Cycling with the parathyroid

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Parathyroid (PT) cells divide infrequently, but they retain the latent ability to proliferate to form large, hyperfunctioning glands. Primary hyperparathyroidism (HPTH), which affects all PT glands, may be due to inactivating mutations in tumor suppressor genes such as the menin gene (*MEN1*), or activating mutations of the RET proto-oncoprotein, the product of the *MEN2a* gene (1). Mutations in *MEN1* are also found in some isolated PT adenomas, while other PT adenomas carry a chromosomal translocation, in which the parathyroid hormone gene (*PTH*) promoter drives a translocated sequence encoding cyclin D1. Indeed, this cyclin, which is now known to be important for the entry of proliferating cells into G1 of the cell cycle, was discovered by Arnold's laboratory through their genetic analysis of PT adenomas. As described in this issue of the *JCI*, this same group has now generated transgenic mice in which expression of cyclin D1 is targeted to the PT, resulting in HPTH (2).

Proliferative and other responses to calcium and phosphate

Factors that can drive the PT cell to leave its dormant state in G0 and enter the cell cycle have been characterized best in patients with secondary HPTH due to chronic renal failure. These studies show that persistently low serum calcium or high serum phosphate levels are the major factors leading to PT cell proliferation. Thus, 1,25(OH)-vitamin D therapy decreases parathyroid hormone (PTH) transcription and PT cell proliferation through its effects on circulating calcium levels, whereas vitamin D deficiency can cause the PT cell to proliferate because of the secondary chronic hypocalcemia.

The PT is geared to respond to hypocalcemia by increasing PT secretion over a timescale of seconds to minutes, by increasing PTH mRNA levels over a period of hours, and by increasing PT cell proliferation over the longer term. The PT calcium-sensing receptor (CaSR) is activated by increases in serum calcium and activates second messengers that lead, through a still poorly understood pathway, to decreased PTH secretion. With hypocalcemia the CaSR is relaxed and PTH secretion is not restrained. Therefore, in the absence of the CaSR constitutive secretion of PTH would be expected, as indeed occurs in knockout mice and patients carrying mutations in *CASR* (3). Similarly, uremic rats given calcimimetic agents that bind to the CaSR show decreased PT cell proliferation demonstrating a role for the CaSR in PT cell proliferation (4).

High serum phosphate levels also increase PTH secretion independently of changes in serum calcium or serum 1,25(OH)-vitamin D levels (5). Phosphate's effects on the PT are mediated at least in part by phospholipase A2, but it is not apparent how the cell senses changes in the extracellular phosphate concentration (6–8). Possibilities include a phosphate-sensing receptor on the cell membrane or changes in levels of phosphorylated intermediary metabolites. PT cell proliferation is increased by chronic hyperphosphatemia and dramatically decreased by hypophosphatemia (9).

Of the various responses to the composition of the extracellular fluid by PT cells, the greatest progress has been made regarding the posttranscriptional regulation of PTH mRNA expression (5, 10). The PTH mRNA 3′-untranslated region (UTR) has a short 26 nucleotide sequence, a *cis* element that binds specific PT cytosolic proteins, the *trans* factors
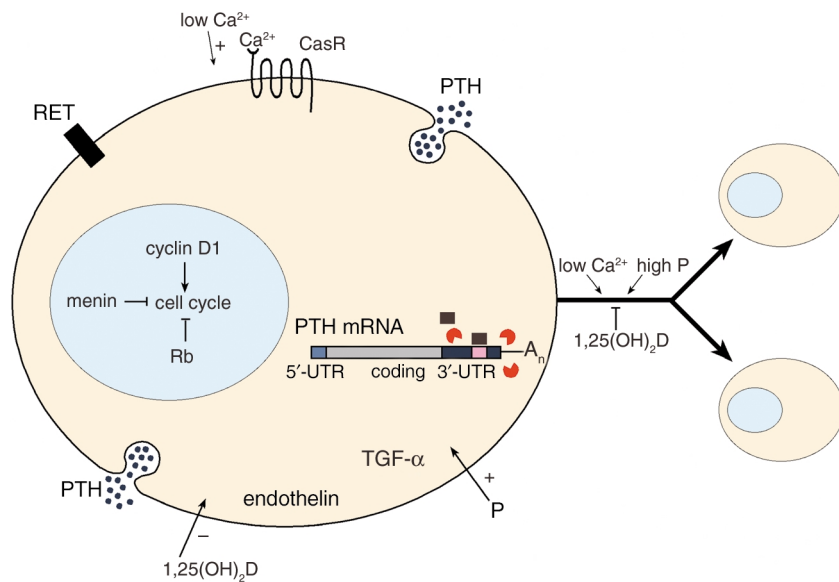
Figure 1

Regulation of PT proliferation, gene expression, and secretion. Cyclin D1 driven by the *PTH* promoter and activating mutations of the *Menin* gene can both cause PT adenomas; germ-line mutations of the latter cause MEN 1. PT carcinomas, which are rare, show lack of expression of the retinoblastoma protein (pRb). Activating mutations of the *RET* proto-oncogene result in MEN 2a. A low serum calcium leads to a decreased activation of the CaSR and results in increased PTH secretion (blue dots), PTH mRNA stability and PT cell proliferation. A high serum phosphate leads to similar changes in each of these parameters. Endothelin and TGF-α are increased in the PTs of proliferating PT glands. 1,25(OH)₂D₃ decreases PTH gene transcription markedly and decreases PT cell proliferation. PTH mRNA stability is regulated by PT cytosolic proteins (*trans* factors, shown here in brown), which bind a short, defined *cis* sequence (pink) in the PTH mRNA 3′-UTR, preventing degradation by ribonucleases (red). One such protective protein is AUF1. In hypocalcemia, there is more binding of the *trans* factors to the *cis* sequence leading to a more stable transcript. A low serum phosphate leads to decreased binding and a rapidly degraded PTH transcript.

(11). In hypocalcemic rats there is an increased protein-PTH mRNA binding that correlates with a stabilization of the PTH transcript. In hypophosphatemic rats there is a marked decrease in this binding that correlates with a marked instability of the PTH transcript and decrease in serum PTH levels. One of the *trans* proteins has been defined as AUF1, a protein that regulates the stability of other mRNAs (12). AUF1 stabilized PTH mRNA in an in vitro degradation assay with PT proteins. Presumably, chronic hyperphosphatemia promotes binding of the *trans* factors to the PTH mRNA 3′-UTR *cis* element, thereby increasing PTH mRNA levels and then serum PTH levels. The combination of chronic hyperphosphatemia and hypocalcemia that is found in many hemodialysis patients leads to markedly increased serum PTH levels and resultant renal bone disease.

There are other factors that regulate PT cell proliferation. Endothelin-1 (ET-1) acts as a mitogen in a variety of cell types, and ET-1 mRNA and protein are highly expressed in the PT chief cells (13), as are ET-1 receptors. In hypocalcemic rats, ET-1 increases in PT cells. Because this effect can be blocked using an ET-1 receptor antagonist (14), it appears that ET-1 acts in an autocrine fashion to induce PT cell proliferation. In human PTs and uremic rats, this PT cell proliferation correlates with an increase in TGF-α levels (15, 16).

A new model
These findings suggest some potential regulators of the proliferative response, but it has been difficult to define the sequence of events precisely, because, in the experimental models available, only a small percentage of the cells enter the cell cycle. The transgenic mice created by Imanishi et al. in this issue of the *JCI* provide a welcome model that may be better suited to studying this response (2). These authors created transgenic mice in which cyclin D1 is specifically expressed in the PT, under control of a 5.1 kb upstream region of *PTH*. As expected, the transgenic mice develop HPTH, with large hyperplastic and, in some cases, adenomatous glands. These mice were then used to study in vivo parameters of PTH physiology. PTH secretion, as measured by the concentration of serum calcium needed to half-maximally suppress PTH secretion (calcium setpoint) is increased in the HPTH mice, similar to the findings in patients with primary or secondary HPTH. Furthermore, expression of the CaSR protein is decreased in the hyperplastic PTs, as has been found in patients. However, now it is clear that this decrease is a secondary phenomenon and not a cause of the HPTH. The bone histomorphometric findings in these animals are of particular interest: Much like patients with primary HPTH, these transgenic mice exhibit a high turnover of bone and substantial cortical bone resorption. In humans, the resultant bone loss often serves as an indication to recommend parathyroidectomy.

There are other questions in PT biology that may now be tackled in this model. Is the vitamin D receptor decreased in the PTs? How does the PT cell progress from hypertrophy to hyperplasia? What is the effect of antiresorptive agents on bone disease? Finally, how might PTH expression be induced for therapeutic purposes? For instance, since PTH is a powerful bone anabolic agent with potential for the treatment of osteoporosis, it might be beneficial to increase the secretion of endogenous PTH rather than relying on daily hormone injections. Advances in basic PT biology, as in the present study, will provide the tools for discovering such therapeutic strategies.

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