Parathyroid Glands in CKD: Anatomy, Histology, Physiology and Molecular Biology in CKD

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Anatomy of Parathyroid Glands

The parathyroid glands develop from endodermal epithelial cells, in conjunction with the thymus. The superior parathyroid glands are derived from the fourth branchial pouch. These glands are closely associated with the lateral lobes of the thyroid and have a short line of embryologic descent [1]. The inferior parathyroid glands are derived from the third branchial pouch. These glands are closely associated with the thymus and have a longer line of embryologic descent, which leads to more variability in their anatomic position [1]. Inferior parathyroids can be found in the upper part of the neck as the carotid sheath and can also be found in the anterior mediastinum or even in the pericardium. However, the majority of inferior parathyroids are located near the inferior pole of the thyroid. The locations of ectopic parathyroid glands are related to the common origins of parathyroid, thyroid, and thymic tissue. The third branchial pouch contributes to thymus development as well as parathyroid and thyroid development. Both the third and fourth branchial pouches also contribute to thyroid development.

Normal parathyroid glands usually have a size of about $5 \times 4 \times 2$ millimeters and weigh 35–50 milligrams. Enlarged parathyroid glands can weigh between 50 milligrams and 20 grams, most often they are approximately 1 gram in weight

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and 1 centimeter in size. When normal in size, these are not usually identified on most imaging studies. In contrast, parathyroid adenomas and gland hyperplasia are larger and more readily identified on imaging studies.

The aspect of parathyroid glands can vary considerably [2, 3]. The color varies from light yellow to reddish-brown, while the shape is oval or spherical in most cases (83%), but can also be elongated (11%). Occasionally the glands are bi-lobated (5%) or multilobated (1%).

The majority (84%) of patients have four parathyroid glands, two superior and two inferior glands [2]. Only three glands are found in a very small number of patients (≤3%) and additional glands are found in 13% of patients [2]. The terms "superior" and "inferior" refers to the embryological origin of the gland, rather than the gland's location in the neck.

Parathyroids are located, in most cases, close to the posterior-lateral surface of the thyroid lobe; however, they can be found along the hyoid bone down to the superior mediastinum [4]. In some cases the parathyroid glands may be included in thyroid parenchima, being intra-thyroidal parathyroid glands [4, 5]. Although there is significant variability in the position of the glands, they are usually symmetric. The superior glands are symmetric in 80% of cases and inferior glands are symmetric 70% of the time [2].

Superior parathyroid glands The anatomic location of the superior parathyroid glands is relatively constant due to the close relationship between these glands and the thyroid gland. They arise from the fourth pharyngeal pouch and descend together with the lateral lobes of the thyroid to contact the thyroid capsule most commonly at the posterior edge of the middle third of the thyroid gland [6]. They lie under the thyroid superficial fascia, posterior to the recurrent laryngeal nerve and can be visualized with an accurate dissection of the thyroid capsule in this region. These glands may also reside inside the thyroid capsule, just superior and medial to the posterior tubercle of Zuckerkandl of the thyroid lobe. The recurrent laryngeal nerve is always anterior to the superior parathyroid gland.

On the basis of anatomic studies, the majority (80%) of normal superior glands are located about 1–2 cm above the junction of the recurrent laryngeal nerve and the inferior thyroid artery and within 1 cm of the entry point for the recurrent laryngeal nerve into the ligament of Berry and the cricoid cartilage [2]. Fewer than 1% are above upper pole of thyroid gland and only 1% along lower pharynx. None have been described at level of carotid bifurcation [7, 8].

Superior parathyroid glands can be undescended, or can be parapharyngeal, retropharyngeal, or retrotracheal within the middle cervical/mediastinal compartment. Enlarged parathyroid glands can travel straight down the tracheoesophageal groove or the retropharyngeal space into the chest.

Inferior parathyroid glands The inferior parathyroid glands have a more variable location due to their embryologic relationship to the thymus, as discussed before. They usually reside in the anterior mediastinal compartment, anterior to the recurrent laryngeal nerve. Fifty percent of the time, the inferior parathyroid

glands are located along the lateral lower pole of the thyroid gland. Fifteen percent of the time, these glands are located 1 cm below the lower thyroid lobe. They can be located anywhere between the angle of the mandible and the upper mediastinum. They are most often found in the thyrothymic tract, or just inside the thyroid capsule on the inferior portion of the thyroid lobes. The incidence of intrathyroidal parathyroid tissue is quite low, approximately 2% [9].

Ectopic parathyroid glands Ectopic parathyroid glands occur because parathyroid tissue may co-locate with tissues that have a similar embryologic development. An ectopic parathyroid gland that fails to have complete migration during normal development is termed "undescended." The ectopic gland may be one of the four parathyroid glands or it may be a supernumerary gland. In a record of 102 patients with persistent or recurrent hyperparathyroidism, who required reoperation, ectopic glands were found in the paraesophageal position (28%), in the mediastinum (26%), intrathymic (24%), intrathyroidal (11%), in the carotid sheath (9%) and in a high cervical position (2%) [10]. These percentages will vary depending whether the ectopic gland is superior or inferior in origin.

Ectopic superior parathyroid glands Ectopic abnormal parathyroid glands in the middle mediastinum, anterior to the main-stem bronchi or in the aortopulmonary window, are considered to be the results of embryologic misplacement of a superior parathyroid gland [11]. It has been hypothesized that these glands develop there because the parathyroid primordium is divided or pushed laterally because of the passage of the carotid artery trunk during developmental events [12]. Such glands may often be supernumerary. Other reported rare locations of possible superior glands include sites within the carotid sheath [13, 14], the lateral triangle of the neck [15, 16], within the esophageal wall [17], or, less commonly than inferior parathyroid glands, intrathyroidal [14, 18].

Ectopic inferior parathyroid glands Undescended inferior glands, although relatively rare, are a well-established embryologic abnormality. The inferior parathyroid gland, which develops from the third branchial pouch, along with the major portion of the thymus, tends to move with the thymus anteriorly in its descent and usually arrests at the level of the inferior pole of the thyroid gland. A significant number (10–40%) descend further and are found in the thyrothymic tract or the upper thymic tongue. Fewer than 2% are pulled into the deep mediastinum at a lower level than the aortic arch. If an inferior gland fails to descend with the thymus, it may remain at its site of embryologic origin at or above the carotid bifurcation. Although anatomic studies suggest that this may occur in 1–2%, the incidence of an undescended abnormal inferior parathyroid gland is less than 1% in most clinical series of primary cervical explorations for hyperparathyroidism [19]. They may also be subcapsular, or completely intrathyroidal in 1% or may reside within or closely to the cervical or anterior mediastinal thymus.

Supernumerary parathyroid glands Supernumerary parathyroid glands occur in 2.5% to 15% of individuals [2, 20]. The majority of supernumerary glands are small, rudimentary, or divided. However, when enlarged, these additional glands

may be responsible for persistent hyperparathyroidism after failed parathyroid exploration, especially in patients with secondary hyperparathyroidism or hyperparathyroidism associated with familial syndromes [2, 21, 22]. In a serie of 137 cases of persistent hyperparathyroidism after parathyroidectomy, supernumerary glands were found in 15% of cases [20]. They can range from 5 to 8 in number [3]. Supernumerary glands can reside anywhere from behind the thyroid down to and including within the thymus, representing the line of descent of thymic tissue during embryologic development. The most common location is within the thymus or in relation to the thyrothymic ligament (two thirds of cases) [3, 22]. The remaining supernumerary glands are usually found in the vicinity of the mid-thyroid lobe between two other glands.

Blood Supply

The arterial supply to both superior and inferior parathyroid glands is provided by the inferior thyroid artery in about 76–86% of cases [23]. Each parathyroid gland usually has its own end-artery. Most parathyroid glands have a single arterial supply (80%), some have a dual artery supply (15%), and a minority have multiple arterial supply (5%) [24]. The venous drainage of the parathyroid glands consists of the superior, middle, and inferior thyroid veins that drain into the internal jugular vein or the innominate vein.

During thyroid surgery, the surgeon should try to preserve all of the parathyroid glands in situ with adequate blood supply whenever possible. However, the blood supply may not be adequate following dissection of the thyroid gland, and the parathyroids are not always clearly identified. It can be difficult to make a reliable intraoperative determination of individual parathyroid function and patients may experience transient hypoparathyroidism despite having all four parathyroid glands preserved.

Superior parathyroid glands The superior parathyroid glands receive most of their blood supply from the inferior thyroid artery and are also supplied by branches of the superior thyroid artery in 15–20% of patients. A superior parathyroid gland that is supplied by the superior thyroid artery will usually be located in close proximity to the superior pole of the thyroid. A subcapsular dissection on the postero-lateral surface can bring to in the identification of parathyroid glands.

Inferior parathyroid glands The inferior parathyroid glands receive their end-arterial blood supply from the inferior thyroid artery. Therefore, gentle medial mobilization of the parathyroid rim from the thyroid capsule and preservation of the lateral arteriole going to the parathyroid gland is important for preserving functioning inferior parathyroid glands. Ligation of the branches of the inferior thyroid artery, close to the thyroid parenchyma and medial to the recurrent laryngeal nerve, may help preserve intact parathyroid vascularity.

Nerve Supply

The innervations of parathyroid glands is sympathetic, consisting of an extensive supply of nerves, derived either directly from the cervical, middle and superior sympathetic ganglia or from an intrafascial plexus located on the posterior lobar side.

It is important to note that these nerves are vasomotor, not secretomotor. Endocrine secretion of parathyroid hormone is controlled hormonally by variations in calcium levels: inhibited by its increase and stimulated by its fall.

Lymphatic Drainage

The lymphatic drainage of the parathyroid glands is carried out by numerous lymphatic vessels which tend to associate with those of the thyroid and thymus. Lymphatic vessels from the parathyroid glands drain into deep cervical lymph nodes and paratracheal lymph nodes.

Anatomy of Parathyroid Glands in ESRD

The overactivity of the parathyroid gland, known as secondary hyperparathyroidism, is a well known feature of chronic renal failure. It is an adaptive process characterized by an increase in the synthesis and secretion of parathyroid hormone (PTH), mainly due to some disturbances of calcium, phosphate, and vitamin D metabolism. The chronic increase in PTH production goes along with an increase in parathyroid gland size [25–27] due to cell proliferation that leads to an increase in cell number [26, 28].

Most ESRD patients with hyperparathyroidism (HPT) have four-gland enlargement. They can enlarge significantly, from 500 to 1000 mg per gland, remaining histologically hypercellular [29, 30]. However, some glands may descend into deep retroesophageal or paratracheal spaces, which makes them difficult to discern [31]. Patients with ESRD may also have enlargement of ectopic parathyroid rests in the cervical thymus [32]. Single or double adenomas are rarely seen in ESRD patients but have been reported in up to 24% of patients post renal transplantation [33].

Histology of Parathyroid Glands

Histologically the parathyroid glands are quite easily recognizable from the thyroid as they are organized in nests and cords of densely packed cells, in contrast with the follicular structure of the thyroid. Each gland is surrounded by a thin

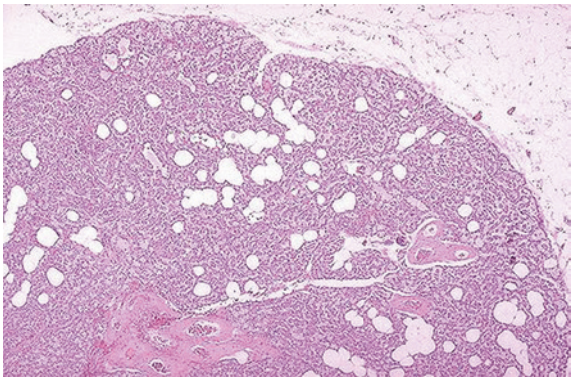

Fig. 1 Normal parathyroid gland

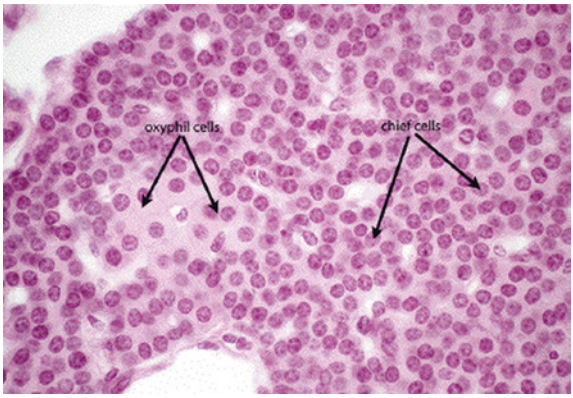

Fig. 2 A high power view of the parathyroid reveals two major cells types, the small chief cells characterized by rather scant, lightly-stained cytoplasm, and the much larger, eosinophilic oxyphil cells, which are often found in small clusters. The chief cells are responsible for the production of parathyroid hormone, while the function of the oxyphil cells is not known

connective tissue capsule but it is not divided into lobules. The parathyroid cells are surrounded and supported by a reticular connective tissue framework, rich in adipocytes which increase in number after puberty, so that the cordonal structure is divided into clusters and cell nests (Fig. 1).

There are two main types of cells in the parathyroid gland: chief or principle cells and oxyphil cells (Fig. 2).

The chief cells are much more prevalent than the oxyphil cells. Their function is to synthesize and release parathyroid hormone (PTH). They are polygonal in shape with a round nucleus and they have a different structure depending on their functional stage: when inactive they contain fewer profiles of rough endoplasmatic reticulum (RER) and Golgi complex and they are filled with glycogen and cytoplasmic lipofuscin; when active they present a lot of RER, Golgi apparatus and secretory vesicles. These cells are small and pale eosinophilic staining. They appear dark when loaded with parathyroid hormone, and paler when the hormone has been secreted, or in their resting state. In adults about 80% of the cells are resting while in children more cells are active. Differently from the thyroid in which the activity of the adjacent cells is coordinated, in parathyroid glands the chief

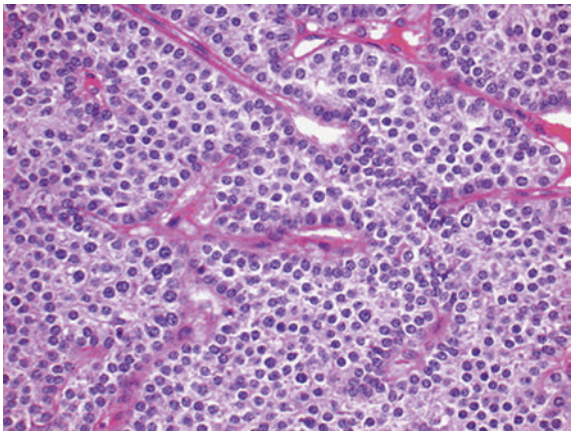

Fig. 3 Chief cells: 6–8 microns, polygonal, central round nuclei, contain granules of parathyroid hormone (PTH); basic cell type, other cell types are due to differences in physiologic activity; 80% of chief cells have intracellular fat; Chief cell is most sensitive to changes in ionized calcium

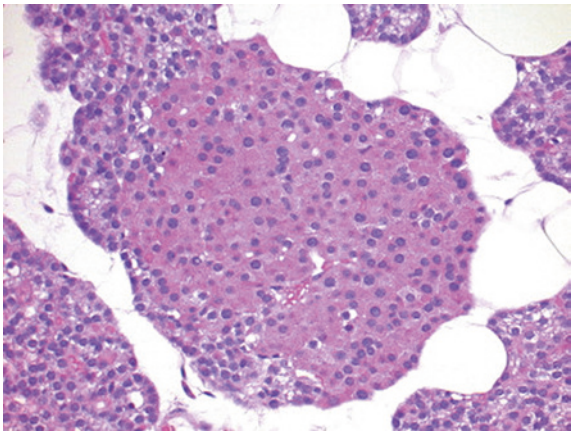

Fig. 4 Oxyphil cells: slightly larger than chief cell (12 microns), acidophilic cytoplasm due to mitochondria; no secretory granules; first appear at puberty as single cells, then pairs, then nodules at age 40

cells undergo cycles of activity and inactivity independently one from another, depending on calcium serum levels (Fig. 3).

The oxyphil cells are bigger and lighter in appearance than chief cells. They contain a small heterochromatic nucleus and their cytosol is eosinophilic, rich in mitochondria and glycogen. They appear in the parathyroid after puberty and increase in number with age. Their function is still unknown, however oxyphil cells have been shown to express parathyroid-relevant genes found in the chief cells and have the potential to produce additional autocrine/paracrine factors, such as parathyroid hormone-related protein (PTHrP) and calcitriol (Fig. 4).

Histology of Parathyroid Glands in ESRD

As already seen before, secondary hyperparathyroidism (HPTs) is an adaptive increase of the parathyroid parenchymal mass due to proliferation of chief cells and

oxyphil cells in multiple parathyroid glands in the presence of a known stimulus for parathyroid hormone secretion. Chronic renal failure is a common cause of secondary hyperparathyroidism, which is one of the most serious complications in long-term haemodialysis patients [34]. The usual histopathological findings in this case is diffusive or nodular hyperplasia. In initial stages of chronic renal failure parathyroid cell proliferation appears to be diffusive and homogeneous, whereas nodular formations develop within enlarged parathyroid glands in advanced stages of renal failure, mostly in chronic dialysis patients with severe secondary hyperparathyroidism.

Compared with normal glands, parathyroid glands in ESRD patients have a preponderance of oxyphilic parathyroid cells, which is significantly more frequent in nodular hyperplasia, more fibrosis with a sclerosed architecture, and more dystrophic calcifications [29, 30]. Such shape and texture changes obscure a clear vascular pedicle, which makes shaping a parathyroid remnant more difficult.

Histopathological studies performed on patients who underwent parathyroidectomy for refractory hyperparathyroidism due to chronic renal failure showed asymmetric enlargement, nodularities and increased number of oxyphil cells. Nodular hyperplasia was the most frequent cause of refractory hyperparathyroidism in uraemic patients; moreover, it indicated more aggressive proliferation since in DNA analysis the relative number of scattered cells in the DNA synthesis phase was significantly greater in nodular than in diffuse hyperplasia. In addition, the calcium set point for the inhibition of PTH secretion was found to be higher in the cells from nodular hyperplasia than in the cells obtained from diffuse hyperplasia. Though there was no difference in expression of PTH mRNA in nodular and diffuse hyperplasia, these data suggest that nodular hyperplasia is more progressively hyperplastic, has more aggressive proliferative activities and show more abnormal regulation of PTH secretion. (Fig. 5).

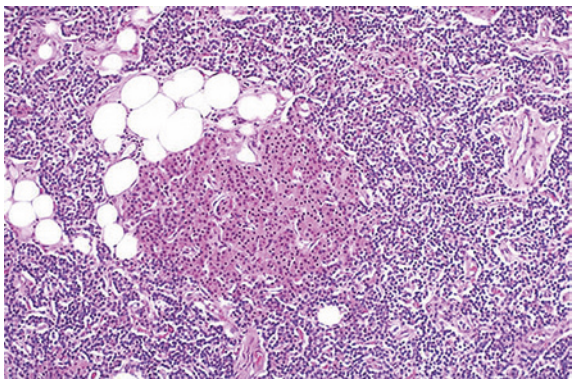

Fig. 5 In parathyroid hyperplasia, there is little or no adipose tissue, but any or all cell types normally found in a parathyroid gland are present. Note the pink oxyphil cells in the nodule seen here. This case shown here is “secondary hyperparathyroidism“ with all parathyroid glands enlarged as a consequence of chronic renal failure with impaired phosphate excretion. The increased serum phosphate tends to drive serum calcium down, which in turn drives the parathyroids to secrete more parathormone

These studies also revealed that patients with higher parathyroid gland mass, longer duration of renal disease and haemodialysis treatment more frequently have nodular hyperplasia, and that oxyphil cells and acinar cell arrangements are marked features of this pattern of hyperplasia [35].

Physiology of the Parathyroid Glands

The main function of the parathyroid glands is to produce parathyroid hormone (PTH). PTH is one of three key hormones modulating calcium and phosphate homeostasis; being the other two calcitriol (1,25-dihydroxyvitamin D) and fibroblast growth factor 23 (FGF23).

PTH is synthesized as a 115-amino acid polypeptide called pre-pro-PTH, which is cleaved within parathyroid cells at the amino-terminal portion, first to pro-PTH (90 amino acids) and then to PTH (84 amino acids). The 84-amino acids form is the stored, secreted and biologically active hormone. The biosynthetic process is estimated to take less than one hour while the secretion by exocytosis takes place within seconds after induction of hypocalcemia. Once secreted, PTH is rapidly cleared from plasma through uptake mainly by the liver and kidney: 1–84 PTH is cleaved into active amino- and inactive carboxyl-terminal fragments that are then cleared by the kidney. Intact PTH has a plasma half-life of two to four minutes while the carboxyl-terminal fragments have half-lives that are 5–10 times greater.

PTH Functions

The primary function of PTH is to maintain the extracellular fluid calcium concentration within a narrow normal range. The hormone acts directly on bone and kidney and indirectly on the intestine through its effects on synthesis of 1,25 OH2D to increase serum calcium concentration; in turn, PTH production is closely regulated by the concentration of serum ionized calcium (Fig. 6).

In particular the increase in PTH release raises the serum calcium concentration toward normal ranges in three ways:

- increased bone resorption, which occurs within minutes after PTH secretion increases
- increased intestinal calcium absorption mediated by increased production of calcitriol, the most active form of vitamin D, which occurs at least a day after PTH secretion increases
- decreased urinary calcium excretion due to stimulation of calcium reabsorption in the distal tubule, which occurs within minutes after PTH secretion increases.

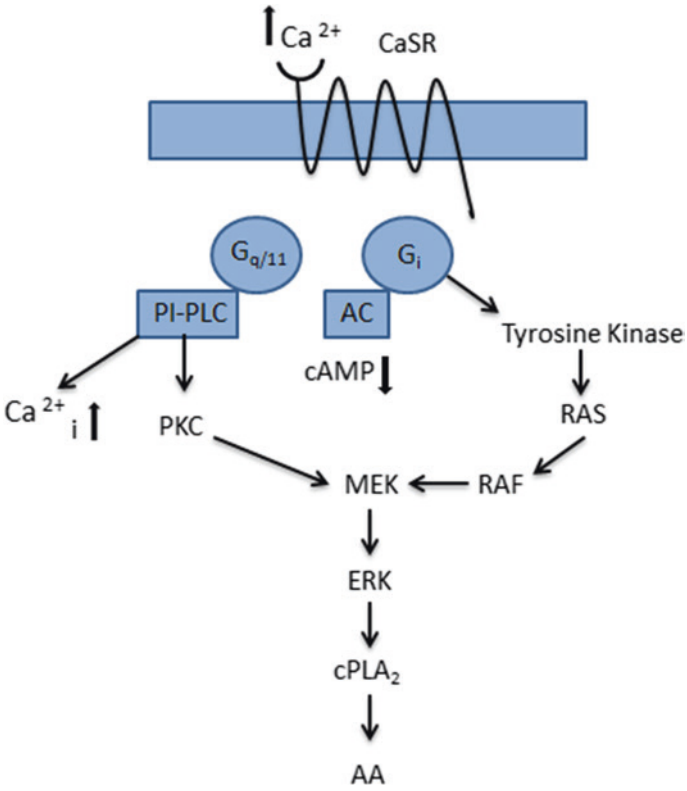

Fig. 6 PTH actions

On a more chronic basis, PTH also stimulates the conversion of calcidiol (25-hydroxyvitamin D) to calcitriol in renal tubular cells, thereby stimulating intestinal calcium absorption as well as bone turnover. Calcitriol inhibits PTH secretion through an indirect negative feedback which consists in its positive action on calcium levels; it also has a direct inhibitory action on PTH biosynthesis and parathyroid cell proliferation.

Skeletal Actions of PTH

PTH acts on bone, the main reservoir of calcium, to release calcium in two different phases [36]. The immediate effect of PTH is to mobilize calcium from skeletal stores that are readily available and in equilibrium with the extracellular fluid. Secondly PTH stimulates the release of calcium and phosphate by activation of bone resorption.

It is known that osteoblasts and not osteoclasts express PTH receptors, so osteoblasts are the main target for bone remodeling. However osteoclasts are indirectly activated in this process.

In fact, under PTH stimulation, pre-osteoblasts mature into bone-forming osteoblasts that produce collagen and subsequently mineralize matrix [37]. Since the remodeling unit is always coupled, once pre-osteoblasts are stimulated, they release cytokines that can activate osteoclasts resulting in bone resorption.

Thus, osteoclast formation requires an interaction with osteoblasts, which may depend upon cell to cell contact or regulators of osteoclast formation such as RANK (the receptor activator of nuclear factor kappa-B), osteoprotegerin, and RANK ligand (RANKL) [38]. PTH, then, can increase osteoclasts in number and activity indirectly through its effects on RANKL and osteoprotegerin [39].

New evidences suggest that PTH can actually bind osteoclasts through a different and incompletely characterized PTH receptor with specificity for the carboxyl-terminal region of PTH (C-PTHRs).

The net effect of PTH on bone can vary according to the severity and chronicity of the PTH excess. Chronic exposure to high serum PTH concentrations, typical of primary or secondary hyperparathyroidism, results in net bone reabsorption, whereas intermittent administration of recombinant human PTH (both full-length 1–84 or a 1–34 amino acids fragment) has been seen to stimulate bone formation more than resorption.

Moreover, specific elements of the PTH molecule seem essential for bone anabolism. PTH fragments 1–31 and 1–34 retain all of the biologic activity of the intact peptide; while amino-terminal truncation of the first two amino acids of PTH eliminates most of the cyclic adenosine monophosphate (cAMP) signaling, a pathway that seems important for the anabolic effect of PTH on bone [40, 41].

Secondary Hyperparathyroidism in ESRD

Secondary hyperparathyroidism (SHPT) is an adaptive and in many cases ultimately maladaptive process that develops in response to declining kidney function, impaired phosphate excretion, and failure to bioactivate vitamin D. Dysregulation of calcium and phosphorous homeostasis leads to decreased renal phosphate excretion, increased serum phosphorous, elevated levels of the phosphatonin fibroblast growth factor 23 (FGF-23), and reduced synthesis of calcitriol, the active form of vitamin D. These changes result into increased synthesis and secretion of parathyroid hormone (PTH) and parathyroid hyperplasia, contributing to the development of a vicious cycle.

Pathogenesis of SHPT

Continuous stimulation of the parathyroid glands because of a combination of elevated extracellular phosphate concentration, decreased extracellular ionized calcium concentration, and markedly reduced serum calcitriol leads to increased PTH

synthesis and release. At the same time, elevated FGF-23 expression downregulates residual renal 25(OH)-1-hydroxylase, which exacerbates the effective deficiency of calcitriol, acting as an additional driver to SHPT. Even at early stages in the development of hyperparathyroidism, these changes are compounded of variable underexpression of the calcium-sensing receptor (CaSR) and vitamin D receptor (VDR), rendering the parathyroid cells unable to respond appropriately to ambient calcium and/or calcitriol. The resulting increase in proliferative activity in the parathyroid glands, eventually, leads to parathyroid hyperplasia.

Recent understanding of the molecular mechanisms behind phosphorous homeostasis has shown FGF-23 and its receptor fibroblast growth factor receptor 1 (FGFR1) to be important players [42]. Increases in both serum FGF-23 and PTH, in patients with chronic kidney disease (CKD), decrease the proximal tubular reabsorption of phosphate and maintain normo-phosphatemia in most patients until the GFR falls below 20 ml/min. Inevitably, as CKD progresses, these negative feedback loops are progressively sabotaged and eventually unable to maintain phosphate homeostasis.

Molecular Biology of Parathyroid Glands (Monoclonality)

As already mentioned in the previous chapters, PTH is synthesized as a precursor, the pre-pro-PTH, from which pro-PTH and the secreted form of PTH are then produced by two proteolytic cleavages. The pre-peptide is made of a central hydrophobic core and, often, charged amino acids at the N-terminal and C-terminal ends. The removal of the pre-peptide to produce pro-PTH is mediated by an enzyme associated with microsomes. After the cleavage the pre-peptide is rapidly degraded so that no labeled pre-peptide can be detected in biosynthetic cells. The proteolytic removal of the pre-peptide probably occurs before completion of the pro-PTH nascent chain, since pre-pro-PTH is difficult to detect in intact cells.

A comparison of the amino acid sequences of PTH showed a highly conserved PTH sequence among different species. In particular there are three regions relatively conserved: the first two regions enclose the biologically active site, in which an addition or loss of a single amino acid greatly reduces biological activity. This region is involved in binding of PTH to the receptor. The third region is located at the C-terminal side and it may have a biological effect on osteoclasts.

The PTH gene contains two introns that divide the gene into 3 exons that code, respectively, for a 5′ untranslated region (the signal peptide), PTH plus a 3′ untranslated region.

PTH, secreted as an 84 amino acid polypeptide, and a related molecule, parathyroid hormone-related protein (PTHrP), act on cells via a common G protein-coupled, seven-transmembrane helix receptor. The activated PTH1 receptor stimulates adenylyl cyclase and phospholipase C pathways.

The natural occurring PTH (1–37) fragment as well as PTH (1–34) maintains all the activities of the intact 1–84 hormone, since it has all the elements necessary to bind and activate PTH1 receptor. In particular the N-terminal region of the peptide is critical for full activation of the receptor; the N-terminal truncated peptide PTH (3–34) is a partial agonist while the further shortened peptide PTH (7–34) is a low affinity antagonist. Also, residues 17 to 31, near the C-terminal side of the PTH (1–34) are necessary for high affinity receptor binding.

PTHrP, physiologically produced in several tissues, is identical in sequence to PTH in the first 13 amino acids so it binds the same G-protein-coupled receptor and its N-terminal fragment has many functions that mimic those of full length PTHrP, PTH (1–34) and PTH (1–84).

In addition to the PTH1 receptor, another receptor named PTH2 has been identified. Its natural ligand, tuberoinfundibular peptide 39 (TIP39), is a structural PTH homolog. The PTH2 receptor shares 70% sequence similarity with the PTH1 receptor, but PTH1 receptor cannot be activated by TIP39 and PTH2 receptor does not respond properly to PTHrP. TIP39 binds to the PTH1 receptor, but its N-terminal domain is unable to stimulate cAMP accumulation, so it works as an antagonist at the PTH1 receptor.

The structure of human PTH (1–34) is a slightly bent helix while the PTH-PTHr complex structure is not fully determined yet.

PTH secretion is regulated by the extracellular calcium concentration, acting on calcium-sensing receptor (CaSR), a G-protein-coupled transmembrane receptor, mainly expressed on parathyroid chief cells, renal distal tubules and thyroid C-cells. CaSR signaling involves phospholipase C (PI-PLC), adenylate cyclase and mitogen-activated protein kinases (MAPK) pathways (ERK 1–2). When extracellular calcium concentration impairs, an acute secretory PTH response starts within few seconds and it can last for 60–90 min, increasing Ca^{2+} renal tubular reabsorption, Ca^{2+} releasing from bone and promoting 1,25(OH)2D3 synthesis in the renal proximal tubules, with increase in intestinal Ca^{2+} absorption.

CaSR, encoded by CaSR gene on chromosome 3 (3q13.3-21), has four main structural domains: a large NH2-terminal extracellular domain, a cysteine-rich domain linking the ECD to the first transmembrane helix, seven transmembrane domains and an intracellular COOH-terminal tail [43]. After its synthesis as a monomer, in the endoplasmic reticulum CaSR dimerizes through intermolecular disulfide bonds between cysteines 129 and 131 within each monomer; non-covalent hydrophobic interactions also contribute to the dimeric CaSR. Before reaching the cell surface, the receptor is glycosylated in the Golgi apparatus. This glycosylation seems to be necessary for right cell surface expression. The extracellular domain has a bilobed structure with a slot between the two lobes that contains the binding site for Ca^{2+}, called venus flytrap (VFT)-like motif. In absence of agonist, the VFT is open and it closes upon binding Ca^{2+}, with conformational changes in the TMD and intracellular domains that initiate signal transduction. When Ca^{2+} binds to CaSR, G proteins Gq/11, Gi, and G12/13 are activated and stimulate phospholipase C (PLC), inhibit adenylate cyclase, and activate Rho

kinase, respectively (Fig. 7). CaSR can also lower cAMP indirectly by increasing intracellular Ca^{2+}, thereby reducing the activity of Ca^{2+}-inhibitable adenylate cyclase or activating phosphodiesterase [44]. Other intracellular signaling systems are involved in CaSR signaling, as ERK 1/2, phospholipases A2 and D, and the epidermal growth factor (EGF) receptor.

PTH secretion is regulated by calcium and phosphate, that regulate the gene expression and parathyroid proliferation too. Ca^{2+} and Pi PTH-regulation acts on PTH mRNA stability, with a post-transcriptional mechanism, by binding the protective trans acting parathyroid cytosolic proteins to a cis instability region in the 3′UTR. When serum Ca^{2+} concentration decreases, increased binding protects PTH mRNA from degradation by cytosolic ribonucleases, improving PTH secretion. When serum Pi concentration decreases, decreased binding stimulates an increased PTH mRNA degradation, impairing PTH secretion and its phosphaturic action. PTH mRNA binding proteins were purified by PTH RNA 3′-UTR affinity chromatography. One of these is identical to AU-rich binding factor (AUF1), involved in the stability of other mRNAs encoding for cytokines, oncoproteins and G-protein coupled receptors. The AUF1 stabilization mechanism is not clear

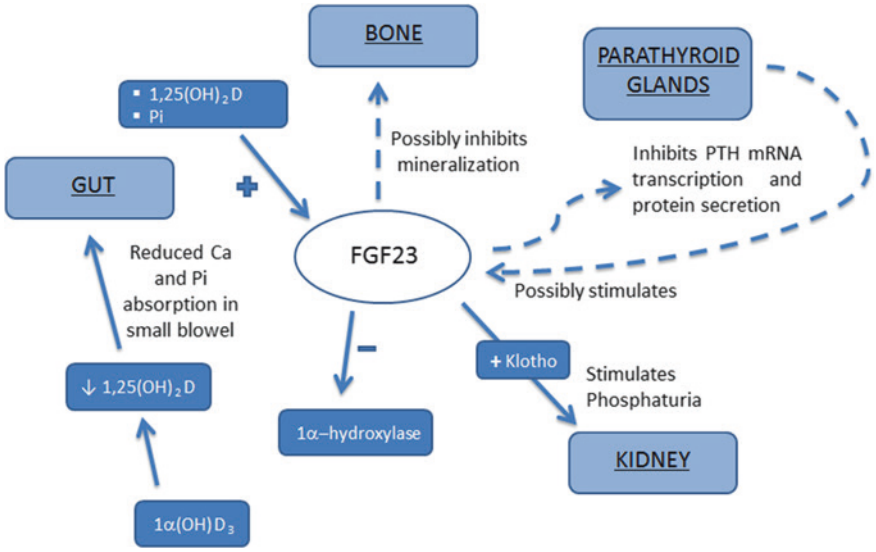

Fig. 7 Model for mechanisms for Ca^2-sensing receptor (CaR)-induced activation in the parathyroid cell. Activation of the 7-membrane-spanning CaR by extracellular Ca^{2+} results in Gq/11-mediated activation of phosphtidylinositol-phosphlipase C (PI-PLC), leading to intracellular Ca^2 (Ca^{2+}i) mobilization, protein kinase C (PKC) activation, and resultant stimulation of the mitogen-activating protein kinase (MAPK) cascade. The CaR also activates MAPK via an isoform of Gi protein, and subsequent downstream activation of a tyrosine kinase-dependent process, involving a RAS- and RAF-dependent series of steps. Activated MAPK then phosphorylates and activates $cPLA_2$, which releases free arachidonic acid (AA) that can be metabolized to biologically active mediators. MEK, ERK/MAPK kinase. AC, adenylate cyclase

(maybe post-translational). Other PTH mRNA binding proteins are hnRNP K. and Up stream of nras (UNR). Another protein, called dynein light chain or LC8 may play a role in the PTH mRNA intracellular localization in the parathyroid cells rather than in PTH mRNA stability.

PTH secretion is also regulated by 1,25-dihydroxyvitamin D [1,25(OH)2D3], that binds a specific PTH gene promoter sequence, called vitamin D response element (VDRE), reducing PTH gene transcription. VDR-RXR heterodimer binds to the VDRE, amplifying PTH-mRNA decrease. 1,25(OH)2D3 decreases PTH secretion also by increasing parathyroid VDR and CaSR concentrations. Calreticulin, a calcium binding protein, protects VDR-RXR from binding to VDRE.

Normally, parathyroid gland is in low turnover. Uremia, hypocalcemia, hyperphosphatemia and calcitriol deficiency induce parathyroid cells to divide by increasing cyclin/Cdk (cyclin dependent kinases) complexes and decreasing Cdk-inhibitors, that regulate mitotic division. Parathyroid hyperplasia is due to changes in the content of cell cycle regulators, like a cyclin D1 overexpression (also connected to human parathyroid adenomas).

In secondary hyperparathyroidism, initially glands growth is polyclonal and diffuse, then it may become monoclonal, with aggressive proliferation, maybe due to cyclin D1 overexpression. Monoclonal proliferation and tumorigenesis are also connected to many genetic factors, like protooncogene amplification (PRAD1/cyclin D1) and tumor suppressor reduction (p27Kip1).

Uremia-induced parathyroid mitoses are also connected and enhanced by high dietary P, meanwhile P restriction prevents parathyroid cells replication, counteracting uremia induced proliferative signals, but not inducing apoptosis. In uremia, parathyroid glands enlarge because of tissue hyperplasia rather than hypertrophy.

Studies in rats demonstrate that P-restriction increases serum calcitriol, that directly activates tumor suppressor p21 gene transcription, explicating an antiproliferative action. Other not known factors may produce post-transcriptional enhancement of p21 protein expression, contributing to its antiproliferative action. p21, with p27 and p57, explicates its function inhibiting G1-cyclin/cdk complexes and arresting G1 cell growth and binding to PCNA (proliferating nuclear cell antigen—a mitotic activity marker) trimers causing DNase-polymerase to lose processivity.

High-dietary P has not effects on p21 reduction; some other factors are involved in high-dietary P-induced parathyroid hyperplasia in uremia, such as TGFα, that increases in hyperplastic glands more in case of high dietary P rather than in case of low dietary P. TGFα induces cell growth through autocrine and paracrine mechanisms upon EGFR activation by the mature, soluble TGFα isoform, and through a juxtacrine pathway involving transmembrane TGFα isoform from an adjacent parathyroid cell. When activated, EGFR signaling involves a ras/MAPK activation that induces cyclin D1 and the cell cycle passes from G1 to S phase. Nuclear EGFR also works as a transcription factor, binding to adenosine-thymidine-rich regions in the cyclin D1 promoter, maybe explaining its

high proliferating activity. In rat parathyroid glands, high dietary P enhances parathyroid EGFR content, while low dietary P reduces EGFR levels.

Calcitriol explicates an antiproliferative action on parathyroid glands via VDR, inducing p21 and decreasing c-myc expression (that normally regulates the progression from G1 to S phase in cell cycle); it limits the increase in parathyroid TGFα and EGFR, too. Therefore, in uremia, like the high dietary P, calcitriol deficiency and the development of a resistance to vitamin D action expose to an increased risk of parathyroid hyperplasia.

Monoclonality plays an important role in parathyroid tumorigenesis. Primary parathyroid hyperplasia, uremic parathyroid hyperplasia, MEN1-associated parathyroid tumors and parathyroid carcinoma (rare) contain a monoclonal component. They all originate from a single clone that undergoes a genetic somatic mutation that leads to a proliferative advantage; then proliferating, other somatic mutations promote and sustain this uncontrolled proliferation. Parathyroid tumorigenesis involves many different factors, such as oncogenes, tumor suppressor genes and other mechanisms.

Among the involved oncogenes can be found PRAD1 (parathyroid adenomatosis gene 1), cyclin D1, normally localized on long arm of chromosome 11 (11q13). In a small percentage of parathyroid tumors (5%), a pericentromeric inversion of an allele of chromosome 11 has been demonstrated, which results in a juxtaposition of the PRAD1 gene to the 5′ region of the PTH gene (11p15). As a consequence, the PRAD1 protein is overexpressed by stimulation by the PTH gene enhancers. Other oncogenes may be FGF-3, EGFR, KGFR (keratinocyte growth factor receptor) and RET proto-oncogene, whose germline missense mutation is involved in the MEN2A syndrome genesis (familial clustering of medullary thyroid carcinoma, pheochromocytoma and parathyroid tumors), but not in sporadic parathyroid tumors [45].

When a bi-allelic inactivation of a tumor suppressor gene occurs, a proliferative advantage is triggered, by which cell proliferates in an uncontrolled manner. In most cases there is an inactivating point mutation of an allele, while other suffers a gross somatic deletion. This somatic deletion is heralded by allelic loss (LOH—loss of heterozygosity). In parathyroid tumors there are nonrandom, chromosomal regions that display LOH and probably they host tumor suppressor genes. One of these regions is 13q11, MEN1 locus, involved in MEN1 syndrome and in sporadic benign parathyroid tumors. Inactivating mutations in the MEN1 gene result in loss of the gene product, menin, an inhibitor of the cyclin D1 proliferative signal; a reduction of the menin in parathyroid tissue involves the binding of NF-kβ to the Cyclin D1 promoter, with an increase in its pro-proliferative activity. Another region showing LOH, in sporadic parathyroid tumors is 1p36, involved in MEN2A. Other tumor suppressor genes marginally involved in parathyroid tumorigenesis could be p53, Rb.

Other molecular pathway could be also involved in parathyroid tumorigenesis, like microsatellite instability and telomerase activity.

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