PARATHYROID GLAND REGULATION: CONTRIBUTION OF THE *IN VIVO* AND *IN VITRO* MODELS

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ABSTRACT

Importance of the field: The current regulation of parathyroid hormone and the development of parathyroid disorders in chronic kidney disease involve complex mechanisms. Factors such as calcium, phosphorous, calcitriol, vitamin D receptor, calcium-sensing receptor and fibroblast growth factor 23 (FGF23), play a key role in the regulatory process in the pathogenesis of secondary hyperparathyroidism.

Areas covered in this review: This review provides an analysis of published results related to the different models and approaches used to study the mechanisms involved in the pathogenesis of secondary hyperparathyroidism. The review includes clinical studies, animal and *ex vivo/in vitro* models which have been extensively used in this area.

What the reader will gain: Readers will have an overview of the main findings and progresses achieved in the knowledge of the parathyroid function combining the results obtained from the different models used to understand the parathyroid gland regulation.

Take-home message: Each of the available models used to study the complex system of parathyroid regulation has advantages and limitations; therefore, it is necessary to combine the information obtained from more than one model in order to have a more complete knowledge of the mechanisms involved in parathyroid hormone regulation.

KEY WORDS

I*n vivo* models*, in vitro* models, parathyroid hormone, calcium, phosphorous, FGF23, parathyroid function, secondary hyperparathyroidism.

1. INTRODUCTION

The progression of chronic kidney disease (CKD) leads to a reduction of 1-alpha hydroxylase in the kidney, which in turn results in low levels of the active form of vitamin D (1.25-dihydroxyvitamin D_3-[$1,25(OH)_2D_3$] or calcitriol) impairing calcium absorption in the intestine favoring the reduction in serum calcium[1]. As a result, the decreases in serum calcium stimulate parathyroid hormone (PTH) synthesis and release, which in turn increase bone turnover, bone resorption and stimulate 1-alpha hydroxylase. All these mechanisms lead to compensatory increases in serum calcium.

In addition, the progressive reduction of the renal function impairs phosphorous excretion, leading to increases in serum phosphorous, which increases fibroblast growth factor 23 (FGF23) and PTH; both mechanisms increase urinary phosphorous excretion. However, FGF23 and PTH exert opposite effects on calcitriol synthesis: FGF23 inhibits 1-alpha hydroxylase, whereas PTH stimulates it.

As the renal function decreases, all these complex and tightly interrelated mechanisms of parathyroid gland regulation fail to adequately control the parathyroid gland function. As a result, low serum levels of calcitriol and calcium and phosphorous retention are present at late stages of CKD. Furthermore, at advanced stages of this disorder, CKD stage 5 patients show severe forms of secondary hyperparathyroidism with diffuse and nodular hyperplasia and a significant reduction in the vitamin D and calcium-sensing receptors (VDR and CaSR) expression with a poor response of the parathyroid glands to calcium changes and vitamin D analogs therapy, and a clear trend towards autonomous (tertiary) parathyroid gland behavior.

Several of the mineral abnormalities end up inducing not only a bone disease, but also several cardiovascular disorders, including vascular calcifications and a greater risk of mortality[2]. The recently coined term “Chronic Kidney Disease-Mineral and Bone

Disorder" (CKD-MBD) encompasses all these abnormalities[3]. CKD-MBD includes either one or a combination of (a) calcium, phosphorous, PTH, or vitamin D metabolism; (b) bone turnover, mineralization, volume, linear growth or strength; and (c) vascular or other soft tissue calcification. There are excellent reviews focused on different aspects of CKD-MBD[4-7]; however, this review will deal mainly with aspects related to the parathyroid gland regulation.

1.1 Parathyroid Hormone

PTH is synthesized by the parathyroid cells; through the blood stream PTH reaches the main target sites: the kidney and bone[8]. The main role of PTH is the regulation of calcium homeostasis. Under physiological conditions, osteoblasts are stimulated by PTH via its specific PTH receptor, which then send signals to bone marrow-derived osteoclast precursors to stimulate their fusion, differentiation and activation. The mature and active osteoclasts resorb bone and release calcium to the blood. In addition, PTH provides an additional calcium homeostatic response in order to preserve normal serum calcium levels acting in the kidneys by increasing tubular calcium reabsorption.

PTH can also exert other anabolic actions in bone. Intermittent or pulsatile injections of recombinant PTH, as well as injections with amino-terminal fragments, are able to increase bone formation and bone mass; the latter became the basis for the use of PTH injections to treat osteoporosis[9]. Besides vitamin D, PTH is the only other anabolic bone agent known.

1.2 Parathyroid gland regulation

PTH regulation involves a complex mechanism in which calcium[10], calcitriol[11], phosphorous[12], and FGF23[13] play a central role.

Both calcium and calcitriol act on the parathyroid cells trough their specific receptors; CaSR and VDR. While the CaSR is a cell-membrane receptor member of the G-protein-coupled receptor family, the VDR is a nuclear receptor that, when bound to vitamin D, acts as a transcription factor. The differences in the nature of the two ligands and their receptors lead to two different mechanisms of action with a complementary function on the parathyroid cells.

On one hand, small decreases in extracellular calcium concentrations are rapidly sensed by the CaSR, triggering, within seconds or minutes, an increase in PTH release. Small increases in calcium are also sensed by the CaSR, yielding opposite results[14-16]. If the stimulus persists for longer periods (hours, days), calcium is able to regulate PTH synthesis post-transcriptionally by modifying the mRNA stability through differences in binding of the parathyroid proteins to an element in its 3'-untranslated region[17-18]. As a result, the decreases in serum calcium reduce mRNA degradation by increasing its stability and the half-life of mRNA PTH. By contrast, the active form of vitamin D (calcitriol) inhibits the PTH gene transcription resulting in a reduction of the PTH synthesis[19-22].

In CKD, the reduction of renal function and active renal mass, together with the increase in serum FGF23[23], decrease 1-alpha hydroxylase synthesis with the consequent reduction in calcitriol synthesis which, in turn, decreases intestinal calcium absorption but also leads a lower PTH gene transcription suppression. Both mechanisms favor the increase of PTH.

High phosphorous is another factor able to act on the parathyroid cells by increasing PTH synthesis[12, 24-25] through a post-transcriptional mechanism stabilizing the PTH mRNA[17]. Finally, calcium, calcitriol and phosphorous, are well-known factors involved in parathyroid cell proliferation, thus, abnormalities of these factors may contribute to the development of parathyroid gland hyperplasia[26-27].

Although the effects of calcium, calcitriol and even phosphorous on the parathyroid function take place through specific mechanisms, these three factors also produce additional complementary effects, mainly through their interaction with the CaSR and VDR parathyroid receptors.

The main function of the CaSR is to sense calcium; however, the CaSR expression in parathyroid glands does not seem to be regulated by the extracellular calcium levels[28-30]. In contrast, it has described that CaSR expression can be regulated by calcitriol, which can increase CaSR even under hypocalcaemic conditions[28, 31-32]. Also, phosphorous may influence the CaSR expression; in fact, a reduction in the expression of CaSR has been described in the presence of hyperphosphataemia[33-35].

Regarding VDR regulation, contrary to what occurs with calcium and CaSR regulation, calcitriol does regulate its own receptor, VDR, stimulating its synthesis and half-life[28, 36-37]. In addition, calcium is also able to modify the VDR expression[28, 32, 38-40].

Finally, FGF23, initially described as a potent phosphatonin[41], is involved not only in the control of phosphorous but also in the regulation of vitamin D metabolism[42] and PTH synthesis[13]. Elegant studies performed by Silver et al.[13] have demonstrated a direct effect of FGF23 on the parathyroid gland through the MAPK pathway, leading to a decrease in PTH synthesis and secretion. In addition, the putative, indirect regulation of PTH by estrogens through FGF23 has been also recently described[43]. The importance of FGF23 in the pathogenesis of secondary hyperparathyroidism is still under

investigation; nevertheless, alternative paradigms for the pathogenesis of secondary hyperparathyroidism in chronic kidney disease involving FGF23 have been recently proposed[44].

1.3 Other important factors involved in parathyroid hormone secretion

Other factors such as calcimimetics and aluminum can also act on the PTH regulation.

Calcimimetics, the CaSR allosteric modulators recently introduced for the treatment of secondary hyperparathyroidism, act by increasing CaSR sensitivity to extracellular calcium, decreasing PTH synthesis and secretion[45-47]. Moreover, calcimimetics are also able to cooperate with VDR activators, increasing the VDR expression in the parathyroid glands[48]. As a result, the use of calcimimetics not only achieves the known effect on the CaSR, but they can also increase the VDR expression.

Finally, several studies have demonstrated the inhibitory effect of aluminum on PTH mRNA levels through complex and combined mechanisms, including a direct action on the CaSR, by reducing its gene expression through a post-transcriptional mechanism[49-50]. It seems clear that the parathyroid gland is a target tissue for aluminum, and part of the direct inhibitory effect of aluminum is also due to its capacity to reduce cell proliferation in the parathyroid gland[50].

Most of the previously described findings have been obtained thanks to the contribution of different *in vivo* and *ex vivo/in vitro* models used to study the regulation of the parathyroid gland function. The remaining part of this review will describe and analyze different models which have been used to investigate the complex mechanisms involved in PTH regulation.

2. MODELS USED TO STUDY THE PARATHYROID GLAND FUNCTION

2.1 Contribution of human clinical studies

The human clinical studies were mostly aimed to obtain information at the functional, morphological, molecular and genomic levels.

Clinical studies carried out in humans have revealed important aspects of the pathogenesis of secondary hyperparathyroidism. Functional studies carried out in humans have clearly shown that hypocalcaemia stimulates PTH secretion, while hypercalcaemia suppresses it. The mathematical model which best relates PTH and serum calcium is a sigmoidal curve[51]. The set-point for calcium has been defined as the calcium concentration at which the maximal PTH is reduced by 50%[52]. In the CKD patients with secondary hyperparathyroidism, the set-point of calcium is shifted to the right, meaning that a higher concentration of calcium is necessary to suppress the PTH. This is the result of a sum of factors which end up increasing the size of the parathyroid gland and decreasing VDR and CaSR expression, the main receptors known to be involved in the parathyroid gland regulation[53-56].

This functional relationship between calcium and PTH has been extensively used in humans to investigate the effect of VDR activators and calcimimetics on the parathyroid gland, assuming that if they are effective they should be able to partly or totally correct the abnormal calcium and parathyroid sigmoidal relationship observed in the advanced forms of secondary hyperparathyroidism. So far, both have been able to shift the set-point of calcium to the left, partly recovering parathyroid sensitivity to calcium [57]. Finally, a successful parathyroidectomy has also proved to be effective in reducing PTH levels and shifting the PTH-calcium curve to the left[58].

The importance and contribution of high phosphorous in the pathogenesis of secondary hyperparathyroidism was experimentally demonstrated many years ago[59].

However, since then, human studies have not been able to fully differentiate and dissect the effect of phosphorous independently from the changes in serum calcium[60] and/or calcitriol levels.

As secondary hyperparathyroidism progresses, the parathyroid glands become refractory to medical treatment and parathyroidectomy is frequently needed. Human parathyroid tissue obtained from parathyroidectomies has been used for genetic, genomic and molecular studies aiming to investigate in depth the mechanisms involved in parathyroid gland deregulation in advanced stages of parathyroid hyperplasia.

The most advanced form of this disorder, nodular hyperplasia, has been associated with chromosomal aberrations[61] and also with severe changes in gene expression[62-63]. To study these specific aspects in diffuse and nodular parathyroid glands from renal patients who underwent parathyroidectomy, several techniques have been used, such as comparative genomic hybridization[61], microarrays and bidirectional subtraction library[62]. The combination of these techniques has allowed to demonstrate that the progression of secondary hyperparathyroidism damages several cell pathways at different levels, a fact which partly explains the multiple, complex and integrated cellular mechanisms involved in the progression of the disease[62].

Cell growth is highly promoted in the nodules; DNA stability is severely compromised because the protective mechanism and repair systems fail; RNA synthesis and stability are also in jeopardy. Finally, protein synthesis, processing and destination become clearly hindered due to failures in the folding, assembly and sorting of the polypeptides. All these striking alterations, dominated by the profile of gene repression found in the severe cases of nodular secondary hyperparathyroidism, are almost impossible to control at this late stage of the disease and alert to the need of an early approach in the management of secondary hyperparathyroidism in CKD patients[62].

In the nodular severe forms of secondary hyperparathyroidism, the monoclonal growth of the parathyroid gland dominates the scene. A study revealed that 64% of hemodialysis patients with refractory secondary hyperparathyroidism showed at least one parathyroid lesion with monoclonal growth[64], whereas another study found that the monoclonal pattern was present in 58% of hyperplastic nodules in females[65]. However, the genes implicated in the genesis and evolution of secondary hyperparathyroidism monoclonality are not those observed in primary hyperparathyroidism[63].

Despite human studies being very useful to understand some aspects of the pathogenesis and progression of secondary hyperparathyroidism, they show several limitations, mainly due to the lack of homogeneity of the studies. As an example, most of the published works have been carried out in patients with different ages and different degrees of severity of secondary hyperparathyroidism receiving different treatments. Thus, the experimental models have been of great value to complement and further expand the investigation on parathyroid regulation.

2.2 Contribution of the *in vivo* animal models

The main advances in the understanding of the pathogenesis of secondary hyperparathyroidism have been possible mainly thanks to the results obtained using experimental animal models of CKD. Partial nephrectomy has been the most common technique used to produce CKD[26, 66-69], although more recently the addition of adenine to the diet has become a current model to study CKD-MBD[47, 70]. Five/six or 7/8 nephrectomy induce a moderate renal insufficiency, which in most cases is not enough to develop severe secondary hyperparathyroidism. To increase the magnitude of the latter, the concomitant use of high phosphorous diet was introduced to provide a

substantial extra stimulus, resulting in a more severe degree of secondary hyperparathyroidism.

The studies performed following the previously described procedures obtained significant increases in PTH gene expression[71] and severe secondary hyperparathyroidism. The stimulatory effect of high serum phosphorous on PTH is powerful enough to exert its action independently of the serum calcium levels[72], achieving PTH levels 20-40 times higher than those obtained using only partial nephrectomy with no phosphorous supplementation[33, 73].

The rat model with CKD has been used to describe that the regulation of the parathyroid function by calcium and phosphorous occurs at posttranscriptional level by regulating the binding of proteins to the 3´-UTR of the PTH mRNA[74]. Furthermore, the addition of high phosphorous levels to the diet not only induced a severe parathyroid hyperplasia, but also triggered a reduction in the expression of CaSR in the same areas of the parathyroid gland where an increased cell proliferation was observed[33]. Similar studies have also shown that the increase in cell proliferation rate produced by a high phosphorous diet precedes the down-regulation of CaSR[75], suggesting that the mechanisms which stimulate the parathyroid cell proliferation precede the reduction in CaSR expression.

The *in vivo* animal studies also have been fundamental to demonstrate the important effects of calcitriol, calcium and calcimimetics. Calcitriol inhibits PTH gene expression and stimulates VDR expression in parathyroid tissue[76-77]. Despite VDR being the specific receptor for calcitriol and other active vitamin D metabolites, serum calcium levels also influence VDR expression; in fact, a recent study has shown that the higher the serum calcium, the higher the expression of VDR levels[32].

The increased PTH gene expression in experimental uremia has been also reversed using calcimimetics which act via a posttranscriptional mechanism involving the trans-acting factor AUF1 present in the parathyroid glands[47].

Regarding regression of the parathyroid hyperplasia after different treatments, the animal models have helped to enhance the knowledge in this area. Calcitriol has shown to decrease cell proliferation and increase apoptosis restoring the levels of CaSR and VDR, leading to a regression of the parathyroid hyperplasia in uremic rats[78]. The direct injection of calcitriol or other vitamin D analogs into hyperplasic glands has also been able to induce cell apoptosis, suggesting this could be a valid, alternative method to reduce the size of the parathyroid gland[79]. Similarly, calcimimetics have proven to be able to reduce parathyroid cell proliferation and gland size in uremic rats with secondary hyperparathyroidism[80].

The animal models used to study secondary hyperparathyroidism have also been used to analyze other aspects of CKD-MBD, such as vascular and other soft tissue calcifications[81-83]. A recent study demonstrated that, after 20 weeks, rats with CKD receiving a high phosphorous diet developed not only severe secondary hyperparathyroidism but also severe vascular calcifications leading to changes in the gene and protein expression profiles of the calcified aortas[73]. In addition, other recent studies have been useful to show the effects and interactions of drugs such as calcitriol, paricalcitol, calcimimetics and biphosphonates in the production and regression of vascular calcifications[84-86].

One important general limitation of the animal models is that even though several parameters cannot be controlled, others frequently associated or related to uncontrolled or unmeasured parameters cannot be controlled, leading to undesirable modifications in other factors involved in PTH regulation. In some studies, this issue

makes it difficult to reach definitive conclusions about the chronology and/or the importance of the factors and mechanisms studied. To limit the influence of all these uncontrolled factors, other models such as knockout models or *ex vivo/in vitro* models have been extensively used to better understand the regulation of the parathyroid hormone secretion.

2.3 Contribution of the knockout models

Knockout models have been of great value to precise the role of several known factors in the pathogenesis of secondary hyperparathyroidism.

CaSR knockout has demonstrated the key role of this membrane protein in calcium homeostasis and PTH regulation[87]. Mice with a partial CaSR knockout are characterized by modest elevations of serum calcium and PTH levels as well as hypocalciuria, whereas mice with complete CaSR knockout show elevated serum calcium and PTH levels, parathyroid hyperplasia, bone abnormalities, retarded growth and premature death, thus demonstrating the great importance of the CaSR in several aspects of parathyroid gland regulation and bone health.

Recently, it has been described the role of the CaSR in maintaining calcium homeostasis in the absence of PTH and consequently the CaSR-regulated PTH secretion, by using single and double knockout mouse models for CaSR and/or PTH[88]. Thus, the double knockout for CaSR and PTH showed high serum calcium levels, similar to those presented in the single CaR-null mice, supporting the fact that CaSR defends against hypercalcemia independently of its regulation of PTH secretion by increasing the urinary calcium excretion.

On the other hand, several papers have tried to demonstrate the effect of VDR on parathyroid gland regulation by using VDR null mice[89-91]. In all cases, VDR null mice

presented severe hypocalcemia and secondary hyperparathyroidism that could be corrected with a high calcium rescue diet, indirectly demonstrating the role of VDR in the normal parathyroid physiology. Furthermore, a recent study with a specific deletion of VDR has provided additional information on the role of this receptor in the control of the parathyroid gland. The deletion induced a moderate increase in PTH levels but also a reduction in the parathyroid CaSR expression, suggesting that the VDR has a limited role in the parathyroid gland regulation[92].

2.4 Contribution of the *ex vivo/in vitro* models

The functional and molecular studies of the response of the parathyroid glands using *ex vivo/in vitro* models have been limited: first, because of the lack of functional parathyroid cell lines and second, because of the limited functional long-term response to calcium observed when isolated parathyroid cells were cultured.

A great number of the *in vitro* studies have been carried out to demonstrate the viability and functionality of primary monolayer parathyroid cell cultures. The methodology followed for this type of culture is simple and there are no important differences in all the published papers[93-98]. Briefly, the parathyroid tissue is minced into small fragments and digested with collagenase in culture medium. Then, the parathyroid cells are released from the tissue to the culture medium, the cells are collected, centrifugated and resuspended in a growth media to work with them.

Unfortunately, despite the simplicity of the procedure to obtain the cells, dispersed or primary monolayer parathyroid cells such as those from bovine often become progressively less responsive to changes in extracellular calcium[93-94] likely due to a rapid decrease in CaSR mRNA and protein levels, a phenomenon observed after a

few hours of culture, which seems not to be influenced by the culture conditions (medium serum, calcium, or calcitriol).

In contrast, another study has found that proliferating bovine parathyroid cells in early passages preserve their functionality, and they were able to respond to calcium and calcitriol even after 72 hours after subconfluency[95]. Under these conditions, it has been found that calcitriol is able to suppress cell proliferation. Similarly, positive results have been also obtained with a human parathyroid cell culture model from uremic patients with secondary hyperparathyroidism[96], in which the parathyroid cells remain viable and functional until the fifth passage, which corresponds approximately to 5 months of follow-up as assessed by persistent responsiveness to changes in extracellular calcium.

In addition, by using this model, it has been found that calcium, calcimimetics and calcitriol were able to decrease parathyroid cell proliferation, whereas phosphorous increased it[97]. Monolayer cultures of bovine parathyroid cells have been also successfully used to study the ability of FGF23 to regulate PTH and 1alpha-hydroxylase expression[98].

In summary, despite the mentioned controversy and limitations, mainly concerning the study of PTH regulation by calcium, primary monolayer cell cultures have been of great usefulness to study the other important factors involved in parathyroid gland regulation, such as calcitriol, calcimimetics, phosphorous and FGF23.

To improve the performance of the parathyroid cell culture model, a new approach has been recently employed. It consists in the coincubation of the bovine parathyroid cells with a t-tail type I collagen; after 1-2 weeks, the parathyroid cells coalesce into a three-dimensional organoid, termed “pseudoglands”. It has been shown that the CaSR mRNA expression in these pseudoglands decreased after 1 day of incubation; however, later on, this negative effect apparently disappears and the CaSR

expression increased becoming almost normal after longer periods of culture. Using this model it was proven that PTH mRNA can be suppressed by extracellular calcium, demonstrating its usefulness to the study of the calcium-mediated control of the parathyroid gland[99].

In addition, it has been recently described that parathyroid cells in culture were able to produce cell aggregates called "spheroids" which secreted parathyroid hormone for more than 150 days[100]. These has been recently used in parathyroid cells obtained from patients with secondary hyperparathyroidism in order to suppress the production of PTH by small interfering RNA (siRNA), a method which provides a useful approach for further studies. The results from the innovative parathyroid spheroid cell culture model stress once more the importance of maintaining a three-dimensional structure in order to have adequate parathyroid cell functionality.

Due to some of the above-mentioned limitations of parathyroid cell culture, non-parathyroid cells have also been used to study some specific mechanisms involved in parathyroid hyperplasia. For example, the human epidermoid carcinoma cell line, A431 which mimics hyperplastic parathyroid cells, has been used to demonstrate that the TGF-alpha/EGFR system is one of the key elements in the regulation of parathyroid hyperplasia[101-102]. In addition, Human Embryonic Kidney cells (HEK293) cotransfected with bovine CaSR have been used to demonstrate that aluminum is a strong agonist of the CaSR[50]. In addition and more recently, the same kidney cells cotransfected with both human CaSR and human PTH plasmids were used to study the regulation of PTH gene expression by the calcimimetic R568[103]. The results indicated that in the cells cotransfected with CaSR, the PTH gene expression was regulated by calcium and the calcimimetic R568; conversely, there was no response in cells without CaSR transfection. The PTH CaSR-dependent decreased gene expression observed in these

engineered cells occurs via the balanced interactions of the trans-acting factors KSRP and AUF1 with PTH mRNA, as already described *in vivo*[47, 74].

Despite all progresses, efforts and sophisticated adaptations such as the one described above, parathyroid cell culture is still a model with some limitations when used to study the PTH regulation by calcium. Therefore, some authors have had to use *ex vivo/in vitro* cultures of isolated parathyroid glands to further explore parathyroid regulation. In fact, the latter has become the reference model to analyze the parathyroid molecular mechanisms in response to different stimuli[12, 25, 104-105].

In this type of *ex vivo/in vitro* culture the whole rat parathyroid glands are extracted and excised from the surrounding thyroid tissue and then immediately placed in well plates containing the experimental culture medium. In the case of human parathyroid glands, slices of tissue are cultured following the same procedure.

By using this model and culturing parathyroid glands from rats, it has been found that calcium is able to acutely suppress PTH secretion as previously described *in vivo*[12, 106]. Similar results were obtained in human hyperplastic parathyroid glands[25]. This model has also allowed for the study of the effect of serum calcium and phosphorous on the parathyroid function[12, 106]. In fact, it has been shown that calcium does not influence the expression of its own receptor (CaSR), but, in contrast, it is able to upregulate the parathyroid VDR[28]. On the other hand, the effect of phosphorous on PTH secretion is slower than that observed for calcium[12], increasing PTH mRNA, as it demonstrated in parathyroid tissue obtained from parathyroidectomies of hemodialysis and kidney-transplant patients[25]. The effect of phosphorous on PTH secretion has also been demonstrated in bovine parathyroid tissue slices but not in dispersed cells, pointing out again the importance of having a three-dimensional architecture in order to obtain adequate functionality in these cells[107].

The use of parathyroid glands from rats has also showed that calcitriol upregulates not only VDR but also CaSR, even in the presence of low calcium levels[28], a finding not described in previous studies[29]. Furthermore, using human parathyroid tissue it has been demonstrated that calcitriol suppresses parathyroid cell proliferation, as long as the phosphorous concentration in the culture medium is normal[108]. In addition, the ability of calcimimetics, alone or in combination with calcitriol, to suppress PTH secretion and to increase VDR expression has also been proven in human and rat hyperplastic parathyroid glands[48], showing that the effect of calcimimetics was exerted, independently of calcium levels, in a concentration-dependent manner.

The *ex vivo/in vitro* parathyroid gland culture model has also been used to demonstrate that aluminum suppresses not only PTH secretion but also PTH mRNA by a posttranscriptional mechanism, acting as a true CaSR agonist[50].

Finally, this model has proven to be useful to test the functionality of fresh and cryopreserved fragments of parathyroid glands which are currently used for the re-implantation of parathyroid tissue[109]. This is a practical matter of great interest because one of the still unsolved problems for surgeons is how to select the best fragments of parathyroid tissue to be re-implanted, either as fresh or cryopreserved. Several techniques have been used to help in this selection but the results have been quite heterogeneous[110-112]. Still, in most cases, the decision has to be made in the operating theatre based only on the macroscopic appearance of the parathyroid gland and, unfortunately, no definitive solid results on this matter have been achieved.

The functional studies with fresh or cryopreserved parathyroid fragments seems to indicate that fresh tissue preserves almost all biological properties whereas after cryopreservation, the parathyroid glands maintain some functionality but their capacity

to fully respond to some effectors, such as calcium, for long periods of time seems to be impaired[113-114].

3. EXPERT OPINION

The study of the parathyroid function regulation is very complex. In the last decades, great advances on the pathogenesis of the disease have been achieved. Human and experimental studies of all types have been crucial to better understand the parathyroid gland behavior in CKD, and also to know that the development and progression of secondary hyperparathyroidism occur due to a combination of factors.

Many aspects of the main factors and feedbacks involved in the parathyroid regulation have been progressively researched and described in the past four decades. The late stages of CKD involve intrinsic molecular and genomic changes which are responsible for the morphological disturbances found in the parathyroid glands. Thanks to the combination of the results obtained from all the experimental models, some of them summarized in this review, the knowledge of the parathyroid function has greatly increased.

However, in more recent years, the molecular biology techniques have been crucial to further the knowledge about the parathyroid function. Fortunately, new findings in this field are published each year which allow us to better specify the role and weight of each known factor in the physiological and pathological regulation of the parathyroid gland at the different stages of CKD.

Phosphorous retention, low levels of calcium and calcitriol, all stimulate PTH production and parathyroid cell proliferation. In addition, FGF23, a recently discovered player, acts not only as a phosphaturic hormone but also as an important vitamin D and PTH regulator. Future studies might help to add new information about the role of

FGF23 in the different stages of CKD. However, with the information available it has been learned that one of the main advantageous and practical results of the FGF23 action is that it prevents the coexistence of high serum phosphorous and calcitriol serum levels, a coincidence that may end up increasing undesirable outcomes, among others, the possibility of having vascular calcifications.

If we had to pick up one topic in which striking improvements have been recently achieved, in no small measure thanks to the described models, we would choose phosphorous. As a result of the combination of results gathered from epidemiological, clinical and experimental studies, the role of phosphorus and the consequences of its overload have greatly increased in recent years, winning the spotlight when it comes to the general impact on health and its role in the parathyroid regulation and other CKD-MBD disorders, such as vascular function and calcification, cardiovascular disease and mortality.

The introduction of the FGF23 and its multiple interactions, mainly with phosphorous, but also with vitamin D and PTH, has enriched the CKD-MBD constellation. In this area of research it should be expected important advances in the coming years which might complete and limit more precisely the role of FGF23 in the parathyroid regulation across the different stages of CKD.

What clearly emerges from this review is that the intelligent use of the results obtained from different but complementary models, exploring the parathyroid function with a “bed to the bench” approach, has allowed for a more comprehensive knowledge about the parathyroid regulation in CKD which may be soon translated into practical measures to improve the daily management of CKD-MBD disorders.

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HIGHLIGHT BOX

- Chronic Kidney Disease Mineral and Bone Disorders are very common in dialysis patients. They are characterized by combinations of abnormalities in (a) calcium, phosphorous, PTH, or vitamin D metabolism; (b) bone turnover, mineralization, volume, linear growth or strength; and (c) vascular or other soft tissue calcification.
- PTH is secreted by the parathyroid glands and it is the main responsible for the control of calcium homeostasis.
- PTH regulation involves calcium, calcitriol, phosphorous, and the recently discovered phosphaturic hormone FGF23.
- Other drugs used for the treatment of CKD-MBD, such as calcimimetics and aluminum, are also able to suppress PTH levels.
- Clinical studies have provided important contributions to the knowledge of the pathogenesis of secondary hyperparathyroidism and also have allowed to identify the cell pathways and mechanisms involved in the progression of the disease.
- Animal models of CKD have been also useful to study the different factors involved in the regulation of the PTH; in addition, they have been crucial to confirm that phosphorous is one of the main players in PTH regulation.
- *In vitro/ex vivo* models have contributed to a better understanding of the molecular mechanisms involved in the pathogenesis and progression of secondary hyperparathyroidism.
- The combination of the results obtained from clinical and experimental studies has been essential to better understand the parathyroid regulation and the effect of the different therapies on CKD-MBD.

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