GCMB and PIN1: regulation of parathyroid function on a transcriptional and post-transcriptional level

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Summary

The extracellular calcium sensing receptor (CaSR) plays a key role in the maintenance of a narrow range of Ca2+ modulating PTH secretion. Moreover, the PTH levels depend from its mRNA stability, and Prolyn isomerase Pin 1 enzyme seems to be closely involved in the stabilization of PTH mRNA. In Pin1-/- animal model, in fact, increased levels of serum PTH protein and PTH mRNA were found, indicating that Pin1 determines basal PTH expression *in vivo*. In this scenario, regulation of parathyroid function is mediated at transcriptional and post-transcriptional level. The parathyroid glands are bilateral organs and the early stages of their organogenesis are closely linked with thymus organogenesis. Normal parathyroid development is compromised by reduced levels of the transcription factor GCMB (glial missing homolog), suggesting that its gene might be important in mediating the effects of extracellular calcium on PTH expression and/or secretion in parathyroid cells. GCMB-deficient mice lack parathyroid glands but show normal parathyroid hormone serum levels. Studies on angiogenesis in parathyroid cells showed the co-expression of endothelial progenitor markers and parathyroid specific genes (GCMB, PTH, CaSR), suggesting their potential role of these so-called parathyroid/endothelial cells as progenitors cells.

KEY WORDS: parathyroid gland; CaSR; GCMB; PIN1.

The calcium sensing receptor

The extracellular Calcium Sensing Receptor (CaSR) plays a key role in the maintenance of a narrow range (1.1-1.3 mM) of the extracellular ionized calcium concentration (Ca^{2+}), primarily by modulating the function of chief cells of parathyroid gland by inhibiting parathyroid hormone (PTH) secretion (1-3), PTH gene expression (4), and parathyroid cellular proliferation (5). In addition, CaSR is expressed in other tissues participating in Ca^{2+} homeostasis, regulating the secretion of parathyroid hormone-related protein (PTHrP) and the transport of calcium in breast epithelial cells (6). Moreover, CaSR is present in bone cells (7-10) as well as in epithelial cells of the small and large intestines (11, 12).
CaSR controls expression of the PTH gene by a post-trascriptional mechanism and may also indirectly inhibit parathyroid function by upregulating Vitamin D Receptor (VDR). It thereby potentiates inhibitory actions of $1,25(OH)_2D3$ on parathyroid cellular proliferation and PTH gene expression (13). Indeed, CaSR acts as a 'calciostat', informing the parathyroid glands and other tissues of the precise level of Ca^{2+}. Both very high and very low levels of Ca^{2+} can lead to serious clinical consequences.
The cells that mainly express CaSR and its roles in regulating bone turnover and intestinal Ca^{2+} absorption in physiological conditions are not fully understood.

Structure and signalling pathways of the CaSR

The CaSR is a member of family C II of the superfamily of 7-TM receptors, also called GPCRs, (14) which is by far the largest group of cell surface receptors.
The human CaSR comprises 1078 amino acid residues and has three structural domains, as all 7-TM receptors. It has a large extracellular domain (ECD) (612 residues), a transmembrane domain (TMD) of 250 amino acids containing the seven membrane-spanning helices, and an intracellular, C-terminal domain (ICD) of 216 amino acids. The receptor exhibits substantial N-linked glycosylation, which is important for normal cell membrane expression of the receptor, but does not appear to modify the function of the receptor (15).
The functional cell surface form of the CaSR is a dimer, and the two monomers within the dimeric CaSR are linked by disulphide bonds involving cysteine residues 129 and 131 within each monomer (16). The active and inactive forms of CaSR are in dynamic equilibrium in the normal physiological range of $[Ca^{2+}]_o$, and the state of the receptor's activity varies in response to the association or dissociation of Ca^{2+} ions in the predicted Ca^{2+}-binding sites.
Under low $[Ca^{2+}]_o$ conditions, the Ca^{2+}- binding sites are largely devoid of ions, and the ECD of CaSR exists as an inactive open form that induces PTH secretion. PTH is responsible for elevating $[Ca^{2+}]_o$. Under high extracellular Ca^{2+} conditions, the binding of Ca^{2+} to all sites leads to conformational changes that promote activation of the receptor, which produces several homeostatic responses designed to normalize $[Ca^{2+}]_o$ level. These include inhibition of PTH secre-

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tion, stimulation of calcitonin (CT) secretion, resulting in $[Ca^{2+}]_o$ reduction, and enhancement of renal Ca^{2+} excretion. Thus, deviation from homeostasis can be restored by the sensing of $[Ca^{2+}]_o$ by these putative Ca^{2+}-binding sites and the resultant reversible conformational changes, than CaSR determines the parathyroid response to $[Ca^{2+}]_o$ at the levels of PTH secretion, PTH gene expression and parathyroid cell proliferation (17, 18).
Increased $[Ca^{2+}]_o$ activates the CaRS, mediating a G-protein-dependent activation of phospholipase-C (PLC), phospholipase-A2 (PLA2) and phospholipase-D (PLD) (19) resulting in the decrease PTH secretion and parathyroid cell proliferation.
Two kinds of drugs act on the allosteric conformation of this receptor: calciomimetics and calciolytics with different action, in fact, calciomimetics bind at the site of transmembrane domain (TMD) of the CaRS, stabilizating the CaSR's active conformation (20, 21), while calciolytics stabilize the inactive one.
Modulation of PTH levels also depends on other factors, acting on PTH mRNA stability. Among them, a key role is played by prolyn isomerase Pin 1 enzyme.

The prolyn isomerase Pin1

The prolyn isomerase Pin1 is a conserved enzyme that is involved in different biological processes and pathological conditions (22). It binds to and isomerizes specific pSer/Thr-Pro motifs in a defined subset of phosphoproteins (23, 24).
Pin's1 functions are cell-cycle control, transcription and splicing regulation, DNA replication checkpoint control, DNA damage response, neuronal survival, and germ cell development (24-26). Aberrant Pin1 function has implicated in several human diseases (25).
Pin1 has a unique role in the regulation of signalling cascades that function via proline-directed phosphorylation. Isomerization of pSer/Thr- Pro motifs between *cis* and *trans* can alter the local or even global structure, and has profound effects on the function and regulation of the target molecule, among them, protein kinases such as Mitogen-Activated Protein Kinases (MAPK) and Cyclin-dependent kinase 2 (Cdk2), and phosphatases such as the protein phosphatase 2 (PP2A), in particular, Pin1 is able to phosphorylate or dephosphorylate only the trans conformation of Ser/Thr-Pro motifs, (27-30) probably because pSer/Thr-Pro motifs in the *cis* conformation are energetically less favourable. By binding and isomering specific pSer/The-Pro motifs, Pin1 regulates phosphorylation signalling. It is overexpressed and activated in many human cancer tissue and cells, can target to the pThr286-Pro motifs in cyclin D1 and stabilize cyclin D1 by preventing its nuclear export and ubiquitin-mediated degradation. Pin1 can also target to pThr 246-Pro motif in β-catenin and to the pThr 254-Pro motif in the p65/RelA subunit of NF-κB, (nuclear factor kappa-light-chain-enhancer of activated B cells) and thereby prevent these proteins from being inhibited by their respective inhibitors Adenomatous polyposis coli- for β-catenin (APC) and IκB -inhibitor of NF-Kb for NF-κB. This complex mechanism induces an increase in nuclear accumulation, protein half-life and transcriptional activity of β-catenin and NF-κB.

PIN1 and β-catenin

Upregulation of the oncogenic transcriptional activator β-catenin has a pivotal role in the development of cancer.
One of the key β-catenin regulators is Adenomatous Polyposis Coli protein (APC). The tumour-suppressing activity of APC controls the nuclear accumulation of the oncogenic transcriptional activator β-catenin. First, APC binds the nuclear β-catenin and exports it to the cytoplasm for protein degradation. Subsequently, APC binds and assembles β-catenin into a multiple protein complex, including glycogen synthase kinase-3b (GSK-3b) and trigger the degradation of β-catenin (31).
Pin1 increased the stability of β-catenin in cells and subsequently increases the nuclear fraction of β-catenin by preventing its interaction with APC.
Moreover, the activation of Wnt signalling inhibits the phosphorylation of β-catenin by GSK-3b, resulting in the stabilization of β-catenin in the cytoplasm and nucleus (31).
It has been shown that Wnt/β-catenin signalling is involved in gene expression control, cell adhesion and cell polarity. Furthermore, the activation of the Wnt/β-catenin signalling pathway is a major feature of human cancers; in fact, the deregulation of this signalling has been found in several subsets of human malignancies that carry mutations in proteins participating in this molecular pathway. The result of the aberrant activation is always the cytosolic stabilization of β-catenin, which enhances the transcription of several target genes, such as cyclin D1 and the transcription factor Myc, leading to oncogenesis (32).
The role of β-catenin in sporadic parathyroid adenomas is controversial but, a specific, homozygous stabilizing mutation (S37A) in exon 3 of β-catenin gene sequence (CTNNB1 gene) has been reported only in 15% of parathyroid adenomas, even if the increased accumulation of non-phosphorylated β-catenin were found in 100% parathyroid adenomas studied (33-36). Interestingly, β-catenin signalling is required for suppression of chondrocyte differentiation and to induce the osteoblastogenesis in fact, multiple genetic evidence established the critical participation of canonical β-catenin activity for early osteoblast differentiation (37).

PIN1 and NF-κB

NF-κB is a family of transcription factors that were first identified as regulators of B-cell differentiation by their ability to bind to the κB site of the κ light-chain gene in B cells. They were also involved in innate and adaptative immunity (38, 39). Moreover, NF-kB drives the expression of genes important for inflammation, immunity, cell migration and cell survival.
There are five members of NF-κB family: RelA(p65), p50, p52, RelB and c-Rel, which share Rel homology domain. pThr 254-Pro motif in p65/RelA subunit is the site of binding of PIN1.
NF-kB activation is dysregulated in some common conditions, including cancer, diabetes, and atherosclerosis (40). Furthermore, it has been shown that NF-κB is involved in osteoclast formation, in skeletal development and renewal of bone throughout life, membranous and endochondral ossification, processes which are tighly regulated by osteogenic pathways as Wnt, TGF-beta/BMP (transforming growth factor β/bone morphogenetic protein), notch signalling and in common bone disease (41-43). In addiction, bone growth and bone remodelling are regulated by parallel molecular pathways involving the PTH receptor 1 (PTH1R) and the frizzled homolog family (FZD) (44-46). Indeed, acting on mature

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osteoblasts, the PTH1R stimulation leads to RANKL (Receptor Activator of Nuclear factor Kappa-B Ligand) production, which binds RANK (Receptor Activator of Nuclear factor Kappa B) on osteoclast precursors and induces osteoclasts differentiation mediated by NF-κB and JNK (JUN N-terminal kinase) signalling (47, 48). Different studies have shown that gene knockout of elements of FZD and the canonical β-catenin signalling pathway likewise produce marked disruption of normal bone formation or bone turnover (46, 49-53).

PIN1 and Wnt

Activation of Wnt signalling induces osteoblasts (OB) proliferation and differentiation, prevents pre-OB and OB apoptosis, and augments osteoprotegerin (OPG) production by OB. Wnt proteins initiate the canonical signalling cascade by binding to receptors of the Frizzled family together with their coreceptors known to be members of the low-density Lipoprotein Receptor-related Protein (LRP) family, among them with LRP5 and LRP6, resulting in the stabilization of cytosolic β-catenin. Recently a new member of this family, LRP4, has been discovered to be expressed on OBs and implicated in the mechanism of action of PTH. LRP4 is expressed in bone and in *in vitro* cultured osteoblasts, able to bind a soluble LRP5 and LRP6 signaling inhibitor, Dickkopf-1 (Dkk1) and sclerostin.
The PTH protein, once bound to PTH-R1, is capable of forming a complex with LRP6 leading to β-catenin activation. Thus, PTH activates Wnt signaling in osteoblastic cells through both, Wnt ligands dependent and Wnt ligands independent mechanisms. Moreover, PTH downregulates the production of sclerostin, an osteocyte-derived Wnt antagonist which acts by binding to LRP5 and LRP6, Dkk1, and Sfrp-4, (secreted frizzled-related protein 4) a factor which binds Wnt proteins, thus antagonizing both canonical and non-canonical Wnt signalling.
The relevance of sclerostin in the mechanism of action of PTH remains uncertain because studies with sclerostin transgenics and sclerostin KO mice have not provided conclusive evidence of the possible role of this osteocytic peptide as a PTH target (54).

PIN1 and PTH

Published data shows that Pin1 plays a crucial role in the regulation of the PTH expression, by modulating PTH mRNA stability. PTH mRNA consists of three exons coding for the 5'-UTR (exon 1), the pre-pro-region (exon 2), and the structural PTH together with the 3'- UTR (exon 3); the PTH mRNA 3'-UTR in all species is rich in A and U nucleotides (55).
A specific sequence at the 3' end of the rat mRNA 3'UTR was identified as a cis-acting type-III AREs (Adenine-and Uridine-Riche elements) that determines PTH mRNA stability and its regulation by calcium and phosphate (56). The ARE of 63 nucleotides contains a 26-nucleotide sequence, conserved among species, suggesting that is an evolutionary preserved functional unit, which is sufficient to regulate PTH mRNA stability and to confer responsiveness of mRNAs to changes in calcium and phosphate levels (19, 57).
It has been shown that the increase of PTH mRNA levels in secondary hyperparathyroidism due to calcium depletion or Chronic Kidney Disease (CKD) is mediated by the interaction of trans-acting RNA-binding proteins with a defined cis-acting ARE in the PTH mRNA 3' UTR. Pin1$^{-/-}$ mice have increased levels of serum PTH protein and PTH mRNA, indicating that Pin1 determines basal PTH expression *in vivo*. In rat animal models fed a calcium depleted diet or CKD rats, Pin1 isomerase activity is decreased in parathyroid extract and, in the same time, PTH mRNA levels and its stability are increased (58).
Interestingly, Pin1 has been observed as a potential suppressor of oncogenesis. In fact, phosphorylation-dependent prolyl-isomerization catalysed by Pin1 is able to induce conformational changes and regulate the function of phosphorylated proteins. Pin1 levels and activity are also upregulated by oncogenic pathways suggesting that the inhibition of Pin1, by disrupting oncogenic pathways, may provide a target for novel anti-cancer therapy (59).

Parathyroid embryogenesis regulation

Epithelial-mesenchymal interactions are a common theme in the development of various organs, therefore the study of parathyroid organogenesis can be useful to understand the mechanisms of function of parathyroid gland.
The parathyroid glands are bilateral organs and the early stages of their organogenesis are closely linked with thymus organogenesis.
The thymus and parathyroid glands originate in a bilateral fashion from endoderm of the third pharyngeal pouch (56, 60). Each third pouch initially forms a common primordium containing thymus and parathyroid specific domains. Studies in mice have shown that, although epithelial cells of the common primordial are morphologically indistinguishable, patterning of third pouch endoderm into organ-specific domains is apparent from the expression of two transcription factors restricted to thymus and parathyroid-fated domains, Forkhead box protein N1 (Foxn1) and glial missing homolog 2 (Gcm2) respectively (61, 62). Gordon et al. proved that Gcm2 expression in mice is located in the anterior and dorsal regions of third pouch endoderm, whereas Foxn1 is expressed in ventral and posterior regions of the third pouch. After the initial patterning is established, the shared primordia detach from the pharyngeal endoderm at approximately embryonic day 12 (E12) (58, 62), then migrate to their final adult locations by about E14.5 (57, 60). The bilateral thymic lobes undergo medial, ventral and caudal migration to reach their final position above the heart (62).
The molecular mechanism that specifies this primordium into distinct organ domains is unknown but, the Gcm2 and Foxn1 transcription factors, required for development of the parathyroid and thymus respectively, seem to be attractive candidates to play this role. Other genes have been identified in development of the parathyroid: in particular, Homeobox A3 (Hoxa3), Paired box proteinPax-1/9 (Pax1/9), transcriptional coactivator and phosphatase1 (Eya1), and Sineoculis homeobox homolog 1/4 (Six1/4), these transcriptional regulators result implicated in the molecular network during the early organogenesis of both organs (62).
Several experimental evidences showed that the transcription factor *Hoxa3* gene is expressed in the third pharyngeal pouch endoderm, and *Pax9* inactivation results in failure of parathyroid formation. Normal parathyroid development is al-

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so compromised by reduced *Gcm2* expression additionally, mice animal model *Eya1* mutants, fail to form the parathyroid gland (62).

The transcription factor GCMB

The human transcription factor GCMB, *glial missing homolog 2*, which belongs to a small family of key regulators of parathyroid gland development, is located on chromosome 6p23- 24 and consists of 5 exons. GCMB encode to transcription factors with a new type of zinc-containing DNA-binding domain, so-called GCM domain, that recognizes the DNA sequence-specific 5'-ATGCGGGT-3' (63-65). The carboxyl terminal portion of GCMB contains a putative inhibitory domain that is located between two TA domains and three PEST motifs which are segments rich in proline (P), glutamic acid (D), aspartic acid (E) and serine (S) or threonine (T) residues and are likely to play a role in protein stability (30). PEST motifs serve as putative intramolecular signals for rapid proteolytic degradation by calpains know to be Ca^{2+}-dependent cysteine proteases able to regulate several enzymes, transcription factors and structural proteins through limited proteolysis (66). The PEST motifs contained in GCMB convey short half-life and low protein stability (67) and influence target gene activation (68).
The GCMB gene is also expressed in adult human parathyroid gland (69), and has responsive elements in the P1 and P2 promoters of the CaSR gene (70). Silencing of the GCMB gene by shRNA in human parathyroid cells led to a decreased expression of the CaSR, but had no significant effect on the PTH, VDR, 25-hydroxyvitamin D (3)-1α-hydroxylase, and Proliferating Cell Nuclear Antigen (PCNA) expression (71).
There are two mammalian orthologs, named Gcm1 and Gcm2 in the mouse, named GCMA and GCMB in humans (63, 72-74).
GCMA has a distinct role in the maintenance, development and turnover of the human trophoblast (75), while recent studies suggest that GCMB expression might be primarily restricted to the parathyroid glands (3, 76, 77), and has been proposed to be a master regulator of mammalian parathyroid development (72). Gunther et al. found that 30% of *Gcm2* -deficient mice die shortly after birth owing to severe hypocalcaemia. The remaining animal models were viable, fertile, with milder hypocalcaemia, associated with hyperphosphatemia and increased calcium elimination in the urine without evidence of renal participation, altogether features are characteristic for hypoparathyroidism. Thus, unlike PTH receptor-deficient mice, Gcm2-deficient mice, despite their lack of parathyroid glands, have PTH serum levels identical to those of wild-type mice, as do parathyroidectomized wild-type animals. Expression and ablation studies identified the thymus, where Gcm1 is expressed, as the additional, downregulable source of PTH. Thus, Gcm2 deletion uncovers an auxiliary mechanism for the regulation of calcium homeostasis in the absence of parathyroid glands (78).
It is possible to hypothesize that during the early stages of embryonal parathyroid organogenesis, the lack of GCM2 gene could alter the process of cellular differentiation, with the consequent preservation of a cell pool belonging to the common primordium with embryonal undifferentiated characteristic. During the following migration of the thymic rudiment, cellular residues which are not thymic but, pseudo-parathyroid undifferentiated cell clusters, will be still able to regulate calcium homeostasis through ectopic production of parathormone. This hypothesis derives from the observation that firstly in the mammalian, thymus and parathyroid glands share a common embryogenic origin and secondly *GCMB*-deficient mice lack parathyroid glands but show normal parathyroid hormone serum levels besides, Gcm1-mediated parathyroid hormone production in the thymus was uncovered as an auxiliary regulatory mechanism for calcium homeostasis in the absence of parathyroid glands (78). In addition, the intrathymic PTH-secreting adenomas expressed GCMB rather GCMA, suggesting that these tumours arose from parathyroid cells that migrated errantly within the thymus during embryological development (79).

GCMB gene and tumorigenesis

Tumorigenesis is usually considered to be a ''developmental disorder'': molecular factors that coordinate cellular proliferation and differentiation in development, when disrupted, lead to uncontrolled cell growth and dedifferentiation. Conversely most endocrine neoplasms retain the normal cellular differentiated function and phenotype. If the GCMB gene, which is a critical regulator of parathyroid development in mammals, were involved in parathyroid tumorigenesis, there should be an overexpression or constitutive activation of this gene in parathyroid neoplasm (77).
The deregulated expression of the GCMB gene in parathyroid neoplasm might result from upstream signals or other transcription factors such as Hoxa3, Pax1, Pax9, and Eya1, which are also important in parathyroid organogenesis by modulating Gcm2 expression in transgenic mouse. The down-regulation of GCMB mRNA expression observed with a low calcium concentration suggests that this gene might be important in mediating the effect of extracellular calcium on PTH expression and/or secretion in parathyroid cells. Hypoparathyroidism can be caused by the mutation of the genes that are required for normal parathyroid physiological functions, including *PTH* (80, 81) and *CaSR* (82). Hypoparathyroidism also can result from the mutation of genes that function in parathyroid development, like *Gata3* (83), *Sox3* (84), and *Gcm2* (77, 85).

Parathyroid-Derived Stem Cells (PDSC)

It has been demonstrated that stem cells exist in several human postnatal tissues, including the bone marrow, adipose tissue, synovium, umbilical cord blood, heart, brain, skeletal muscles, pancreas, skin, and dental pulp. Recently, Shih et al. have isolated and characterized Mesenchymal Stem Cells even from human parathyroid tissue (*Parathyroid-Derived Stem Cells*, PDSC). The parathyroid cells can be isolated and cultured *in vitro* and are able to regulate parathyroid hormone secretion through the presence of CaSR responding to extracellular calcium (86).
PDSC are capable of self-renewal and could differentiate into mesenchymal lineages and demonstrated similar growth characteristics of postnatal mesenchymal stem cells from bone marrow (BM-MSC) (87) and umbilical cord blood (88) in *in vitro* cultures.
PDSCs' spindle-shaped morphology, plate adherence, the ability to be sub-cultured *in vitro,* and cell surface phenotype

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characterization showed the MSC-related markers subset expression, such as CD73 and CD105, while endothelial and haematopoietic lineage markers were absent in addition, the presence of telomerase activity in the isolated cells showed their distinction from terminally differentiated somatic cells.
CaSR gene expression in PDSCs was evident under culture in keratinocyte medium with or without calcium or vitamin D suggesting that, under determinate culture conditions, these cells could be induced to express CaSR on their cell membranes and might respond to extracellular calcium (89, 90).

Cellular physiology of the parathyroid gland

The cellular constitution of the mature parathyroid gland appears stable under basal conditions, with an estimated steady cell turnover of about 5% for year (91), consistent enlargement of the gland size may occur in several pathological conditions. Another peculiar characteristic of parathyroid cells is the ability of spontaneously inducing angiogenesis in both *in vitro* and *in vivo* animal models.
Studying angiogenesis in parathyroid glands, it has been detected the presence of a subpopulation of parathyroid derived $CD34^+$ cells (92, 93) isolated and characterized in normal and tumoral parathyroid glands (93). A small proportion of parathyroid-derived $CD34^+$ cells co-expressed both endothelial progenitors and parathyroid specific genes. The parathyroid/endothelial cells showed a different phenotype in parathyroid tumours compared with normal parathyroid, suggesting their involvement in parathyroid tumorigenesis. Most $CD34^+$ cells around small vessels co-expressed CD146, (cluster of differentiation 146), laminin, isolectin, and vWVIII (von Willebrand factor) resembling the endothelial cell phenotype (94).
Conversely, the $CD34^+$ cells scattered throughout the parathyroid parenchyma were negative for some markers of mature endothelium while $CD34^-$ cells consisted with the parathyroid chief cells.
Moreover, the amount of $CD34^+$ scattered cells was higher in parathyroid adenomas and hyperplasia than in normal tissue. Corbetta et al. found two subpopulations of $CD34^+$ cells which differed for location and co-expression of other endothelial markers, one displaying the phenotype of differentiated endothelial cells expressing vWVIII, isolectin, laminin, and CD146, while the other cells derived from parenchyma, were negative for the expression of endothelial cells lineage., Interestingly, $CD34^+$ fraction contained a population of cells expressing highly specific parathyroid genes, such as *GCMB*, *PTH* and *CaSR* (93). These parathyroid/endothelial cells resulting more abundant, less committed, and more functionally active in parathyroid tumours with respect to normal glands. Although their role in parathyroid tumour development remains still undefined, $CD34^+$ cells displayed some properties suggestive for potential progenitors (93).

Concluding remarks

The concentration of $[Ca^{2+}]_o$ is controlled with subtle modulation of the expression of several genes. Considering that both, very high and very low levels of this ion can lead to serious clinical consequences. The first modulation starts by the early parathyroid organogenesis that is linked with thymus organogenesis from endoderm of the third pharyngeal pouch (56, 60). GCMB is a master transcriptional factor that specifies development and differentiation of the parathyroid cells. This gene is also expressed in adult parathyroid gland (69) and plays a key role in the maintenance of CaSR gene expression on the membrane surface of the different cell types, especially on parathyroid and bone cells, being the main regulator in the maintenance of the narrow range of Ca^{2+}. The functional active form of CaSR is a dimer, but this form is in equilibrium with the monomeric inactive form, and the receptor status varies in response to association or dissociation of Ca^{2+}. When Ca^{2+} binds to CaSR, this receptor is in the activate form that inhibits synthesis and secretion of PTH and down regulates the parathyroid cells proliferation.
The second regulation mechanism is at a transcriptional level. In fact, the 3'UTR of PTH mRNA is rich in A and U nucleotide (55) and a specific sequence cis-acting type III AREs has been identified at the 3' end of rat PTH mRNA sequence. This sequence determines PTH mRNA stability and its regulation by calcium and phosphate (56). Pin1 has a unique role in the regulation of signalling cascades operating via-proline-directed phosphorylation. Isomerizarion of pSer/Thr-Pro motifs between *cis* and *trans* has profound effects on the function and regulation of target molecule, such as MAPK; CdK2; PP2A; NF-κB; β-catenin/WNT pathway, cyclinD1. These molecules are involved in the osteoblastogenesis pathway, acting on regulation of phosphate and $[Ca^{2+}]$ levels. Moreover, Pin1 determines basal PTH expression *in vivo* (43) and aberrant Pin1 function has been found implicated in several human diseases.
Angiogenesis studies in parathyroid cells showed the co-expression of endothelial progenitor markers and parathyroid specific genes (GCMB, PTH, CaSR) (93), named parathyroid/endothelial cells with different phenotype in parathyroid tumours compared to normal parathyroid, considering that they resulting more abundant, less committed and more functionally active in the parathyroid tumours with respect to normal glands. Their role in parathyroid tumour development remains undefined but could play a role of potential progenitor cells (93).

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Competing interests

The Authors declare that they have no competing interests.

Author's contributions

All Authors meet the criteria for authorship.

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