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Review

The calcium-sensing receptor and the hallmarks of cancer☆

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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.

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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.

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