Differential Gene Expression by Oxyphil and Chief Cells of Human Parathyroid Glands

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Context: Parathyroid oxyphil cells, whose function is unknown, are thought to be derived from chief cells. Oxyphil cells increase in number in parathyroid glands of patients with chronic kidney disease (CKD) and are even more abundant in patients receiving treatment for hyperparathyroidism with calcitriol and/or the calcimimetic cinacalcet.

Objective: We examined oxyphil and chief cells of parathyroid glands of CKD patients for differential expression of genes important to parathyroid function.

Design/Setting/Participants: Parathyroid tissue from CKD patients with refractory hyperparathyroidism was immunostained for gene expression studies.

Main Outcome Measure: Immunostaining for PTH, PTHrP, calcium-sensing receptor, glial cells missing 2, vitamin D receptor, 25-hydroxyvitamin D-1-OHase, and cytochrome c was quantified and expression reported for oxyphil and chief cells.

Results: Expression of all proteins analyzed, except for the vitamin D receptor, was higher in oxyphil cells than in chief cells.

Conclusion: Human parathyroid oxyphil cells express parathyroid-relevant genes found in the chief cells and have the potential to produce additional autocrine/paracrine factors, such as PTHrP and calcitriol. Additional studies are warranted to define the secretory properties of these cells and clarify their role in parathyroid pathophysiology. (J Clin Endocrinol Metab 97: E1499–E1505, 2012)

The parathyroid glands of young adults consist primarily of chief cells. However, with age or after excessive functional stress, another cell type appears in the parathyroid gland, the oxyphil cell (1, 2). Histologically, oxyphil cells are larger than chief cells (12–20 vs. 6–8 μm), and the cytoplasm is more eosinophilic due to a high mitochondrial content. The observation of transitional oxyphil cells, which are more eosinophilic but similar in size to the chief cells, suggests that oxyphil cells are derived from chief cells. Additional evidence for a chief cell-to-oxyphil cell transdifferentiation is that transitional and oxyphil cells express PTH (3) and glial cells missing 2 (GCM2) (4), a parathyroid-specific transcription factor that is essential for parathyroid gland development.

Parathyroid oxyphil cells are markedly increased in chronic kidney disease (CKD). Interestingly, recent studies have found an association between treatment of secondary hyperparathyroidism with calcitriol and/or cinacalcet and an even higher oxyphil content of the parathyroid compared with no treatment (5–7). Although the function of the oxyphil cell is not known, it has been shown that oxyphils from CKD patients synthesize and secrete PTH (3). However, it is unclear how much PTH they secrete or whether the secretion is regulated. The expression of the calcium-sensing receptor (CaR) and vitamin D receptor (VDR) have not specifically been examined in oxyphil cells.

Parathyroid oxyphil cells have been reported to express PTHrP (8, 9), although it is unclear whether these cells

Abbreviations: CaR, Calcium-sensing receptor; CKD, chronic kidney disease; GCM2, glial cells missing 2; H&E, hematoxylin and eosin; IOD, integrated OD; 1αOHase, 25-hydroxyvitamin D-1α-hydroxylase; VDR, vitamin D receptor.
secrete the hormone systemically. Given the increased mass of oxyphil cells in CKD patients, if PTHrP is secreted by these cells in high enough concentration, it could potentially contribute to renal osteodystrophy, because it acts through the same receptor as PTH. Alternatively, PTHrP could act as an autocrine/paracrine factor, regulating parathyroid growth (10) or PTH secretion (11, 12). It is noteworthy that in other cell types, PTHrP gene transcription is repressed by calcitriol (13), whereas CaR activation enhances PTHrP production in a variety of cells, including malignancies such as human prostate and breast cancer and rat Leydig cell tumor cell lines (14, 15). In contrast, in normal mammary epithelial cells, high calcium suppresses PTHrP gene expression and secretion (16–18).

Here, we compared the expression of key genes involved in parathyroid function in oxyphil and chief cells of hyperplastic parathyroid glands from patients with secondary hyperparathyroidism.

Materials and Methods
Human parathyroid gland immunostaining

As approved by the Human Research Protection Office of Washington University School of Medicine, archived parathyroid tissue obtained from 20 patients having undergone parathyroidectomy due to uremic secondary hyperparathyroidism was used for immunohistochemical analysis. A portion of tissue (formalin fixed and paraffin embedded) from a single parathyroid gland per patient was studied. Of the 20 patients analyzed, 10 were female, and 10 were male; average age was 46.1 yr old (range 29–69 yr). Race is as follows: African-American (n = 10), Caucasian (n = 8), Asian (n = 1), unknown (n = 1). Within a year before surgery, the patients had received the following treatment for secondary hyperparathyroidism: Sensipar (n = 1), unknown (n = 2), a combination of Sensipar and Zemplar (n = 4), no treatment (n = 7), or unknown (n = 1).

Immunostaining was performed using the following antibodies: CaR (1:500;developed in our laboratory) (19), VDR c-20 (sc-1008, 1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), cytochrome c (A-8, 1:50; Santa Cruz Biotechnology), 25-hydroxyvitamin D-1α-hydroxylase (1αOHase; provided by Dr. Eduardo Armbrecht (St. Louis University, St. Louis, MO) followed by streptavidin-horseradish peroxidase conjugate (Invitrogen). The immune complexes were visualized with 3-αmino-9-α-ethylcarbazole substrate-chromogen (Invitrogen, Carlsbad, CA). For PTH, tissue sections were incubated for 30 min with biotinylated rabbit antichicken second antibody (1:100; Sigma-Aldrich, St. Louis, MO) followed by streptavidin-horseradish peroxidase conjugate (Invitrogen). The immune complexes were visualized with 3-αmino-9-α-ethylcarbazole substrate-chromogen.

Tissue sections from 20 patients with CKD were analyzed. Oxyphil cells were identified by their large size and eosinophilic cytoplasm as revealed by hematoxylin and eosin (H&E) staining and, when possible, by positive staining for cytochrome c. An encapsulated or well-defined area of chief or oxyphil cells was designated as nodular. Nonnodular areas of chief cells were designated as diffuse; nonnodular areas of oxyphil cells were designated as oxyphil clusters.

Immunostaining was quantified as previously described (19). Briefly, ×200 images of stained tissue sections were captured, converted to grayscale, and analyzed using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The intensity of staining was quantified using the OD function of the software. The integrated OD (IOD) and area were obtained for each immunostained image, and IOD/area was calculated to express the intensity of staining per unit area. The IOD/area of the negative control immunostaining (i.e. control serum or IgG) was subtracted from IOD/area for the antibody-stained sections to obtain the corrected IOD/area. For each gene, the immunostaining of areas of diffuse chief cells in each section was considered the baseline staining. The corrected IOD/area for chief nodules, oxyphil clusters, or oxyphil nodules, if present in that section, was then calculated as the percentage of the diffuse chief cell immunostaining. Generally, each patient had three to five genes analyzed on serial tissue sections. For each gene, results were reported as a percentage of baseline staining.

Serum PTHrP measurement

To determine whether parathyroid glands release detectable amounts of PTHrP, plasma was obtained from eight CKD patients before and after parathyroidectomy and from six control subjects and analyzed by Active PTHrP immunoradiometric assay from Diagnostic Systems Laboratories (DSL, Webster, TX), a Beckman Coulter Co. Blood was collected into tubes containing EDTA/aprotinin/leupetin, as recommended by DSL. The plasma samples were also analyzed in a PTHrP enzyme immunoassay (Peninsula Laboratories, LLC, San Carlos, CA).

Statistics

One-way ANOVA with Tukey post hoc test (GraphPad Software, Inc., La Jolla, CA) was used for analysis of quantitation of the immunostaining for differential gene expression. Results (average ± SEM) were reported as percent baseline staining of the chief diffuse cells.
Expression of quantitated immunostaining is reported as percentage of diffuse chief. A portion of one gland per patient was analyzed; n = number of patients analyzed for that particular gene and cell type.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Chief diffuse</th>
<th>Oxyphil cluster</th>
<th>Chief nodule</th>
<th>Oxyphil nodule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1αOHase</td>
<td>100 (n = 17)</td>
<td>264.0 ± 26.7a (n = 13)</td>
<td>68.8 ± 16.5ab (n = 6)</td>
<td>191.6 ± 23.7abc (n = 7)</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>100 (n = 10)</td>
<td>921.3 ± 239a (n = 7)</td>
<td>335.9 ± 195.6ab (n = 5)</td>
<td>1076.3 ± 306.0abc (n = 5)</td>
</tr>
<tr>
<td>PTH</td>
<td>100 (n = 9)</td>
<td>126.4 ± 20.2a (n = 5)</td>
<td>76.4 ± 10.3ab (n = 6)</td>
<td>117.8 ± 15.0a (n = 4)</td>
</tr>
<tr>
<td>PTHrP</td>
<td>100 (n = 14)</td>
<td>225.5 ± 34.3a (n = 10)</td>
<td>103.8 ± 23.3b (n = 7)</td>
<td>168.4 ± 23.9abc (n = 6)</td>
</tr>
<tr>
<td>VDR</td>
<td>100 (n = 11)</td>
<td>124.9 ± 39.5a (n = 8)</td>
<td>118.8 ± 23.6 (n = 6)</td>
<td>128.8 ± 23.9abc (n = 6)</td>
</tr>
<tr>
<td>CaR</td>
<td>100 (n = 11)</td>
<td>128.4 ± 17.9a (n = 9)</td>
<td>94.9 ± 13.7ab (n = 6)</td>
<td>134.8 ± 29.3abc (n = 5)</td>
</tr>
<tr>
<td>GCM2</td>
<td>100 (n = 12)</td>
<td>171.5 ± 21.1a (n = 8)</td>
<td>72.2 ± 13.7ab (n = 5)</td>
<td>96.0 ± 19.6a (n = 6)</td>
</tr>
</tbody>
</table>

Expression of quantitated immunostaining is reported as percentage of diffuse chief. A portion of one gland per patient was analyzed; n = number of patients analyzed for that particular gene and cell type.

a P < 0.05 vs. chief diffuse.
b P < 0.05 vs. oxyphil cluster.
c P < 0.05 vs. chief nodule.
oxyphil cells, a markedly elevated expression was observed for the 1αOHase, the mitochondrial cytochrome P450 that catalyzes the formation of 1,25-dihydroxyvitamin D₃ (calcitriol), the active form of vitamin D (Table 1). Figure 3D shows the staining for 1αOHase in oxyphil clusters compared with diffuse chief cells. Chief cell nodules exhibited the lowest expression of 1αOHase (not shown).

The parathyroid-specific transcription factor GCM2 has been shown to be essential for completion of parathyroid gland development (24) and continues to be expressed in mature parathyroids (25, 26). We found GCM2 to be significantly increased in oxyphil clusters (Table 1 and Fig. 4), suggesting it may be responsible, in part, for the higher CaR and PTH in these cells. As indicated in Table 1, chief cell nodules expressed the least amount of GCM2.

Serum PTHrP measurement

Plasma PTHrP was measured by immunoradiometric assay and enzyme immunoassay. Levels in all normal subjects, and before and after parathyroidectomy in the CKD patients, were undetectable in both assays (data not shown).

Discussion

The chief cells of the parathyroid glands play a central role in calcium homeostasis by sensing changes in extracellular calcium and releasing the appropriate amount of PTH to correct or maintain normal blood calcium levels. Although chief cells are the major cell type of the parathyroid glands of young, healthy subjects, another cell type, the oxyphil cell, appears with age and increases dramatically in number in patients with CKD (1). More recently, evidence indicates that treatment of hyperparathyroidism in CKD patients increases the content of oxyphil cells in the glands (5–7). Importantly, oxyphil content was highest in patients receiving the calcimimetic cinacalcet, suggesting a role for CaR activation in the genesis of these cells. Also of note is the report by Allen and Thorburn (27) that oxyphil cell content is positively associated with mean serum calcium in cases of primary multiple-gland hyperparathyroidism, consistent with a role for CaR activation in their formation. Although these data support the hypothesis of the involvement of the CaR in oxyphil development, additional studies are needed to fully define the factors involved in the transdifferentiation of chief to oxyphil cell.

The function of oxyphil cells is not known, but it has been speculated that they may be deactivated chief cells. The parathyroid glands become hyperplastic when the existing chief cells cannot produce sufficient PTH to correct external stimuli (i.e., low blood calcium, high blood phosphate, or low calcitriol levels). However, when these stimuli are corrected, or masked in the case of cinacalcet treatment for secondary hyperparathyroidism, the (apparent) need for the additional chief cells declines. Parathyroid chief cells do not readily undergo apoptosis, and therefore, conversion to a non-PTH-secreting cell type could prevent excessive secretion of the hormone. However, our current findings indicate that parathyroid oxyphil cells contain high levels of immunoreactive PTH, indicating that these cells continue to produce the hormone. Furthermore, CaR and VDR are expressed by oxyphil cells, suggesting continued regulation of PTH by calcium, calcimimetics, and vitamin D compounds. Tanaka et al. (3) previously showed that heterotransplantation of human parathyroid nodules that consisted exclusively of oxyphil or chief cells into nude mice were able to secrete intact human PTH. Both cell types were determined to have similar PTH secretory activity. In the present study, we show that immunoreactive PTH is significantly higher in oxyphil cells compared with chief cells, with chief cell nodules expressing the least amount of PTH. It is not known whether this increased expression of PTH in oxyphil cells is reflective of
intact PTH, inactive PTH fragments, or antagonistic fragments [e.g. PTH (7–84)]. Future studies with isolated oxyphil cells will better assess their secretory properties.

Although the overall levels of expression of the CaR and VDR are decreased in hyperplastic parathyroid tissue compared with normal tissue (19, 28–33), they are still highly expressed, although heterogeneously, throughout the tissue. Grzela et al. (34) found that protein expression of the CaR and VDR in tertiary hyperparathyroidism was expressed in a markedly diverse manner throughout the parathyroid, with areas of very intense staining for CaR or VDR being surrounded by areas of minimal staining. Likewise, Valimäki et al. (35) found that expression of CaR, VDR, and PTH mRNA were heterogeneously expressed in parathyroid glands of patients with secondary hyperparathyroidism, varying widely not only within the same gland but also between glands. However, neither study reported expression with respect to cell type. In the present study, we found that the CaR was differentially expressed in hyperplastic parathyroid tissue and was significantly higher in oxyphil cells. Expression of the VDR was also heterogeneously expressed in the tissue, but the difference between chief and oxyphil cells was found not to be significant. It is tempting to speculate that treatments for hyperparathyroidism (i.e. calcitriol, calcimimetics, or vitamin D analogs) may alter expression of the CaR and/or VDR, resulting in less variation of expression in the tissue. In fact, Mizobuchi et al. (36) found that the calcimimetic compound NPS R-568 up-regulates CaR expression levels in parathyroid glands of rats with chronic renal insufficiency. Additional studies correlating differential gene expression with treatment for hyperparathyroidism in human glands are in progress.

Consistent with the finding of CaR and PTH in oxyphil cells is our observation that they also express GCM2. This parathyroid-specific transcription factor is critical for parathyroid development (24) and functions to maintain high levels of expression of PTH and CaR (21). Silencing GCM2 in human parathyroid cells reduces the expression of the CaR (21) and PTH (Brown, A. J., and C. S. Ritter, manuscript in preparation), indicating a continued role for GCM2 in parathyroid function. Nonaka (4) recently investigated GCM2 as a diagnostic marker of parathyroid lesions, and found positive expression in chief, oxyphil, and clear cells. However, they reported that expression was uniform throughout the parathyroid tissue. In the present study, we found a heterogeneous expression of GCM2, with expression highest in oxyphil clusters and lowest in chief cell nodules. To see whether this difference in observed expression (uniform vs. heterogeneous) was due to different GCM2 antibodies, we tested our parathyroid tissue with two additional GCM2 antibodies (Santa Cruz; GCM2 S-19 and C-20) and still found the expression to be heterogeneous (unpublished observation).

**FIG. 3.** Immunostaining of PTHrP, cytochrome c, and 1αOHase in parathyroid oxyphil and chief cells. A, Chief (ch) and oxyphil (oxy) cells were identified by H&E stain in a representative image of parathyroid tissue from a patient with CKD; B–D, serial sections show differential expression of PTHrP (B), cytochrome c (C), and 1αOHase (D), with higher levels in the oxyphil cells compared with chief cells. Magnification, ×200.

**FIG. 4.** Immunostaining of GCM2 in parathyroid oxyphil and diffuse chief cells. Chief (ch) and oxyphil (oxy) cells were identified by H&E stain in a representative image of parathyroid tissue from a CKD patient (left). An adjacent section (right) shows differential expression of GCM2, with higher levels in the oxyphil cells. Even smaller groups of oxyphil cells (asterisks) interspersed among the chief cells express higher levels of GCM2. Magnification, ×200.
Parathyroid oxyphil cells were previously reported to express PTHrP (8, 9), and our findings confirm this observation. However, it is unclear whether the PTHrP is secreted by the cells. Given that the mass of oxyphil cells in these enlarged parathyroid glands can be substantial, we investigated whether PTHrP was released systemically in concentrations that had the potential to influence bone turnover. We found that plasma PTHrP levels in eight CKD patients studied were undetectable both before and after parathyroidectomy, the same as for normal individuals. This was true even in two of the CKD patients who each had an estimated 1.2 g oxyphilic parathyroid tissue (one patient had a total of 6 g parathyroid tissue removed, 20% of which was estimated to be oxyphilic according to H&E evaluation; the second patient had a total of 3 g parathyroid tissue removed, 40% of which was estimated to be oxyphilic). Thus, if PTHrP is secreted by oxyphil cells, it does not appear to leave the parathyroid glands and may act only in an autocrine/paracrine fashion. In fact, exogenous PTHrP has been shown to enhance PTH release in response to low calcium in rats and in rat parathyroid gland cultures (11, 12), indicating the potential of PTHrP actions in human glands as well.

Oxyphil cells have abundant mitochondria, suggesting a need for energy production. Mitochondria are also the site of vitamin D metabolism, and we recently reported that the vitamin D 1αOHase is very highly expressed in oxyphil cells (20). Calcitriol produced by extrarenal tissues does not typically enter the circulation to regulate mineral metabolism. Instead, it is usually made in response to cell-specific stimuli and carries out autocrine/paracrine functions. We have recently demonstrated that high calcium increases the production of 1αOHase in human parathyroid monolayers and that this enzyme is active (20). Therefore, we speculate that parathyroid oxyphil cells, formed by transdifferentiation in response to CaR activation, may produce calcitriol to reduce PTH synthesis, providing an additional potential mechanism for control of PTH by calcimimetics.

In conclusion, the parathyroid oxyphil cells that accumulate in substantial numbers in CKD patients express parathyroid-relevant genes found in the chief cells (PTH, VDR, CaR, and GCM2), strongly suggesting that oxyphil cells are not simply deactivated chief cells. The presence of VDR and CaR further suggests that oxyphil cells release PTH in a regulated fashion, but this must be determined empirically using enriched cell populations. Parathyroid oxyphil cells also have the potential to produce autocrine/paracrine factors, such as PTHrP and calcitriol. Comparative analysis of the proteins released by oxyphil and chief cells (secretomes) may identify additional novel secreted factors of pathophysiological importance. The fact that oxyphil cells are increased in the parathyroid glands of CKD patients, being highest in patients receiving conventional treatment for hyperparathyroidism, suggests that they have a function distinct from that of chief cells. Additional studies are required to clearly define the role of these cells in parathyroid pathophysiology.

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References


