Parathyroid cell growth in patients with advanced secondary hyperparathyroidism: vitamin D receptor and cyclin-dependent kinase inhibitors, p21 and p27

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Abstract
Uraemic patients with advanced secondary hyperparathyroidism (2HPT) have nodular hyperplastic glands with a decreased vitamin D receptor (VDR) density. Previous studies have shown that nodular hyperplasia expressed a significantly lower VDR density as compared with diffuse hyperplasia, and the VDR density negatively correlated with both the glandular weight and the marker of cell proliferation. However, the mechanism by which the decreased VDR density leads to parathyroid cell proliferation remains unclear. In the myelomonocytic cell line, active vitamin D3 is known to activate the transcription of both p21 and p27, cyclin-dependent kinase inhibitors (CDKIs), regulating the transition from the G1 to the S phase of the cell cycle, in a VDR-dependent manner. Moreover, the overexpression of p21 and p27 inhibits cell proliferation. In order to elucidate the mechanism of parathyroid cell proliferation, the expression of CDKIs, p21 and p27, and the VDR was analysed immunohistochemically, and compared among nodular and diffuse hyperplastic parathyroid glands, and histologically normal parathyroid glands. The VDR expression in nodular hyperplasias was significantly decreased compared with either diffuse hyperplasias or normal parathyroid glands. The expression of both p21 and p27 was also significantly lower in nodular hyperplasias than in diffuse hyperplasias or normal parathyroid glands. Sections of parathyroid glands with a high expression of nuclear VDR highly expressed both p21 and p27. In nodular hyperplasias, the expression of both p21 and p27 correlated either positively with the nuclear VDR expression or inversely with the glandular weight. Therefore, the reduced expression of p21 and p27, being VDR dependent, is a major pathogenic factor for nodular parathyroid gland growth in advanced 2HPT.

Keywords: cyclin-dependent kinase inhibitor; haemodialysis; p21; p27; secondary hyperparathyroidism; vitamin D receptor

Introduction
A number of pathogenic factors, including hypocalcaemia, phosphate retention, vitamin D deficiency, reduced density of both vitamin D receptors (VDRs) and Ca\(^{2+}\)-sensing receptors (CaRs) in the parathyroid cells, contribute to the development of secondary hyperparathyroidism (2HPT) in patients with chronic renal failure [1–3]. The characteristic findings of renal hyperparathyroidism are the asymmetrical enlargement as well as the nodularity of the parathyroid glands, and an increase in both oxyphilic and transitional oxyphilic cells. In spite of the identical environmental factors, such as the serum concentrations of calcium, phosphorus and active vitamin D3 (1,25D), the growth of the parathyroid glands varies in the same patient, suggesting that the respective parathyroid glands have asymmetrical abnormalities for sensing environmental factors.

Resistance to the physiological concentration of serum 1,25D in nodular hyperplasia

The inhibitory effects of 1,25D on 2HPT have been shown during either oral or intravenous 1,25D pulse therapy [4–6], which decreases the concentration of serum parathyroid hormone (PTH). In patients with severe 2HPT, the hyperplastic parathyroid glands often
show resistance to the physiological concentration of serum 1,25D [5,7–9], and this can be partly explained by VDR deficiency in hyperplastic glands [10–12].

Receptor abnormalities and cell proliferation in the hyperplastic parathyroid glands

Parathyroid hyperplasia in patients with chronic renal failure has been classified into two major patterns, diffuse and nodular, and the weight of nodular hyperplasia is usually heavier. It has been postulated that the pattern of hyperplasia may change from diffuse to nodular as the parathyroid gland becomes heavier [13,14]. Fukuda et al. reported that nodular hyperplasia had a significantly lower VDR density than diffuse hyperplasia, and that the VDR density negatively correlated with both the weight and proliferative activity of the hyperplastic parathyroid glands in patients with advanced 2HPT [2].

Antiproliferative effect of 1,25D

The antiproliferative effect of 1,25D on the parathyroid glands has also been demonstrated in 2HPT patients undergoing oral 1,25D pulse therapy, leading to a decrease in the size of the hyperplastic glands [4]. Kremer et al. showed that 1,25D abolished the expression of a proto-oncogene, c-myc, and delayed the subsequent proliferation of bovine parathyroid cells in primary culture [15]. However, nodular hyperplastic glands, expressing a lower VDR density, are often resistant to 1,25D pulse therapy as compared with diffuse hyperplasia [2].

Regulation of parathyroid cell growth by 1,25D via the VDR

Parathyroid cell proliferation is reduced by 1,25D, which decreases the expression of the proto-oncogene, c-myc [15]. This gene modulates the progression from the G1 to the S phase of the cell cycle. A decrease in the plasma concentration of 1,25D and/or a disturbance of its action at the level of the parathyroid cell, which are both observed frequently in uraemic patients, can decrease the inhibition of c-myc expression and lead to progression of the cell cycle. Futhermore, Liu et al. found that p21, a cyclin-dependent kinase inhibitor (CDKI), is transcriptionally induced by 1,25D in a VDR-dependent manner, but not in a p53-dependent manner, and p27 is also induced by 1,25D in the myelomonocytic cell line, U937 [16]. It is known that the p21 and p27 genes coding CDKI regulate progression from the G1 to the S phase of the cell cycle by inhibiting cyclin-dependent kinase [17–19]. Moreover, Cozzolino et al. recently demonstrated that in uraemic rats, the efficacy of 1,25D, and its less calcaemic analogue 19-nor-1,25D, in preventing high phosphorus-induced parathyroid hyperplasia could be partially explained by the induction of parathyroid p21 expression. They also reported that the increases in p21 correlated inversely with reduced Ki67 [20]. Their findings indicate another possible mechanism by which calcitriol may regulate the proliferation of parathyroid cells.

We have therefore hypothesized that the reduced p21 and p27 production via decreased nuclear VDR expression leads to parathyroid cell proliferation, particularly in nodular hyperplasia.

Relationship between the VDR and CDKI, p21 and p27

Immunohistochemical staining of VDR protein revealed mainly nuclear localization and, to a lesser degree, cytoplasmic localization of each histological type. In our study, nodular hyperplasias showed a significantly lower VDR density than diffuse hyperplasias or normal parathyroid glands, being compatible with previous reports [2]. Therefore, the effects of 1,25D through the VDR might be limited in nodular hyperplasia. Expression of both p21 and p27 proteins revealed nuclear localization, and the semi-quantitative analyses showed that the expression of both p21 and p27 in nodular hyperplasias was also significantly lower than in diffuse hyperplasias or normal parathyroid glands, as in the case of the VDR. Using serial sections, the distribution of the VDR and either p21 or p27 was examined. It was revealed that the sections of parathyroid glands with high nuclear VDR expression elicited high p21 and p27 expression, whereas the sections that lacked the VDR had no detectable p21 or p27. Moreover, a significant positive correlation between VDR expression and the expression of either p21 or p27 was found only in nodular hyperplasia [21]. Our results suggest that p21 and p27 expression may be regulated in a VDR-dependent manner in hyperplastic parathyroid glands (Figure 1), as observed in the myelomonocytic cell line, U937 [16]. Indeed, a functional vitamin D response has been identified in the p21 promoter area [16], but not in the p27 promoter area.

Relationship between CDKI, p21 and p27, and parathyroid cell growth

Both p21 and p27 proteins participate in regulating the transition from the G1 to the S phase of the cell cycle [17,18]. Dusso et al. [22] elegantly demonstrated the direct effect of phosphorus on parathyroid cell proliferation in the early phase of uremia in 5/6 nephrectomized rats. Low dietary phosphorus induced p21 expression, whereas high phosphorus intake enhanced transforming growth factor-α (TGF-α) with a subsequent stimulation of parathyroid cell proliferation independent of the changes in concentration of serum 1,25D. In their study, there was a significant correlation between decreased p21 expression and enhanced
proliferating cell nuclear antigen (PCNA) expression, showing a direct relationship between p21 and parathyroid cell proliferation. In our study, the weight of the resected glands inversely correlated with the expression of either p21 or p27 in nodular hyperplasias. Semi-quantitatively, the expression of Ki67 antigen was significantly higher in nodular hyperplasias than in both diffuse hyperplasias and normal parathyroid glands. The expression of Ki67 antigen did not correlate with the expression of both p21 and p27 in our study, although the expression of Ki67 antigen was significantly higher in nodular hyperplasia [21]. Therefore, our results also support the hypothesis that decreased expression of both p21 and p27 can lead to parathyroid cell proliferation in advanced 2HPT (Figure 1). Thus, the transcriptional up-regulation of both p21 and p27 genes due to 1,25D may be one of the mechanisms of the antiproliferative effects of the 1,25D–VDR complex which block G1–S transition.

Possible management of 2HPT by modulating CDKI, p21 and p27

The decreased VDR expression and the deficiency of 1,25D play a major role in parathyroid cell proliferation and continuous oversecretion of PTH, even after correction of the calcium–phosphorus imbalance. For the prevention and management of 2HPT, it is thus crucial to understand the mechanisms regulating the VDR-dependent inhibition of cell growth. Induction of p21 arrests growth in monocyte–macrophages [16], keratinocytes [23] and human cancer cells [24], as well as suppressing tumorigenicity in vivo [25]. Induction of p27 also induces growth arrest in monocyte–macrophages [16].

In situ immunohistochemical analyses of p21 and p27 expression in tissue samples of hyperplastic parathyroid glands, obtained by a needle biopsy, may be a useful technique for estimating both the responsiveness to 1,25D therapy and an indication for parathyroidectomy. Up-regulation of the VDR content by 1,25D administration could correct the parathyroid VDR density, and therefore normalize p21 and p27 levels.

Conclusion

The higher mitogenic properties observed in nodular hyperplasia of the parathyroid glands in patients with advanced 2HPT can be attributed to the reduced expression of the CDKIs, p21 and p27, being associated
with the down-regulated VDR expression in parathyroid cells. Based on the present results and other published reports, we conclude that the decreased VDR-dependent expression of both p21 and p27 is a major cause of the advanced parathyroid cell growth in 2HPT.

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References