Chapter

Evaluation of Parathyroid Pathophysiology via Cell Distribution and Expression Patterns

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Abstract

The parathyroid tissue is composed of the chief, oxyphil, and water-clear cells. The cell type in each parathyroid gland is highly heterogeneous between different pathologies. The parathyroid oxyphil cells are markedly increased in secondary hyperparathyroidism due to chronic kidney diseases. These cells include more eosinophil than oxyphil cells, but they are closer in size to the chief cells. Studies reported that the oxyphil cells are derived from chief cells, and this presents another cell type that occurs as "transitional oxyphilic cells." As is known, calcium-sensing receptor (CaSR) is expressed abundantly in the chief cells. Expression of CaSR is elevated in disparate parathyroid tissues, which is possibly related to differential expression levels of parathyroid-specific transcription factors including *GCM2* (Glial Cells Missing Transcription Factor 2), *MAFB* (V-maf musculoaponeurotic fibrosarcoma oncogene homolog B), *GATA3* (GATA Binding Protein 3), *RXR* (The retinoid X receptor), and even *VDR* (Vitamin D Receptor). The pathways that connect CaSR to parathyroid cell proliferation are precisely not known yet. Evaluation of oxyphil and chief cells of parathyroid glands and their differential expression patterns are important to understand the parathyroid function and its behavioral changes due to related diseases. This chapter presents a summary of the current literature on the cell type distribution of parathyroid and pathophysiology by comparing the expression patterns.

Keywords: parathyroid gland, hyperplasia, adenoma, oxyphil cells, chief cells, water-clear cells

1. Introduction

Restoration of calcium levels is essential for the human body, which leads to proper function such as enzyme activity, hormone secretion, neurological stimulation, and/or muscle function. Calcium homeostasis is in relationship with different tissues and structures such as bone, parathyroid gland, and kidney. Regulation of calcium-homeostasis-related events begins with rapid changes in the parathormone (PTH) release from parathyroid gland cells. Hypocalcemic or hypercalcemic

responses are controlled by a specific receptor that senses the changes in serum calcium levels [1]. If calcium levels decrease, the mechanism provides a rapid increase in the PTH level to maintain proper calcium levels. Once calcium is balanced to the normal values, continual calcium release suppresses the PTH through a negative-feedback mechanism. Major and pulsatile events occur during the process itself through calcium-sensing receptors (CaSRs) [2]. This G-protein-coupled receptor is a unique part of the parathyroid gland function to monitor changes in serum calcium levels. This process is carried out by two main mechanisms which are, respectively, stimulation of the kidneys and intestine to increase the absorption of calcium and stimulation of the bones to release calcium into the blood [3, 4].

PTH mRNA expression levels are suppressed by the 1-25OH_2D modulation, despite CaSR expression with its effect on the modulation of the PTH release. Ritter et al. report that PTH release decreased by 1–25$(OH)_2$D, 1 hydroxy-vitamin D, and 25(OH) D in mice parathyroid cells in culture [5–11]. Earlier in this study, Kim et al. showed a lack of upregulation of PTH transcription in the Vitamin D Receptor (VDR) knockout mice, and lack of suppression of PTH transcription by 1,25$(OH)_2$D administration [12]. Consistent with both researcher and current literature, the 1–25$(OH)_2$D reduces the PTH mRNA expression and has an antiproliferative effect on parathyroid cells from uremic rats, subsequently enhancing the VDR expressions. Acute changes in the calcium levels of sHPT patients enhance the CaSR and Klotho expressions by Vitamin D [5, 13].

The location of the parathyroid glands is on the posterior side of the thyroid gland. Mostly there are four glands and rarely supernumerary glands [14]. Starting from the early phases of embryogenesis, the pharyngeal pouches give rise to parathyroid glands along with many organs [15, 16]. The third and the fourth pharyngeal pouches particularly emerge as parathyroid glands [17]. Inferior and superior parathyroid glands develop with thymus and a portion of the thyroid respectively [15]. Different cell adhesion after separation from the pharyngeal pouch modulates the formation of parathyroids, while the thymus continues to migrate above the heart [16]. Separation of the superior parathyroid glands is carried out during the seventh week of the development and after detaching the pharyngeal wall, the parathyroid fuses with the posterior surface of the thyroid [14].

The parathyroid glands are derived from the endoderm during embryogenesis. Endoderm consists of a high amount of actin fibers that provide formation and expansion to the ectoderm. Numerous signaling molecules and proteins maintain the progression of morphogenesis. Particularly, Glial cell missing 2 (*GCM2*), Homeobox A3 (*HOXA3*), Forkhead box protein N1 (*FOXN1*), Eyes Absent Homolog 1 (Transcriptional Coactivator And Phosphatase 1—*EYA1*), T-Box Transcription Factor 1 (*TBX1*), GATA binding protein 3 (*GATA3*), Paired box 1 (*PAX1*), and Paired box 9 (*PAX9*) genes are the leading transcription factors that initiate the parathyroid morphogenesis [16, 17]. Among them, *GCM2* is particularly important as the early transcription factor of parathyroid formation [18]. The developmental stage includes another important transcription factor: V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (*MAFB*) [19].

In order to understand the development of the parathyroid gland, extensive research on murine models is performed. To identify the involving molecules and signaling processes, researchers have proven most of the downstream and upstream mediators. The remaining unknown factors remain to be elucidated. In current practice, the known factors involving the development and maturation of the parathyroid glands are evaluated in murine models and related parathyroid disorders. This chapter

summarizes the molecular findings based on the cell type distribution of parathyroid and pathophysiology up to the present.

2. Cellular distribution of parathyroid gland

The vascular structure of the parathyroid glands is separated from the outside by a fibrous thin capsule. Such capsules contain thin fibrous bands that disperse toward the inner parts by fibrous structures. This provides access to the blood vessels, lymphatics, and nerves to provide nutrients to the tissue [20, 21]. Macroscopic appearances may vary depending on cell type, cell/organelle amount, blood supply status, and the amount of fat content of the tissue [1, 22].

In literature, cell-type distribution in the parathyroid tissue has been reported based on either two or three different cell types. In some reports, it has been stated as two: oxyphil and chief cells [14], while another group of researchers has elucidated histological evidence that there are water-clear cells besides oxyphil and chief cells [23]. Besides, there is another group of cell types that are observed with disease and/or age-related changes, which are not yet classified as a specific cell type. Although this type is not yet classified, the transitional chief cells are present and particularly reported as *chief cell-to-oxyphil cell transdifferentiated cell* group [23, 24]. These four types of cells do not have distinctive markers yet. Their variations in size and number per tissue originate from different parathyroid pathophysiology, which is the main unknown aspect of parathyroid cell biology. Hence, this chapter prioritizes the three main cell types in the parathyroid gland.

2.1 Water-clear cells

Water-clear cells contain many glycogen granules in their cytoplasm and are rarely seen. These cells are clinically encountered in the tissues of patients with secondary hyperparathyroidism (sHPT) and primary hyperparathyroidism (pHPT) [25]. In 1992, Emura's study on rabbit parathyroid tissue stated that water-clear cells contained a large number of vacuoles in the parathyroid tissue sections, which were observed with electron microscopy and were found scattered around the chief cells. Water-clear cells reside in between the perivascular space and the basal lamina that have been observed to have a desmosomal connection with chief cells [26]. In 2013, Ezzat et al. showed that these cells were not associated with PTH secretion and serum calcium levels. In addition, they reported that only 0.3% of pHPT cases had "water-clear cell hyperplasia" or "water-clear cell adenomatosis" [27]. A recent report presented that the water-clear cell hyperplasia ratio is less than 1% of all pHPT cases [28]. Distinct features or changes in the water-clear cell accumulation in parathyroid tissue or relation with disorders remain to be elucidated.

2.2 Chief cells

The chief cells constitute the cellularly dominant cell type of parathyroid tissue, and it was described by Baker in 1942 [29]. Baker describes these cells as the dark "primary cells" with distinctive cytoplasmic structures. Their rod-shaped mitochondria are homogeneously distributed throughout the cytoplasm [20]. In addition, Trier reported that he did not observe the dark and pale stained oxyphil cells, neither with light nor with electron microscopy, which Baker mentioned in his notes in 1942.

The chief cells are usually rich in intracellular fat content. Cells are supported by a thin connective tissue and accordingly are located more closely to the capillaries [30]. Although the chief cells may contain more than one nuclei (multinucleic cells), the nuclear matrix structures are densely arranged [31]. Chief cells are considered the main cell type, and cells are mostly spherical or oval-shaped with long nuclei and narrow cytoplasm [32]. The knowledge about the cell shapes of the chief cells is mostly obtained from histological examinations. Besides, observations by using live imaging or confocal microscopy are either absent or limited. Since it has been reported that single chief cell diameters are 0.2 μm wide according to electron microscope images [33, 34] and 6–8 μm histologically [23]. The agranular membrane structure of the Golgi body of the chief cells was first visualized in 1957 [35]. Due to the eosinophilic cytoplasm, they may appear dark or light in color at the time of staining.

To date, the parameters such as age, disease history, and drug use, which are the definitive features of parathyroid function, may affect cytoplasm amount or changes in the cytoplasm content or nucleus size for all processes [32, 36, 37]. In brief, functional activity and cytoplasm content are the two related main chief cell behaviors of the parathyroid. The most distinctive feature of the chief cells is that they contain a large number of secretory vesicles. These membrane-covered vesicles mostly contain PTH [38].

As is known, the main function depends on the chief cells, which are responsible cell type for PTH release. These cells play an important role in calcium homeostasis, thanks to the CaSR expression on its surface. The receptor detects the low amount of extracellular calcium changes and releases the appropriate amount of PTH to balance the calcium in the blood [23, 39].

2.3 Oxyphil cells

Oxyphil cells are the cells with well-circumscribed eosinophilic cytoplasm and pycnotic nuclei [1, 20, 39]. Between 1952 and 1953, Parade compared monkey, equine, and human parathyroid tissues and reported that the size and number of mitochondria in oxyphil cells varied between species. In addition, the variability in the number of mitochondria in human oxyphilic cells is also associated with age [23, 33, 34]. As of note, rat parathyroid tissue does not contain oxyphil, oxyphil-like, or mitochondria-rich cell groups [32]. In 1958, Trier observed that some of the oxyphilic cells were stained “dark” and some were “pale.” He described pale-stained oxyphilic cells as having “low” mitochondrial content, and dark-staining oxyphil cells as having “excessive” mitochondrial content [20]. Both studies have confirmed the outcome of oxyphil cells.

In 1981, Allen and Thorburn examined the activity of oxyphil cells in abnormal parathyroid tissue of 114 patients with sHPT due to chronic kidney disease. The absence and presence of oxyphil cells in human parathyroid tissues were evaluated in this retrospective study, in which histological evaluation was associated with clinical practice. They reported that hyperparathyroidism was seen in more than one parathyroid tissue in 55% of the cases, and adenoma was found in one of the four parathyroid glands in 69% of the cases. They also reported that oxyphil cells were found in 91% of the cases, and the number of oxyphilic cells was positively correlated with serum calcium level [40].

In 1990, Suzuki et al. reported oxyphil cell function in 148 parathyroid tissues from patients who are taking hemodialysis. They calculated for each tissue by proportioning the area occupied by oxyphilic cells by morphometric measurements

concerning the total parathyroid cross-sectional area (oxyphil cell area/total area). Additionally, it has been reported that serum PTH levels do not have a statistically significant relationship with age and the dialysis duration; however, the total tissue size is positively correlated with PTH release. Based on the results, they concluded that the oxyphil cell/total tissue area was not effective in PTH release in patients with chronic renal failure [41].

In 1996, Tanaka et al. used 22 sHPT tissues to understand oxyphil cell function. The study reported that 10 of these tissues had oxyphil cells and the mRNA expression of PTH was found to increase. Then, performed heterotransplantation in mice to determine oxyphilic cell function by evaluating serum PTH levels. As a result, the change in PTH levels was positively correlated with transplanted tissue size, not the cell number or type [42].

Despite the reported studies, there is no definite information about the exact function of oxyphil cells, but this question was clarified in many ways by Ritter and Brown et al. [5, 6, 23, 39]. Histologically, increased eosinophilic content from the chief cell through oxyphil cell, suggesting that oxyphil cells are formed by "transition." As evidence, the oxyphil cells have been shown to express PTH [42] and *GCM2*, which is a parathyroid-specific transcription factor [18] and has a role in parathyroid tissue development. It has been observed that oxyphil cells are more numerous in patients with chronic renal failure, and the amount of oxyphil cells is much higher than in the tissues of healthy individuals [43, 44]. Although studies have shown that oxyphil cells express the parathormone-dependent protein (PTHrP) [45, 46] and that protein is involved in PTH release [42], the amount/mechanism of PTH release is not yet known. The proposed function of the PTHrP on parathyroid cells may be responsible for autocrine or paracrine signaling while affecting PTH release or parathyroid maturation [23].

On the one hand, the CaSR expression levels of oxyphil cells are statistically found higher than other parathyroid cells. On the second hand, there was no significant difference in Vitamin D Receptor (VDR) expression [5, 39]. The higher mitochondrial content of oxyphil cells indicates that energy requirements are much higher than in chief cells. Mitochondria are also responsible for VDR function. One study by Ritter et al. elucidated that 25-hydroxyvitamin D-1α-hydroxylase (1αOHase) is highly expressed and this is the inactive form of Vitamin D [5]. In addition, the amount of 1αOHase in human parathyroid tissue was directly proportional to calcium levels. The study reported that calcimimetic therapy in patients with chronic renal failure causes a significant increase in the amount of 1αOHase in oxyphil cells [5, 6].

A recent commentary also highlighted Ritter's findings after 5 years. Metabolic changes of parathyroid tissue are significantly affected by changes in tissue volume and/or cell type, cell count rate due to drug use, or changes in serum calcium level. Concomitant CaSR induction and its persistence are also known to affect the expression profile [47]. Thus, in terms of PTH expression, it has been clarified that oxyphil cells contain more PTH than chief cells. Some of these data also confirm the findings of Allen and Thorburn in 1981 [40].

3. Cellular variations in parathyroid research and related diseases

Calcium or di-/trivalent cations induce the activation of CaSR, which triggers the PTH release [48]. Definitive research studies have demonstrated the outcomes from direct or indirect approaches so far. The comparisons and the evaluations were

mainly performed with the diseased tissues, not the healthy parathyroids due to the difficulties in retrieval processes. There is only a limited number of papers that compare/evaluate healthy parathyroid tissue expression profiles of cellular content. Particularly, the location and the size of the parathyroid gland make it difficult to obtain from healthy individuals. Researchers, surgeons, and physicians reported different approaches to finding and/or distinguishing the parathyroid tissue during thyroid operation [49–51]. This challenge still exists, and suggested techniques such as near-infrared autofluorescence [52] are not readily available for the use of numerous surgeons. Essentially, it is a fact that even if healthy tissue is donated, it will take a long time to reach the appropriate sample size required to conduct various studies. Therefore, a limited number of healthy parathyroid tissues either used or to be used in studies for comparisons.

The further part of this chapter continues with parathyroid tissue from the primary and secondary hyperparathyroidism patients (adenoma and hyperplasia tissues respectively) were compared according to their cellular content. The changes in their expression profiles were evaluated with different stages of the related diseases.

Starting 25 years ago, most of the papers evaluated the CaSR, PTH, proliferation markers, and transcription factors expression changes by mRNA and/or protein levels (mostly immunohistochemistry, western blot, ELISA methods). The clinical characteristics and the cell content of the parathyroid tissue were compared by Yamaguchi et al. in 1997. Samples are retrieved from patients who have secondary hyperparathyroidism and cell proliferation specific marker PCNA (proliferating cell nuclear antigen) expression was compared between normal, adenoma, and hyperplasia parathyroid tissues. This study divided the cell content in each tissue group including dark-stained chief cells, clear chief cells, vacuolated chief cells, transitional oxyphil cells, and oxyphil cells [53]. This divided cell type classification was very similar to the report by Trier in 1958 [20]. Among 27 out of 40 normal parathyroid tissues were found positive for PCNA, and no correlation between age and expression levels is observed. Cell content was reported as clear-chief>dark-chief>oxyphil cells, respectively. However, the normal parathyroid tissue was obtained from thyroid cancer patients, and a definite interpretation should not be made without ignoring this situation. Adenoma tissue showed remarkably higher PCNA expression levels, and the cell content included mostly clear chief cells and less commonly transitional oxyphil cells. In 129 parathyroid hyperplasia tissue samples, all of the divided cell types of this study were found distinguishable. The PCNA expression was found significantly higher in the nodular type from the glandular structure of the parathyroid. The authors concluded that clear chief cells are the most proliferative cell group in all samples, and the dark chief cell group was found the lowest proliferative group [53]. Yamaguchi's study alone is one of the rare studies that evaluate the highest number of parathyroid tissues in its related field.

In 2000, Corbetta et al. demonstrated that 27 parathyroid adenoma tissues contain only chief cells. Cell isolation, cultivation under different calcium concentrations, PTH levels, and CaSR expression levels were evaluated. Additionally, it is stated that there was no correlation between different calcium sensitivity and pathology. Furthermore, PTH and CaSR mRNA and protein levels were significantly reduced when compared with normal tissue, and the authors concluded that defective calcium sensing occurs in abnormal parathyroid tissue [54]. This study may be considered as a starting point for understanding the defects in the sensing mechanism of the CaSR. Failure to obtain the expected changes in the PTH level under different calcium concentrations should not be interpreted as a resistance mechanism.

In 2006, Brown et al. stated that three different Vitamin D prodrugs, which are lacking side-chain hydroxyl groups, were treated with bovine parathyroid cells and showed that PTH levels decreased based on the related concentrations. The prodrugs have different affinities to the VDR; however, utilization of the VDR decreases PTH synthesis for treatment of secondary hyperparathyroidism [7]. The inhibition of PTH synthesis was performed in vitro using prodrugs at that time of the work, and this indicates a new aspect of parathyroid research. Continued with Ritter et al. in 2006, by the same research group, competitive VDR binding of the vitamin D analogs was examined. In this report, two main conclusions were included. One is the $25(OH)D_3$ has the highest affinity to the VDR among other analogs, and direct action mechanisms through VDR suppress PTH [8]. The PTH regulation versus VDR activation may be explained by a compensatory mechanism model. Although the authors did not exclude other regulatory systems such as the negative feedback mechanism of the PTH [10]. Studies conducted between 2005 and 2011 mostly did not focus on cell-type-specific changes. Instead, the relationships between VDR and PTH were investigated in terms of regulation mechanism, especially in bovine, rat, and other knockout-animal models.

In 2012, Ritter et al. defined the differential mRNA expression of parathyroid glands by cell types. In this paper, histological examination was provided and the size of the chief, oxyphil, and transdifferentiated oxyphil cells was reported. Consistent with previous reports, the high oxyphil cell amount was reported in chronic kidney diseased patients, accordingly higher PTH and CaSR expression was elucidated as well. In addition, oxyphil cells showed *GCM2* expression, which is a specific parathyroid transcription factor. Therefore, in order to understand parathyroid pathophysiology in patients with secondary hyperparathyroidism, oxyphil cell secretomes may help to define their role other than chief cells [23]. This study still has important outcomes that shaped the perspective to a particular point for chief and oxyphil cell features. Future studies including isolation of oxyphil and chief cells separately with the assessment of secretory features will justify each cell type's function.

In 2014, Shi et al. demonstrated a flow cytometric cell sorting of 20 parathyroid adenoma tissues from primary hyperparathyroidism patients. They divided the cells into three distinct populations including the chief, oxyphil, and lymphocytes. The cutting-edge research from Shi et al. provided electron microscopy images of each population and also demonstrated the immunofluorescence staining of CaSR and mRNA expressions of *CASR* and *PTH* by comparing oxyphil and chief cells. At end of this unique study, they reported that oxyphil cells respond to calcium faster than chief cells by releasing higher PTH and did not find differences in their CaSR expression profiles. They also reported that the feature of oxyphil cells provides an important function to the parathyroid tissue as a piece of solid evidence [55].

In 2015, Howson et al. investigated the oxyphil-cell-rich adenoma tissues from primary hyperparathyroidism patients. During the 10-year follow-up period, among obtained 2739 tissues, 91 of the parathyroid adenoma were found oxyphil cell adenoma type. About 80% of these patients were symptomatic and most commonly had higher serum calcium and PTH levels than the classical type of adenoma. On the contrary, the frequency of oxyphilic adenomas was not rare, and patients trend toward a higher rate of morbidity and potential mortality if left untreated [56].

In 2017, two different groups presented water-clear cell-type adenoma and hyperplasia cases. The clinical symptoms of the adenoma patient were unintentionally led and treated for hyperparathyroidism due to the clinical features. However, after surgical removal of the two adenoma tissues from the same patient, the histopathological

evaluation showed water-clear cell double parathyroid adenomas [57]. This is followed by another case report that presents primary hyperparathyroidism clinically. Contrary to the clinic, the histopathological results showed enormous unilateral water-clear cell hyperplasia in parathyroid [28]. Both of the cases concluded that despite ultrasonography, biochemical, and clinical follow-up, these extremely rare cases unintentionally misled the physicians [28, 57]. In the same year, another study by Ritter et al. was reported. In that study, parathyroid hyperplasia tissues were retrieved from chronic kidney patients and grouped according to their calcimimetic treatment (cinacalcet versus paricalcitol versus cinacalcet+paricalcitol). The effects of treatment processes on the cell type of the parathyroid tissue were compared with four healthy parathyroid tissues. According to the histopathological evaluation, parathyroid oxyphil cell content was found to increase significantly for the cinacalcet-treated patients [58]. The role of the CaSR activation possibly led to a new comprehension to understand the outcome of conventional treatment with vitamin D analogs or calcimimetics on the cellular composition of the parathyroid.

In 2020, Ding et al. provided a comparison of clinical characteristics and oxyphil cell proportion through 78 patients. In total, 295 parathyroid tissue samples were retrieved from 78 patients who did not have cinacalcet treatment. Clinical characteristics included serum calcium, phosphorus, alkaline phosphatase, age, dialysis duration, and preoperative PTH levels. The samples were divided based on the mean oxyphil cell ratio (high oxyphil cell content and low oxyphil cell content, respectively). Subsequently, etiopathogenesis and histological examinations were evaluated. They reported that preoperative PTH levels of the patients were found lower than the oxyphil cell-rich group [37]. This finding contradicts the previous study by Howson [56]. Furthermore, Ding et al. reported that if parathyroid tissue contains more oxyphil cells, it became lighter than the tissue with fewer oxyphil cells. As of note, weight comparison was performed between parathyroid hyperplasia tissues [37].

At the beginning of 2021, Altinay et al. demonstrated the cellular composition of the hyperplasia and adenoma tissues while comparing normal parathyroid glands. Furthermore, PTH, GATA3, and PAX8 levels were evaluated histologically. As a result, expression of GATA3 and PTH was found more prominent in pathologic parathyroid tissues when compared with normal. Particularly the GATA3 staining has shown positive only for parathyroid, not thyroid tissue. Chief cell amount was high in adenoma and hyperplasia tissues; however, PTH staining was found low when compared with normal tissue. In addition, adenoma displayed less PTH and GATA3 expression histologically [59].

Another study from Rodriguez et al. reported the clinicopathological outcome of the oxyphil cell clusters, which were detected in parathyroid adenoma tissue. The main idea is to define the particular effect of the oxyphil cell content-rich/poor tissue composition and localization. Despite clinic versus histopathological comparisons so far, this study has a similar manner with distinct oxyphil cell subgroups [60]. Rodriguez's team investigated histopathological function with symptomatology, and this could depend on the changes in the percentage of the oxyphil cells. Clinically, observations included age, sex, body mass index, and symptomatic reasons for surgical initiation such as nephrocalcinosis, osteoarticular and/or neuropsychiatric and/or cardiovascular symptoms. Biochemical parameters were as follows: ionic calcium, corrected serum calcium, albumin, PTH, 25-OH Vitamin D, alkaline phosphatase, creatinine, glomerular filtration rate (GFR), and urinary calcium excretion. Additionally, oxhyphil cell percentages were divided

into the following four categories: 0–24, 25–49, 50–74, and 75–100% [60]. In terms of variables, this is the most comprehensive evaluation to date associated with clinical (including both biochemical and symptomatic) and parathyroid cell groups. In total 261 parathyroid tissues obtained from 238 patients were used. Eventually, 77% of tissues have less than 25% in the percentage of oxyphil cells and 8% of tissues are greater than 75% in terms of percentage of oxyphil cells. No significant difference was found in terms of biochemical parameters such as calcium, phosphorus, alkaline phosphatase, PTH, and 25-OH vitamin D. In addition, the localization of the adenoma tissues did not show significant changes when compared between inferior and superior glands. Different distributions of the oxyphil cell clusters among varied cell percentages showed no significant changes. Although, increased thyroid nodularity and higher prevalence in cardiovascular symptoms are shown within the less oxyphil cell groups (<25%). Imaging tests also justified a correlation between better localization with increased oxyphil cell group (>75%). Nevertheless, preoperative GFR and urinary calcium excretion significantly worsen the patients' symptoms that were altered in parathyroid tissues containing high oxyphil cells. Findings in the non-pathological tissue samples from normocalcemic patients showed an absence/low level in the oxyphil cells [60]. These conditions are still controversial.

3.1 Exception: parathyroid carcinoma

Parathyroid carcinomas influence the laboratory values, which are similar to those with hyperparathyroidism (primary or secondary). For instance, increased serum PTH levels are around thousands, and a palpable mass may be detected in the neck region. Currently, carcinoma can be observed in any (upper or lower) of the glands and is not considered to have any priority within the four existing glands while the invasion of the adjacent thyroid gland is observed [61]. The histological resemblance of parathyroid adenoma causes a challenge for diagnosis. Observations in parathyroid carcinoma tissue include increased mitotic potential, necrosis, formation of encapsulated structures, and spread from the capsule through adjacent tissues [62].

Studies on parathyroid cancer are gaining attention. A recent study evaluated whether tumor volume and tumor size were associated with disease severity [63]. Another study determined circulating miRNA expression levels as a potential diagnostic biomarker in parathyroid carcinomas [64, 65].

Despite all these findings, in terms of cell type, water-clear, oxyphil, and chief cells do not provide a differential diagnosis. The prime reasons are a rarity, and it is not possible to impose a limitation in terms of cell composition.

4. Conclusion

To further understand the parathyroid gland function and development, studies were carried out by numerous researchers. Indicated contributions already shaped the required future studies for this composite tissue. The parathyroid gland is a relatively small tissue while the behavior and changes in its composition provide a fine balance in terms of its function. Thus, this has enabled us to come a little closer to elucidating the actual mechanisms. Foremost, the mechanisms that maintain certainty in all research results can be listed as the following:

- Parathyroid cells mostly contain three types of cells; chief, oxyphil, and water-clear cells.

- Depending on the circumstances such aging and diseases may affect and increase the oxyphil cell amount.

- The cells that switch between chief through oxyphil cells can affect different mechanisms, which are still uncategorized.

- Categorization of the differentiating cells may explain the influence of autocrine/paracrine effect on tissue behavior.

- So-called "transdifferentiated cells" can be classified as another cell type that will provide a separate diagnosis criterion such as prognosis, degree, or etiology of parathyroid-related-diseases.

Expression patterns of the same transcription factors such as GCM2, MAFB, and RXR are the most difficult part of distinguishing the cell-type-specific features. Even with all the findings in the literature, one question still remains; where does the border of differential cellular diagnosis end? The answer to this question is still unknown.

The role of autocrine and paracrine effects in parathyroid cell differentiation cannot be ignored. Each person's metabolism balances this process individually; therefore, studies should include larger cohorts. More collaborative studies are required between researchers. The lack of oxyphil cells in some murine models is another challenge. This indicates that understanding the cellular composition/regulation of parathyroid tissue behavior is mandatory, particularly for primary human parathyroid tissue cell studies.

The emergence of oxyphilic cells is perhaps a defense mechanism that is developed from the parathyroid tissue against external signalings, which was first reported by Christi in 1955 [31]. However, this is yet to be confirmed. Another remark can be made from the state of immunogenicity, which is considered as a defense mechanism or after pathological disturbances leads to adverse circumstances, and as a result, the oxyphil cells differentiate from chief cells. Nevertheless, several studies have focused on the presence of other immunological markers for parathyroid tissue. Verified studies to date have limited definitions for the expression of immunological markers such as human leukocyte antigens (HLAs) [66–69]. Revealing the possible relationship of immunological and/or defense mechanisms in parathyroid cell composition requires more studies such as different co-culture models. This can be a starting point for future studies.

A few important subjects that have not been finalized yet in terms of parathyroid tissue are:

- Histological studies are contributing to the field; however, more biomarker research is required for the specific differential prognosis of related diseases.

- The inverse relationship between the increased oxyphilic cell and the lighter tissue structure is still not understood, whereas the studies that determine weight through cell composition of the parathyroid tissue could provide paramount importance.

In conclusion, the approaches that have been proposed in the literature paved the way for future research objectives. Differentiating points obtained by comparing the outcomes and the data of different researchers raise new questions. The exact mechanism for basic parathyroid biology requires new *in vitro* and *in vivo* approaches, and mostly, primary tissue culture systems are essential to understand such a mechanism.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Baloch ZW, LiVolsi VA. Parathyroid glands, pathology. In: Reference Module in Biomedical Sciences. 3rd ed. Philadelphia, PA, USA: Elsevier; 2014. p. 474

[2] Chiavistelli S, Giustina A, Mazziotti G. Parathyroid hormone pulsatility: Physiological and clinical aspects. Bone Research. 2015;**3**:14049

[3] Hall JE. Guyton and Hall Textbook of Medical Physiology. 13th ed. Vol. xix. Philadelphia, PA: Elsevier; 2016. p. 1145

[4] Lofrese JJ, Lappin SL. Physiology, Endocrine, Parathyroid. Treasure Island (FL): StatPearls; 2018

[5] Ritter CS, Haughey BH, Armbrecht HJ, Brown AJ. Distribution and regulation of the 25-hydroxyvitamin D3 1alpha-hydroxylase in human parathyroid glands. The Journal of Steroid Biochemistry and Molecular Biology. 2012;**130**(1-2):73-80

[6] Brown AJ, Ritter CS, Weiskopf AS, Vouros P, Sasso GJ, Uskokovic MR, et al. Isolation and identification of 1alpha-hydroxy-3-epi-vitamin D3, a potent suppressor of parathyroid hormone secretion. Journal of Cellular Biochemistry. 2005;**96**(3):569-578

[7] Brown AJ, Ritter CS, Knutson JC, Strugnell SA. The vitamin D prodrugs 1alpha(OH)D2, 1alpha(OH)D3 and BCI-210 suppress PTH secretion by bovine parathyroid cells. Nephrology, Dialysis, Transplantation. 2006;**21**(3):644-650

[8] Ritter CS, Armbrecht HJ, Slatopolsky E, Brown AJ. 25-hydroxyvitamin D(3) suppresses PTH synthesis and secretion by bovine parathyroid cells. Kidney International. 2006;**70**(4):654-659

[9] Brown AJ, Ritter CS. The vitamin D analog 1alpha,25-dihydroxy-2beta-(3-Hydroxypropyloxy) vitamin D(3) (eldecalcitol) is a potent regulator of calcium and phosphate metabolism. Calcified Tissue International. 2011;**89**(5):372-378

[10] Ritter CS, Brown AJ. Direct suppression of Pth gene expression by the vitamin D prohormones doxercalciferol and calcidiol requires the vitamin D receptor. Journal of Molecular Endocrinology. 2011;**46**(2):63-66

[11] Ritter CS, Brown AJ. Suppression of PTH by the vitamin D analog eldecalcitol is modulated by its high affinity for the serum vitamin D-binding protein and resistance to metabolism. Journal of Cellular Biochemistry. 2011;**112**(5):1348-1352

[12] Kim MS, Fujiki R, Murayama A, Kitagawa H, Yamaoka K, Yamamoto Y, et al. 1Alpha,25(OH)2D3-induced transrepression by vitamin D receptor through E-box-type elements in the human parathyroid hormone gene promoter. Molecular Endocrinology. 2007;**21**(2):334-342

[13] Bienaime F, Prie D, Friedlander G, Souberbielle JC. Vitamin D metabolism and activity in the parathyroid gland. Molecular and Cellular Endocrinology. 2011;**347**(1-2):30-41

[14] Rosen RD, Bordoni B. Embryology, Parathyroid. Treasure Island (FL): StatPearls; 2021

[15] Peissig K, Condie BG, Manley NR. Embryology of the parathyroid glands. Endocrinology and Metabolism Clinics of North America. 2018;**47**(4):733-742

[16] Miles B, Srinivasan VN. Embryology, Pharyngeal Pouch. Treasure Island (FL): StatPearls; 2021

[17] Parvari R, Diaz GA, Hershkovitz E. Parathyroid development and the role of tubulin chaperone E. Hormone Research. 2007;**67**(1):12-21

[18] Nonaka D. Study of parathyroid transcription factor Gcm2 expression in parathyroid lesions. The American Journal of Surgical Pathology. 2011;**35**(1):145-151

[19] Naveh-Many T, Silver J. Transcription factors that determine parathyroid development power PTH expression. Kidney International. 2018;**93**(1):7-9

[20] Trier JS. The fine structure of the parathyroid gland. The Journal of Biophysical and Biochemical Cytology. 1958;**4**(1):13-22

[21] Delattre JF, Flament JB, Palot JP, Pluot M. Variations in the parathyroid glands. Number, situation and arterial vascularization. Anatomical study and surgical application. Journal de Chirurgie (Paris). 1982;**119**(11):633-641

[22] Brown MB, Limaiem F. Histology, Parathyroid Gland. Treasure Island (FL): StatPearls; 2019

[23] Ritter CS, Haughey BH, Miller B, Brown AJ. Differential gene expression by oxyphil and chief cells of human parathyroid glands. The Journal of Clinical Endocrinology and Metabolism. 2012;**97**(8):E1499-E1505

[24] Roussanne MC, Gogusev J, Hory B, Duchambon P, Souberbielle JC, Nabarra B, et al. Persistence of Ca^{2+}–sensing receptor expression in functionally active, long-term human parathyroid cell cultures. Journal of Bone and Mineral Research. 1998;**13**(3):354-362

[25] Chen H, Emura S, Shoumura S. Ultrastructure of the water-clear cell in the parathyroid gland of SAMP6 mice. Tissue & Cell. 2006;**38**(3):187-192

[26] Emura S, Shoumura S, Isono H. Ultrastructure of the water-clear cell in the rabbit parathyroid gland. Archives of Histology and Cytology. 1992;**55**(2):159-166

[27] EzzatT, MacleanGM, ParameswaranR, Phillips B, Komar V, Mihai R, et al. Primary hyperparathyroidism with water clear cell content: The impact of histological diagnosis on clinical management and outcome. Annals of the Royal College of Surgeons of England. 2013;**95**(3):e60-e62

[28] Boutzios G, Sarlanis H, Kolindou A, Velidaki A, Karatzas T. Primary hyperparathyroidism caused by enormous unilateral water-clear cell parathyroid hyperplasia. BMC Endocrine Disorders. 2017;**17**(1):57

[29] Rives JD, Baker DD. Anatomy of the attachments of the diaphragm: Their relation to the problems of the surgery of diaphragmatic hernia. Annals of Surgery. 1942;**115**(5):745-755

[30] Ovalle WK, Nahirney PC, Netter FH. Netter's Essential Histology. 2nd ed. Vol. xv. Philadelphia, PA: Elsevier/ Saunders; 2013. p. 517

[31] Christie AC. A histochemical property of the oxyphil cells of the human parathyroid glands. Journal of Clinical Pathology. 1955;**8**(4):302-309

[32] Mense MG, Rosol TJ. Chapter 34—Parathyroid gland. In: Suttie AW, editor. Boorman's Pathology of the Rat. 2nd ed. Boston: Academic Press; 2018. pp. 687-693

[33] Palade GE. An electron microscope study of the mitochondrial structure.

The Journal of Histochemistry and Cytochemistry. 1953;**1**(4):188-211

[34] Palade GE. The fine structure of mitochondria. The Anatomical Record. 1952;**114**(3):427-451

[35] Lever JD. Fine structural appearances in the rat parathyroid. Journal of Anatomy. 1957;**91**(1):73-81

[36] Javadov M, Karatay E, Ulusan K, Ozpek A, Idiz O, Duren M, et al. Number of cells in parathyroid tissue in primary hyperparathyroidism cases and its relationship with serum calcium value. Medicine (Baltimore). 2021;**100**(46):e27530

[37] Ding Y, Zou Q, Jin Y, Zhou J, Wang H. Relationship between parathyroid oxyphil cell proportion and clinical characteristics of patients with chronic kidney disease. International Urology and Nephrology. 2020;**52**(1):155-159

[38] Eroschenko VP, Fiore MSH. DiFiore's Atlas of Histology with Functional Correlations. 11th ed. Vol. xvii. Philadelphia: Wolters Kluwer Health/ Lippincott Williams & Wilkins; 2008. p. 532

[39] Mizobuchi M, Ritter CS, Krits I, Slatopolsky E, Sicard G, Brown AJ. Calcium-sensing receptor expression is regulated by glial cells missing-2 in human parathyroid cells. Journal of Bone and Mineral Research. 2009;**24**(7):1173-1179

[40] Allen TB, Thorburn KM. The oxyphil cell in abnormal parathyroid glands. A study of 114 cases. Archives of Pathology & Laboratory Medicine. 1981;**105**(8):421-427

[41] Suzuki Y, Suzuki M, Hirasawa Y, Morita T, Shimizu T, Arakawa M. Oxyphil cells of parathyroid glands in chronic renal failure may not secrete parathyroid hormone. Nephron. 1990;**56**(1):62-65

[42] Tanaka Y, Funahashi H, Imai T, Seo H, Tominaga Y, Takagi H. Oxyphil cell function in secondary parathyroid hyperplasia. Nephron. 1996;**73**(4):580-586

[43] Lomonte C, Martino R, Selvaggiolo M, Bona RM, Cazzato F, Milano R, et al. Calcitriol pulse therapy and histology of parathyroid glands in hemodialysis patients. Journal of Nephrology. 2003;**16**(5):716-720

[44] Sumida K, Nakamura M, Ubara Y, Marui Y, Tanaka K, Takaichi K, et al. Histopathological alterations of the parathyroid glands in haemodialysis patients with secondary hyperparathyroidism refractory to cinacalcet hydrochloride. Journal of Clinical Pathology. 2011;**64**(9):756-760

[45] Kitazawa R, Kitazawa S, Maeda S, Kobayashi A. Expression of parathyroid hormone-related protein (PTHrP) in parathyroid tissue under normal and pathological conditions. Histology and Histopathology. 2002;**17**(1):179-184

[46] Kitazawa R, Kitazawa S, Fukase M, Fujita T, Kobayashi A, Chihara K, et al. The expression of parathyroid hormone-related protein (PTHrP) in normal parathyroid: Histochemistry and in situ hybridization. Histochemistry. 1992;**98**(4):211-215

[47] Basile C, Lomonte C. The function of the parathyroid oxyphil cells in uremia: Still a mystery? Kidney International. 2017;**92**(5):1046-1048

[48] Conigrave AD. The calcium-sensing receptor and the parathyroid: Past, present, future. Frontiers in Physiology. 2016;**7**:563

[49] Aydin H, Ferahman S, Abdullayev S, Sahbaz NA, Dural AC, Guzey D, et al. Technological advances have improved surgical outcome in thyroid surgery: Myth or reality? Acta Endocrinologica (Buchar). 2021;**17**(1):1-6

[50] Caglia P, Puglisi S, Buffone A, Bianco SL, Okatyeva V, Veroux M, et al. Post-thyroidectomy hypoparathyroidism, what should we keep in mind? Annali Italiani di Chirurgia. 2017;**6**:371-381

[51] Zobel MJ, Long R, Gosnell J, Sosa JA, Padilla BE. Postoperative hypoparathyroidism after total thyroidectomy in children. The Journal of Surgical Research. 2020;**252**:63-68

[52] Paras C, Keller M, White L, Phay J, Mahadevan-Jansen A. Near-infrared autofluorescence for the detection of parathyroid glands. Journal of Biomedical Optics. 2011;**16**(6):067012

[53] Yamaguchi S, Yachiku S, Morikawa M. Analysis of proliferative activity of the parathyroid glands using proliferating cell nuclear antigen in patients with hyperparathyroidism. The Journal of Clinical Endocrinology and Metabolism. 1997;**82**(8):2681-2688

[54] Corbetta S, Mantovani G, Lania A, Borgato S, Vicentini L, Beretta E, et al. Calcium-sensing receptor expression and signalling in human parathyroid adenomas and primary hyperplasia. Clinical Endocrinology. 2000;**52**(3):339-348

[55] Shi Y, Hogue J, Dixit D, Koh J, Olson JA Jr. Functional and genetic studies of isolated cells from parathyroid tumors reveal the complex pathogenesis of parathyroid neoplasia. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(8):3092-3097

[56] Howson P, Kruijff S, Aniss A, Pennington T, Gill AJ, Dodds T, et al. Oxyphil cell parathyroid adenomas causing primary hyperparathyroidism: A clinico-pathological correlation. Endocrine Pathology. 2015;**26**(3):250-254

[57] Yazar FM, Karaagac M, Isler A, Bulbuloglu E, Ezberci F. An unusual cause of hypercalcemic crisis: Water-clear cell double parathyroid adenoma. Turkish Journal of Surgery. 2017;**33**(4):243-247

[58] Ritter C, Miller B, Coyne DW, Gupta D, Zheng S, Brown AJ, et al. Paricalcitol and cinacalcet have disparate actions on parathyroid oxyphil cell content in patients with chronic kidney disease. Kidney International. 2017;**92**(5):1217-1222

[59] Altinay S, Erozgur B, Dural AC, Volante M, Papotti MG. Monoclonal/polyclonal PAX-8, PTH and GATA3 immunohistochemistry in parathyroid lesions. Journal of Endocrinological Investigation. 2021;**44**(9):1997-2008

[60] De la Hoz RA, Munoz De Nova JL, Munoz Hernandez P, Valdes de Anca A, Serrano Pardo R, Tovar Perez R, et al. Oxyphil cells in primary hyperparathyroidism: A clinicopathological study. Hormones (Athens, Greece). 2021;**20**(4):715-721

[61] Byrd C, Kashyap S, Kwartowitz G. Parathyroid Cancer. StatPearls Publishing, Treasure Island (FL); 2021

[62] Machado NN, Wilhelm SM. Parathyroid cancer: A review. Cancers (Basel). 2019;**11**(11):1676-1693

[63] Calapkulu M, Sencar ME, Unsal IO, Sakiz D, Duger H, Ozbek M, et al. Tumor volume can be used as a parameter indicating the severity of disease in parathyroid Cancer. Endocrine Practice. 2021;**27**(7):706-709

[64] Krupinova J, Mokrysheva N, Petrov V, Pigarova E, Eremkina A, Dobreva E, et al. Serum circulating miRNA-342-3p as a potential diagnostic biomarker in parathyroid carcinomas: A pilot study. Endocrinol Diabetes Metab. 2021;**4**(4):e00284

[65] Verdelli C, Forno I, Morotti A, Maggiore R, Mari G, Vicentini L, et al. miR-126-3p contributes to parathyroid tumor angiogenesis. Endocrine-Related Cancer. 2021;**28**(1):53-63

[66] Bjerneroth G, Juhlin C, Gudmundsson S, Rastad J, Akerstrom G, Klareskog L. Major histocompatibility complex class II expression and parathyroid autoantibodies in primary hyperparathyroidism. Surgery. 1998;**124**(3):503-509

[67] Goncu B, Yucesan E, Aysan E, Kandas NO. HLA class I expression changes in different types of cultured parathyroid cells. Experimental and Clinical Transplantation. Apr 17, 2019. DOI: 10.6002/ect.2018.0388 [Epub ahead of print]

[68] Goncu B, Yucesan E, Ersoy YE, Aysan ME, Ozten Kandas N. HLA-DR, -DP, -DQ expression status of parathyroid tissue as a potential parathyroid donor selection criteria and review of literature. Human Immunology. 2022;**83**(2):113-118

[69] Natali PG, Bigotti A, Nicotra MR, Viora M, Manfredi D, Ferrone S. Distribution of human Class I (HLA-A,B,C) histocompatibility antigens in normal and malignant tissues of nonlymphoid origin. Cancer Research. 1984;**44**(10):4679-4687