diverse type of cancer (Rozengurt, 2007) (Figure 1).

CaSR and Metastasis

According to "Seed and Soil" hypothesis formulated by Stephen Paget suggests the high incidence of tumor metastasis in bone, while bone is inhospitable environment for tumor growth (Cumano and Godin, 2007). Bone extracellular matrix (ECM) serves as the reservoir of growth factors such as insulin like growth factor, fibroblastic growth factor, bone morphogenetic proteins, and transforming growth factor β (TGF-β) (Hofer and Brown, 2003). Osteolysis releases growth factors and calcium autocrine signaling for the tumor growth and invasion (Kingsley et al., 2007).

CaSR is expressed on normal mammary epithelial and response to low Ca^{2+} by increasing production of PTHrP production (VanHouten et al., 2004). For further investigation Liao et al incubated three prostate cancer cell lines that have different tumorigenic and metastatic potential in low versus high calcium media (Liao et al., 2006). Knockdown of CaSR by RNA interference decreased the tumor metastatic potential (Dvorak-Ewell et al., 2011). CaSR activation increased attachment of cells to extracellular matrix (Sanders et al., 2001). Increased adherence was reduced by the addition of pertussis toxin, a CaSR antagonist (Theman and Collins, 2009). CaSR signals, in part, through MAP kinase to stimulate PTHrP (Chattopadhyay, 2006). Blocking CaSR induced MAP kinase pathway elements inhibited calcium-induced PTHrP release (Chattopadhyay, 2006). TGF-β, one of
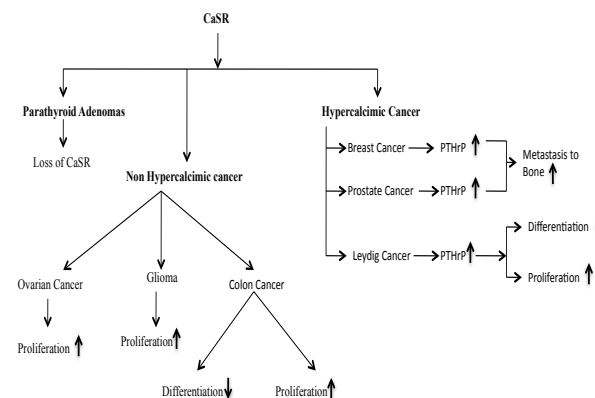

Figure 1. Summary of Various Functions of Casr in Different Types of Cancer Cells.

the growth factors released from bone matrix, also acts synergistically with CaSR agonists to stimulate PTHrP production (Sanders et al., 2000). Tumor cells express a number of "bone specific" markers including CaSR, osteopontin, osteocalcin and bone sialoprotein (Koeneman et al., 1999; Bellahcene et al., 2008). The chemokine CXCL12/SDF-1, which is highly expressed in bone marrow may also be important (Adams et al., 2006). CaSR-/-HSCs successfully localized to the endosteal niche using a SDF-1 gradient, though they could not lodge, suggesting that CaSR functions downstream of SDF-1 in HSC homing to bone. Blocking of SDF-1 signaling reduced metastasis (Theman and Collins, 2009).

CaSR in Parathyroid Adenoma

The primary site of CaSR regulation is the chief cells of parathyroid gland, where CaSR an increase in blood calcium concentration into decreased parathromone secretion (Rodland, 2004). CaSR protein and mRNA are more abundantly expressed in the parathyroid than in most other tissues (Rodland, 2004). Therefore, it is logical to examine CaSR expression in parathyroid adenomas (Liu et al., 2011). Under normal physiological conditions, one of the functions influenced by CaSR is cellular proliferation (Saidak et al., 2009). Thus CaSR in parathyroid may serve as tumor suppressor gene by virtue of its ability to suppress parathyroid cell growth. Recently, it has been reported about the human CaSR gene, which is encoded by seven exons, was shown to have two promoters and two 5' untranslated exons, and the alternative utilization of exon 1A abd 1B leads to different mRNA (Chikatsu et al., 2000). The expression of exon 1A, contains TATA and CAAT boxes, is reduced in parathyroid adenomas and is expressed at levels only 60% of those in normal glands, whereas of exon 1B is not different between adenomas and normal glands (Garrett et al., 1995; Chikatsu et al., 2000). However, this finding does suggest that CaSR exon 1A is regulated differently than the exon 1B transcripts, and may be correlated with the ability of CaSR to inhibit proliferation in the parathyroid cells. The reduced expression of exon 1A in parathyroid cells demonstrates an alternation that may contribute to tumorigenesis. CaSR expression was decreased primarily in areas of active cell proliferation (Sanders et al., 2001).

The mechanism behind CaSR activation leading to reduced parathyroid cell proliferation is not completely understood and the intracellular pathways involved still remain to be elucidated. One possible candidate in parathyroid cancer that as an oncogene is Cyclin D1, having a role in parathyroid cell proliferation and dysregulated PTH secretion (Motokura et al., 1991). The gene of cyclinD1 is under the influence of regulatory region of PTH gene in parathyroid adenomas, as a result of chromosomal traslocation there is over expression (Arnold et al., 1989). Corbetta et al. have demonstrated that EGF and FGF induced increases in cyclin D1 expression and ERK1/2 phosphorylation were inhibited by CaSR agonists in parathyroid adenomas. This shows that expression of cyclin D1 (Imanishi et al., 2012) of parathyroid cells is modulated by CaSR activation in presence of the growth factors mimicking normal physiological conditions. In absence of growth factors the CaSR activation was not observed. It strongly suggests that there is transactivation between CaSR and the growth factor receptors which is reported in other cell lines (Sanders et al., 2001; Yano et al., 2004). Thus it can be concluded that the activation of CaSR may play an inhibitory role in parathyroid tumorigenesis through cyclin D1 expression. Due to the down regulation of CaSR in many thyroid adenomas, the normal inhibitory effect of parathyroid cells is not able to proceed, leading to detrimental events promoting the development of parathyroid tumors.

CaSR in Colon Cancer

Calcium in case of colon cancer is considered as a chemopreventive agent (Garland et al., 1985; Lipkin, 1999; Kampman et al., 2000; Wargovich et al., 2000). Most epidemiological studies have reported that the colon cancer is inversely related to the dietary Ca^{2+} consumption (Garland et al., 1985; Sellers et al., 1998; Kampman et al., 2000; Wu et al., 2002; Lamprecht and Lipkin, 2003). In vitro studies have demonstrated that maintenance of human colon carcinoma cells in Ca^{2+} free medium results in increased proliferation of cells that are loosely attached to the substratum. Conversely elevated Ca^{2+} decreases the rate of growth, and the cells take on a flattened appearance and behave as a cohesive epithelial unit(Bhagavathula et al., 2005). Studies conducted by Cross et al. have also demonstrated the proliferative potential is inversely proportional to Ca^{2+} concentration in case of intestinal Caco-2 cells . The expression of CaSR in colon is not unexpected because numerous other CaSR agonists occur naturally in the colon, such as polyamines, suggestive of a physiological function of the CaSR. In the healthy gut, a gradient of CaSR expression exists in the colonic crypts, and rapidly proliferating epithelial cells at the bottom of the crypt do not express the CaSR, whereas cells in the middle and top of the crypt do express this receptor, with the highest level of expression at the top. Cells of the colonic crypt have been shown to acquire the CaSR expression as they differentiate and move towards the apex of the crypt (Chakrabarty et al., 2005). Thus it is well established that Ca^{2+} concentration is a direct modulator of colonocyte proliferation and differentiation. This Ca^{2+} concentration gradient was hypothesized to be responsible for the differential CaSR expression, as well as the enhanced differentiation and decreased proliferative activity of the cells from the bottom to top of the crypt.

Chakraborty et al. have shown that Ca^{2+} ion acting on the CaSR, promotes the expression of E-cadherin in several human colon carcinoma cell lines, leading to suppression of their malignant properties (Chakrabarty et al., 2003). E-cadherin belongs to the superfamily of intercellular adhesion molecules expressed by the intestinal epithelial cells. Dysregulation of E-cadherin played an important role in the transition from adenoma to carcinoma (Birchmeier and Behrens, 1994; Birchmeier, 1995) leading to increased metastasis and invasiveness (Oka et al., 1993; Pignatelli et al., 1994). In rapidly proliferating epithelial cells the E-cadherin expression

is very low, at times of differentiation it is up- regulated (Birchmeier and Behrens, 1994). The tumor-suppressing effects of E-cadherin are believed to be produced through its interaction with β-catenin, a protooncogene (Morin, 1999), which is a member of the Wnt pathway family (Moon et al., 1997). E-cadherin along with β-catenin forms a complex which is then linked to the actin-based cytoskeleton (Brembeck et al., 2006). When it is present as a complex at the cell surface, tissues acts as cohesive unit, otherwise the single cells are able to move and invade surrounding tissues (Kuroda et al., 1998). The activity of β-catenin (Rey et al., 2012) is controlled by E-cadherin by sequestering the protein from cytoplasm to the cell membrane, thus limiting its availability for signaling, hence antagonizing its functions (Fagotto et al., 1996). Activation of CaSR by Ca^{2+} leads to decreased binding of β-catenin to TCF4 and suppression of this malignancy-promoting pathway (Chakrabarty et al., 2003). These finding suggest that induction of E-cadherin expression and suppression of the β-catenin/TCF4 pathway may contribute to the chemopreventive action of Ca^{2+} in colon cancer through activation of the CaSR (Chakrabarty et al., 2003).

A downstream event following the activation β-catenin/TCF4 stimulates the proto-oncogenes, c-*myc* and cyclin D1 (Tetsu and McCormick, 1999; Wong and Pignatelli, 2002). Bhagavathula et al. (2007) demonstrated relationship between activation of CaSR and cell surface β-catenin localization on one hand and reduced expression of c-*myc* and cyclin D1 on the other, suggesting that the quiescence induced by Ca^{2+} may be produced through inhibition of β-catenin/TCF/c-*myc*/cyclin D1 sequence of events. Apart from these the association of β- catenin with TCF family of transcription factor leads to increased expression of MMP7 and γ-catenin, which have role in development and progression of colon cancer (Wong and Pignatelli, 2002). Additionally, Ca^{2+} has been seen to activate the cyclin-dependent kinase inhibitors p21 and p27, which potently induce differentiation in intestinal epithelial cells (Quaroni et al., 2000; Deschenes et al.,

Figure 2. The Role of E-cadherin and the β-catenin / TCF4 Pathway in CaSR Mediated Effects in Colon Cancer

2001). The expression of p21 and p27 is lost in the early stages of colon tumorigenesis is observed along with the reduction of CaSR expression (Polyak et al., 1996). (Figure 2)

CaSR in Prostate and Breast Cancer

Prostate and breast cancer are the two most frequently occurring cancers after lung cancer in men and women respectively, which cause death. Both prostate and breast cancer preferentially metastasize to bone (Coleman, 1997). Approximately 75% of patients who develop advanced breast cancer will have secondary tumors in the bone with the majority being osteolytic, while in case of prostate cancer about 90% of patients die of advanced prostate cancer develop bone metastases (Roodman, 2004). The preferential metastasis of prostate and breast cancer to bone indicates that bone microenvironment provides a favorable niche (Liao et al., 2006). Multiple growth factors favorable chemotactic and growth promoting functions required for tumor localization and owing to bone turnover by remodeling in the skeleton such as the femur, pelvis, and humerus (Eaton and Coleman, 2003; Schneider et al., 2005).

Due to higher remodeling rate trabecular bone are more active than the cortical bone. Ca^{2+} is one of the major inorganic factors released as a result of bone remodeling (Parkinson and Fazzalari, 2003). This may lead to numerous adverse effects like sever pain, spinal cord compression, higher risk of fractures and hypercalcemia (Chakravarti et al., 2009). Therefore, CaSR can serve as a major target of Ca^{2+} in facilitating the formation and growth of skeletal metastasis of prostate and breast cancer. CaSR are expressed in osteoblasts, osteoclasts (Fukumoto, 1998) and its precursor cells, which can provide groundwork for local cross-talk in event that cancer cells localized in the bone (Sun et al., 2005). Chemoattractant likes TGF-β, IGF-I, IGF-II and platelet derived growth factor are believed to attract cancer cells to their metastatic location and increase their survival and proliferation (Imbriaco et al., 1998; Yin et al., 1999; Greenlee et al., 2001; Morony et al., 2001; Neudert et al., 2003; Yin et al., 2005; Cicek and Oursler, 2006; Le Gall et al., 2007; Zheng et al., 2007). During bone formation these factors are released by bone resorption thereby contributing to the propensity of the prostate and breast cancer to metastasis.

Another important contributor is the PTHrP, which initiates the vicious circle of metastatic breast and prostate cancer in bone (Grill et al., 1991). In normal cells of breast and prostate the secretion of PTHrP is monitored by the CaSR, they are antagonist in nature (Saidak et al., 2009). Recently, it was shown that inhibition and stimulation of PTHrP occurs due to the activation of G-protein by the cancerous cells (Mamillapalli et al., 2008). In the normal condition the CaSR couples to $G_{\alpha i}$, thus inhibition of cAMP formation and consequently PTHrP secretion, whereas in cancerous cells the CaSR activates $G_{\alpha s}$ thus promotes to PTHrP release (Sanders et al., 2000; Yano et al., 2004). Elevated Ca^{2+} level in serum activate the CaSR, produces additional release of PTHrP
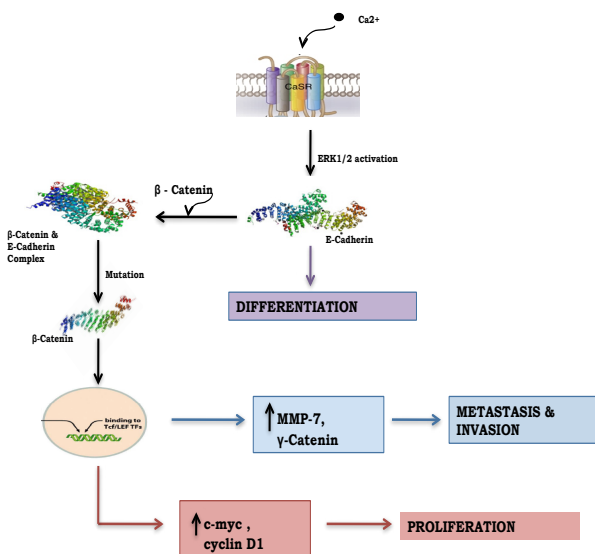
from cancerous cells which stimulates osteoclastogenesis (Sanders et al., 2000). Activated CaSR transactivate the epidermal growth factor receptor, which is then followed by ERK1/2 activation, implying that the transactivation is mediated by the activation of MMP (Prenzel et al., 1999; Gschwind et al., 2001). The mechanism behind activation of TGF-β is unclear but it might be involved in upregulation of CaSR expression or its signaling pathways thereby increasing the amount of PTHrP. PTHrP stimulates osteoclastogenesis by increasing the expression of RANKL on osteoblasts, thereby stimulating the formation of mature osteoclasts (Roodman, 2001). This is how the PTHrP contributes to the increased bone resorption and releases the chemoattractants like TGF-β, IGF-I, IGF-II and platelet derived factor implicating in the induction and development of the vicious circle (Sanders et al., 2000) (Figure 3).

CaSR in Other Cancers

Angiogenesis is one of the six hallmarks of cancer. Leydig tumor cells exhibit high level of angiogenesis (Tfelt-Hansen et al., 2003). CaSR activation enhances new blood vessel formation via the production of angiogenic factor bFGF. Increased Ca^{2+} concentration regulates VEGFR which stimulates nitrix oxide systhesis, this promotes neovascularization in tumors (Lala and Chakraborty, 2001). Again it has been seen that high Ca^{2+} produced, CaSR mediated stimulation of proliferation as well as resistance to apoptosis. Thus it not only enhances tumor growth but also increases invasiveness and metastasis (Cheng et al., 1998; Thomsen and Miles, 1998).

It has been known that the fibroblastic cells require Ca^{2+} rich media for their proliferation while epithelial cells require less Ca^{2+} for optimal proliferation and differentiation (Hennings et al., 1980a: 1980b). The surface epithelium of the ovary is contiguous with the mesothelial lining of the peritoneum. Thus the ovarian surface epithelial (OSE) cells are actually derived from a mesodermal lineage (Auersperg et al., 1998; Auersperg et al., 1994). Increasing extracellular calcium from 0.2mM to 5mM has a marked proliferative response in normal ovarian surface epithelial cells (Saxena et al., 2012). CaSR may mediate the growth of human ovarian surface epithelial cells by extracellular calcium (McNeil et al., 1998).
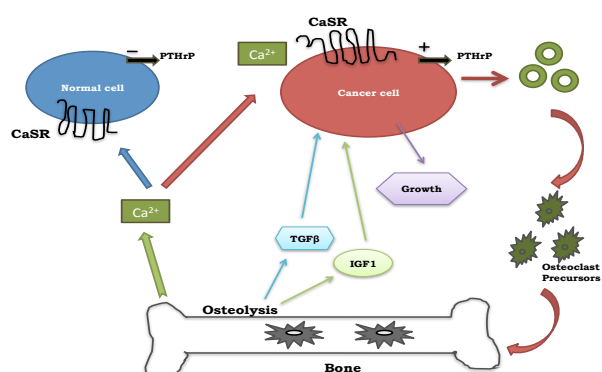

Figure 3. The Role of CaSR in Normal As Well As in Cancerous Cells.

Glioma is a collective term for glial cell-derived tumor, including astrocyte, oligodendrocyte and ependymal cell derived tumors. Gliomas are the most common primary neoplasm of the central nervous system (Chattopadhyay et al., 1998). The activation of CaSR via Ca^{2+} in turn activates the K^+channel which results in cell proliferation and secretion of PTHrP (Chattopadhyay et al., 1999). It generally shows imbalance or disturbance in calcium homeostasis (Bandyopadhyay et al., 2010). The expression of the CaSR in glial cells indicates potential roles in the maintenance of local ionic homeostasis as well as in tumor formation.

Conclusion

CaSR is neither a potent oncogene nor tumor supressor, it does play an important role in Ca^{2+} homeostasis, which indirectly maintains a balance between proliferation and differentiation in response to change in extra cellular calcium level. Hence there should be a balanced intake of calcium rich diet, like patients suffering from colon or ovarian cancer should increase the calcium intake, while patients with prostate or breast cancer should decrease the intake of calcium rich food.

CaSR can be considered as a molecule that can either promote or prevent tumor growth depending on the type of cancer. Targeting of receptor based on the cancer may offer a cure in designing new therapies, which might complement the existing therapies.

Acknowledgement

References

Adams GB, Chabner KT, Alley IR, et al (2006). Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. *Nature*, **439**, 599-3.

Al-Haddad S, Zhang Z, Leygue E, et al (1999). Psoriasin (S100A7) expression and invasive breast cancer. *Am J Pathol*, **155**, 2057-6.

Arnold A, Kim HG, Gaz RD, et al (1989). Molecular cloning and chromosomal mapping of DNA rearranged with the parathyroid hormone gene in a parathyroid adenoma. *J Clin Invest*, **83**, 2034-0.

Auersperg N, Edelson MI, Mok SC, Johnson SW, Hamilton TC (1998). The biology of ovarian cancer. *Semin Oncol*, **25**, 281-04.

Auersperg N, Maines-Bandiera SL, Dyck HG, Kruk PA (1994). Characterization of cultured human ovarian surface epithelial cells: phenotypic plasticity and premalignant changes. *Lab Invest*, **71**, 510-8.

Bandyopadhyay S, Tfelt-Hansen J, Chattopadhyay N (2010). Diverse roles of extracellular calcium-sensing receptor in the central nervous system. *J Neurosci Res*, **88**, 2073-2.

Barrett PQ, Kojima I, Kojima K, et al (1986). Short term memory in the calcium messenger system. Evidence for a sustained activation of protein kinase C in adrenal glomerulosa cells. *Biochem J*, **238**, 905-2.

Bellahcene A, Castronovo V, Ogbureke KU, Fisher LW,

Fedarko NS (2008). Small integrin-binding ligand N-linked glycoproteins (SIBLINGs): multifunctional proteins in cancer. *Nat Rev Cancer*, **8**, 212-26.

Bhagavathula N, Hanosh AW, Nerusu KC et al (2007). Regulation of E-cadherin and beta-catenin by Ca^{2+} in colon carcinoma is dependent on calcium-sensing receptor expression and function. *Int J Cancer*, **121**, 1455-2.

Bhagavathula N, Kelley EA, Reddy M, et al (2005). 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**, 1364-1.

Birchmeier W (1995). E-cadherin as a tumor (invasion) suppressor gene. *Bioessays*, **17**, 97-9.

Birchmeier W, Behrens J (1994). Cadherin expression in carcinomas: role in the formation of cell junctions and the prevention of invasiveness. *Biochim Biophys Acta*, **1198**, 11-26.

Breitwieser GE (2012). Minireview: The Intimate Link Between Calcium Sensing Receptor Trafficking and Signaling: Implications for Disorders of Calcium Homeostasis. Mol Endocrinol.

Brembeck FH, Rosario M, and Birchmeier W. (2006). Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. *Current Opinion in Genetics & Development*, **16**, 51-9.

Brown EM, Gamba G, Riccardi D, et al (1993). Cloning and characterization of an extracellular Ca($^{2+}$)-sensing receptor from bovine parathyroid. *Nature*, **366**, 575-0.

Brown EM, MacLeod RJ (2001). Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev*, **81**, 239-97.

Capiod T, Shuba Y, Skryma R, Prevarskaya N (2007). Calcium signalling and cancer cell growth. *Sub-cellular biochemistry*, **45**, 405-27.

Chakrabarty S, Radjendirane V, Appelman H, Varani J (2003). 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**, 67-1.

Chakrabarty S, Wang H, Canaff L, et al (2005). Calcium sensing receptor in human colon carcinoma: interaction with Ca($^{2+}$) and 1,25-dihydroxyvitamin D(3). *Cancer Res*, **65**, 493-8.

Chakravarti B, Dwivedi SK, Mithal A, Chattopadhyay N (2009). Calcium-sensing receptor in cancer: good cop or bad cop? *Endocrine*, **35**, 271-84.

Chattopadhyay N (2006). 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**, 761-0.

Chattopadhyay N, Mithal A, Brown EM (1996). The calcium-sensing receptor: a window into the physiology and pathophysiology of mineral ion metabolism. *Endocr Rev*, **17**, 289-07.

Chattopadhyay N, Ye C, Singh DP, et al (1997). Expression of extracellular calcium-sensing receptor by human lens epithelial cells. *Biochem Biophys Res Commun*, **233**, 801-5.

Chattopadhyay N, Ye CP, Yamaguchi T, et al (1998). Extracellular calcium-sensing receptor in rat oligodendrocytes: expression and potential role in regulation of cellular proliferation and an outward K+ channel. *Glia*, **24**, 449-8.

Chattopadhyay N, Ye CP, Yamaguchi T, Vassilev PM, Brown EM (1999). Evidence for extracellular calcium-sensing receptor mediated opening of an outward K^{+} channel in a human astrocytoma cell line (U87). *Glia*, **26**, 64-2.

Cheng I, Klingensmith ME, Chattopadhyay N, et al (1998). Identification and localization of the extracellular calcium-sensing receptor in human breast. *J Clin Endocrinol Metab*, **83**, 703-7.

Chikatsu N, Fukumoto S, Takeuchi Y, et al (2000). 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**, 7553-7.

Cicek M, Oursler MJ (2006). Breast cancer bone metastasis and current small therapeutics. *Cancer Metastasis Rev*, **25**, 635-4.

Coleman RE (1997). Skeletal complications of malignancy. *Cancer*, **80**, 1588-4.

Cumano A and Godin I (2007). Ontogeny of the hematopoietic system. *Annu Rev Immunol*, **25**, 745-85.

Deschenes C, Vezina A, Beaulieu JF, Rivard N (2001). Role of p27(Kip1) in human intestinal cell differentiation. *Gastroenterology*, **120**, 423-38.

Dores MR, Trejo J (2012). Ubiquitination of GPCRs, Functional Implications and Drug Discovery. Mol Pharmacol.

Drueke TB (2006). Haematopoietic stem cells--role of calcium-sensing receptor in bone marrow homing. *Nephrol Dial Transplant*, **21**, 2072-4.

Dvorak-Ewell MM, Chen TH, Liang N, et al (2011). Osteoblast extracellular Ca^{2+} -sensing receptor regulates bone development, mineralization, and turnover. *J Bone Miner Res*, **26**, 2935-47.

Eaton CL, Coleman RE (2003). Pathophysiology of bone metastases from prostate cancer and the role of bisphosphonates in treatment. *Cancer Treatment Reviews*, **29**, 189-8.

Fagotto F, Funayama N, Gluck U, Gumbiner BM (1996). Binding to cadherins antagonizes the signaling activity of beta-catenin during axis formation in Xenopus. *J Cell Biol*, **132**, 1105-4.

Fukumoto S (1998). [Localization and function of calcium-sensing mechanism in bone cells]. *Nihon Rinsho*, **56**, 1419-4.

Garland C, Shekelle RB, Barrett-Connor E, et al (1985). Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet*, **1**, 307-9.

Garrett JE, Capuano IV, Hammerland LG, et al (1995). Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. *J Biol Chem*, **270**, 12919-5.

George GP, Ramesh V, Mittal RD (2011). Impact of total and ionized serum calcium on prostate cancer risk in North Indian men. *Asian Pac J Cancer Prev*, **12**, 1257-0.

Greenlee RT, Hill-Harmon MB, Murray T, Thun M (2001). Cancer statistics, 2001. *CA: A Cancer J Clinicians*, **51**, 15-36.

Grill V, Ho P, Body JJ, et al (1991). Parathyroid hormone-related protein: elevated levels in both humoral hypercalcemia of malignancy and hypercalcemia complicating metastatic breast cancer. *J Clin Endocrinol Metab*, **73**, 1309-5.

Gschwind A, Zwick E, Prenzel N, Leserer M, Ullrich A (2001). Cell communication networks: epidermal growth factor receptor transactivation as the paradigm for interreceptor signal transmission. *Oncogene*, **20**, 1594-0.

Haylock DN, Nilsson SK (2006). Osteopontin: a bridge between bone and blood. *Br J Haematol*, **134**, 467-4.

Hennings H, Holbrook K, Steinert P, Yuspa S (1980a). Growth and differentiation of mouse epidermal cells in culture: effects of extracellular calcium. *Curr Probl Dermatol*, **10**, 3-25.

Hennings H, Michael D, Cheng C, et al (1980b). Calcium regulation of growth and differentiation of mouse epidermal cells in culture. *Cell*, **19**, 245-4.

Hofer AM, Brown EM (2003). Extracellular calcium sensing and signalling. *Nat Rev Mol Cell Biol*, **4**, 530-8.

Holmbeck K, Bianco P, Chrysovergis K, Yamada S, Birkedal-Hansen H (2003). MT1-MMP-dependent, apoptotic remodeling of unmineralized cartilage: a critical process in skeletal growth. *J Cell Biol*, **163**, 661-1.

House MG, Kohlmeier L, Chattopadhyay N, et al (1997). Expression of an extracellular calcium-sensing receptor in human and mouse bone marrow cells. *J Bone Miner Res*, **12**, 1959-70.

Imanishi Y, Nagata Y, Inaba M (2012). Parathyroid Diseases and Animal Models. *Frontiers in Endocrinology*, **3**, 78.

Imbriaco M, Larson SM, Yeung HW, et al (1998). A new parameter for measuring metastatic bone involvement by prostate cancer: the Bone Scan Index. *Clin Cancer Res*, **4**, 1765-2.

Kampman E, Slattery ML, Caan B, Potter JD (2000). Calcium, vitamin D, sunshine exposure, dairy products and colon cancer risk (United States). *CCC* , **11**, 459-6.

Kingsley LA, Fournier PG, Chirgwin JM, Guise TA (2007). Molecular biology of bone metastasis. *Mol Cancer Ther*, **6**, 2609-7.

Koeneman KS, Yeung F, Chung LW (1999). Osteomimetic properties of prostate cancer cells: a hypothesis supporting the predilection of prostate cancer metastasis and growth in the bone environment. *Prostate*, **39**, 246-61.

Kuroda S, Fukata M, Nakagawa M, et al (1998). Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin- mediated cell-cell adhesion. *Science*, **281**, 832-5.

Lala PK, Chakraborty C (2001). Role of nitric oxide in carcinogenesis and tumour progression. *The lancet oncol*, **2**, 149-6.

Lamprecht SA, Lipkin M (2003). Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. *Nat Rev Cancer*, **3**, 601-14.

Le Gall C, Bellahcene A, Bonnelye E, et al (2007). A cathepsin K inhibitor reduces breast cancer induced osteolysis and skeletal tumor burden. *Cancer Res*, **67**, 9894-2.

Liao J, Schneider A, Datta NS, McCauley LK (2006). Extracellular calcium as a candidate mediator of prostate cancer skeletal metastasis. *Cancer Res*, **66**, 9065-3.

Lipkin M (1999). Preclinical and early human studies of calcium and colon cancer prevention. *Ann N Y Acad Sci*, **889**, 120-7.

Liu J, Lv F, Sun W, et al (2011). The abnormal phenotypes of cartilage and bone in calcium-sensing receptor deficient mice are dependent on the actions of calcium, phosphorus, and PTH. *PLoS Genet*, **7**, 1002294.

Mamillapalli R, VanHouten J, Zawalich W, Wysolmerski J (2008). 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**, 24435-47.

McNeil SE, Hobson SA, Nipper V, Rodland KD (1998). Functional calcium-sensing receptors in rat fibroblasts are required for activation of SRC kinase and mitogen-activated protein kinase in response to extracellular calcium. *J Biol Chem*, **273**, 1114-0.

Moon RT, Brown JD, Yang-Snyder JA, Miller JR (1997). Structurally related receptors and antagonists compete for secreted Wnt ligands. *Cell*, **88**, 725-8.

Morin PJ (1999). beta-catenin signaling and cancer. *Bioessays*, **21**, 1021-0.

Morony S, Capparelli C, Sarosi I, et al (2001). Osteoprotegerin inhibits osteolysis and decreases skeletal tumor burden in syngeneic and nude mouse models of experimental bone metastasis. *Cancer Res*, **61**, 4432-6.

Motokura T, Bloom T, Kim HG, et al (1991). A novel cyclin encoded by a bcl1-linked candidate oncogene. *Nature*, **350**, 512-5.

Neudert M, Fischer C, Krempien B, et al (2003). Site-specific human breast cancer (MDA-MB-231) metastases in nude rats: model characterisation and in vivo effects of

ibandronate on tumour growth. *Int J Cancer*, **107**, 468-7.

Nilsson SK, Johnston HM, Coverdale JA (2001). Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. *Blood*, **97**, 2293-9.

Nilsson SK, Johnston HM, Whitty GA, et al (2005). Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. *Blood*, **106**, 1232-9.

Oka H, Shiozaki H, Kobayashi K, et al (1993). Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. *Cancer Res*, **53**, 1696-1.

Parkinson IH, Fazzalari NL (2003). Interrelationships between structural parameters of cancellous bone reveal accelerated structural change at low bone volume. *J Bone Miner Res*, **18**, 2200-5.

Pignatelli M, Ansari TW, Gunter P, et al (1994). Loss of membranous E-cadherin expression in pancreatic cancer: correlation with lymph node metastasis, high grade, and advanced stage. *J Pathol*, **174**, 243-8.

Pin JP, Galvez T, Prezeau L (2003). Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. *Pharmacol Ther*, **98**, 325-54.

Polyak K, Hamilton SR, Vogelstein B, Kinzler KW. (1996). Early alteration of cell-cycle-regulated gene expression in colorectal neoplasia. *Am J Pathol*, **149**, 381-7.

Prenzel N, Zwick E, Daub H, et al (1999). EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature*, **402**, 884-8.

Quaroni A, Tian JQ, Seth P, Ap Rhys C (2000). p27(Kip1) is an inducer of intestinal epithelial cell differentiation. *Am J Physiol Cell Physiol*, **279**, 1045-57.

Ray JM, Squires PE, Curtis SB, Meloche MR, Buchan AM. (1997). Expression of the calcium-sensing receptor on human antral gastrin cells in culture. *J Clin Invest*, **99**, 2328-3.

Ray K, Hauschild BC, Steinbach PJ, et al (1999). 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**, 27642-0.

Rey O, Chang W, Bikle D, et al (2012). Negative cross-talk between calcium-sensing receptor and beta-catenin signaling systems in colonic epithelium. *J Biol Chem*, **287**, 1158-7.

Rodland KD (2004). The role of the calcium-sensing receptor in cancer. *Cell Calcium*, **35**, 291-5.

Roodman GD (2001). Biology of osteoclast activation in cancer. *J Clin Oncol*, **19**, 3562-1.

Roodman GD (2004). Mechanisms of bone metastasis. *N Engl J Med*, **350**, 1655-4.

Rozengurt, E (2007). Mitogenic signaling pathways induced by G protein-coupled receptors. *J Cell Physiol*, **213**, 589-02.

Ruat M, Molliver ME, Snowman AM, Snyder SH (1995). Calcium sensing receptor: molecular cloning in rat and localization to nerve terminals. *Proc Natl Acad Sci U S A*, **92**, 3161-5.

Saidak Z, Mentaverri R, Brown EM (2009). The role of the calcium-sensing receptor in the development and progression of cancer. *Endocr Rev*, **30**, 178-5.

Sanders JL, Chattopadhyay N, Kifor O, Yamaguchi T, Brown EM (2001). Ca$^{(2+)}$-sensing receptor expression and PTHrP secretion in PC-3 human prostate cancer cells. *Am J Physiol Endocrinol Metab*, **281**, 1267-4.

Sanders JL, Chattopadhyay N, Kifor O, et al (2000). Extracellular calcium-sensing receptor expression and its potential role in

regulating parathyroid hormone-related peptide secretion in human breast cancer cell lines. *Endocrinology*, **141**, 4357-4.

Saxena H, Deshpande DA, Tiegs BC, et al (2012). The GPCR OGR1 (GPR68) mediates diverse signalling and contraction of airway smooth muscle in response to small reductions in extracellular pH. *Br J Pharmacol*, **166**, 981-0.

Schneider A, Kalikin LM, Mattos AC, et al (2005). Bone turnover mediates preferential localization of prostate cancer in the skeleton. *Endocrinology*, **146**, 1727-6.

Sellers TA, Bazyk AE, Bostick RM, et al (1998). Diet and risk of colon cancer in a large prospective study of older women: an analysis stratified on family history (Iowa, United States). *CCC*, **9**, 357-7.

Squires PE, Harris TE, Persaud SJ, et al (2000). The extracellular calcium-sensing receptor on human beta-cells negatively modulates insulin secretion. *Diabetes*, **49**, 409-7.

Sun YX, Schneider A, Jung Y, et al (2005). 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**, 318-9.

Tetsu O, McCormick F (1999). Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature*, **398**, 422-6.

Tfelt-Hansen J, Schwarz P, Terwilliger EF, et al (2003). Calcium-sensing receptor induces messenger ribonucleic acid of human securin, pituitary tumor transforming gene, in rat testicular cancer. *Endocrinology*, **144**, 5188-3.

Tharmalingam S, Daulat AM, Antflick JE, et al (2011). Calcium-sensing receptor modulates cell adhesion and migration via integrins. *J Biol Chem*, **286**, 40922-33.

Theman TA, Collins MT (2009). The role of the calcium-sensing receptor in bone biology and pathophysiology. *Curr Pharm Biotechnol*, **10**, 289-01.

Thomsen LL, Miles DW (1998). Role of nitric oxide in tumour progression: lessons from human tumours. *Cancer Metastasis Rev*, **17**, 107-18.

VanHouten J, Dann P, McGeoch G, et al (2004). The calcium-sensing receptor regulates mammary gland parathyroid hormone-related protein production and calcium transport. *J Clin Invest*, **113**, 598-8.

Ward DT, Riccardi D (2012). New concepts in calcium-sensing receptor pharmacology and signalling. *Br J Pharmacol*, **165**, 35-48.

Wargovich MJ, Jimenez A, McKee K, et al (2000). Efficacy of potential chemopreventive agents on rat colon aberrant crypt formation and progression. *Carcinogenesis*, **21**, 1149-5.

Wong NA, Pignatelli M (2002). Beta-catenin--a linchpin in colorectal carcinogenesis? *Am J Pathol*, **160**, 389-1.

Wu K, Willett WC, Fuchs CS, et al (2002). Calcium intake and risk of colon cancer in women and men. *J Natl Cancer Inst*, **94**, 437-6.

Yano S, Macleod RJ, Chattopadhyay N, et al (2004). Calcium-sensing receptor activation stimulates parathyroid hormone-related protein secretion in prostate cancer cells: role of epidermal growth factor receptor transactivation. *Bone*, **35**, 664-2.

Yin JJ, Pollock CB, Kelly K (2005). Mechanisms of cancer metastasis to the bone. *Cell Res*, **15**, 57-62.

Yin JJ, Selander K, Chirgwin JM, et al (1999). TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. *J Clin Invest*, **103**, 197-6.

Zheng Y, Zhou H, Modzelewski JR, et al (2007). Accelerated bone resorption, due to dietary calcium deficiency, promotes breast cancer tumor growth in bone. *Cancer Res*, **67**, 9542-8.