Abstract Parathyroid hormone-related peptide (PTHrP), which frequently causes the humoral hypercalcemia of malignancy syndrome, is an autocrine/paracrine regulator of chondrocyte proliferation and differentiation that acts through the PTH/PTHrP receptor (PTH1R). PTHrP is generated in response to Indian hedgehog (Ihh), which mediates its actions through the membrane receptor patched, but interacts also with hedgehog-interacting protein (Hip). Mice lacking PTHrP show accelerated chondrocyte differentiation, and thus premature ossification of those bones that are formed through an endochondral process, and similar but more-severe abnormalities are observed in PTH1R-ablated animals. The mirror image of these skeletal findings, i.e., a severe delay in chondrocyte differentiation and endochondral ossification, is observed in transgenic mice that overexpress PTHrP under the control of the \(\alpha 1(II)\) procollagen promoter. Severe abnormalities in chondrocyte proliferation and differentiation are also observed in two genetic disorders in humans that are most likely caused by mutations in the PTH1R. Heterozygous PTH1R mutations that lead to constitutively activity were identified in Jansen metaphyseal chondrodysplasia, and homozygous or compound heterozygous mutations that lead to less-active or completely inactive receptors were identified in patients with Blomstrand lethal chondrodysplasia. Based on the growth plate abnormalities observed in these human disorders and in mice with abnormal expression of either PTHrP or the PTH1R, it appears plausible that impaired expression of PTHrP and/or its receptor contributes to the growth abnormalities in children with end-stage renal disease. In fact, mild-to-moderate renal failure leads in animals to a reduction in PTH1R expression in growth plates and impaired growth, but it remains uncertain whether this contributes to altered chondrocyte growth and differentiation.

Key words Parathyroid hormone-related peptide · Indian hedgehog · Skeletal development

Introduction

Through the development of genetically manipulated animals and through the molecular definition of rare genetic disorders in humans, remarkable progress has been made in understanding some of the factors that are involved in the regulation of growth plate chondrocytes, and thus bone growth and elongation. The parathyroid hormone (PTH)/parathyroid hormone-related protein (PTHrP) receptor (also termed PTH1R) plays a central role in this process by regulating chondrocyte proliferation and differentiation. It belongs to a distinct family of G protein-coupled receptors, mediates the actions of two peptides hormones, PTH and PTHrP, and signals through at least two different second messenger pathways, cAMP/PKA and Ca\(^{2+}/IP_3/PKC\). The PTH1R is most abundantly expressed in kidney and bone, where it mediates the endocrine actions of PTH, and in growth plate cartilage, where it mediates the autocrine/paracrine actions of PTHrP [1].

PTH is, besides 1,25-dihydroxyvitamin vitamin D\(_3\), the major regulator of mammalian calcium and phosphorus homeostasis [1]. A second hormone with biological properties that are largely indistinguishable from those of PTH was first proposed in the early 1940s [2] and led to the molecular cloning of PTHrP from several different tumors that cause the hypercalcemia of malignancy syndrome [3–7]. Despite this important breakthrough in understanding the pathogenesis of tumor-associated humoral hypercalcemia, only limited conclusions could be drawn in these studies about the physiological role(s) of PTHrP in normal fetal and adult life.

Insights into its normal biological role(s) were gained through the development of transgenic and gene-ablated animals, and through the molecular definition of two rare genetic disorders in humans, Jansen metaphyseal chondrodysplasia and Blomstrand lethal chondrodysplasia, which are both characterized by striking abnormalities in
growth plate development. Based on these studies, it is now obvious that PTHrP and the PTH1R have major roles in normal endochondral bone formation and bone elongation. Furthermore, some other proteins, namely Indian hedgehog (Ihh), patched (Ptc) and smoothened (Smo), and hedgehog-interacting protein (Hip) have been identified, and their importance in chondrocyte growth and differentiation has been established. These advances in understanding growth plate biology are likely to have significant implications for understanding and exploring the growth abnormalities in children with end-stage renal disease.

**Parathyroid hormone-related peptide**

As indicated above, PTHrP was discovered as the humoral factor that causes the hypercalcemia of malignancy syndrome [4–7]. Its amino-terminal portion, but not its mid- and carboxyl-terminal regions, resembles that of PTH. Due to this limited structural homology, PTH and PTHrP are able to activate with indistinguishable efficacy a common G protein-coupled receptor, the PTH/PTHrP receptor (Fig. 1) [8, 9]. Consequently, amino-terminal fragments of both peptides have largely indistinguishable biological properties in vivo, at least with respect to their roles in regulating calcium and phosphorus homeostasis [10–13].

A secreted, mid-regional PTHrP fragment, PTHrP (38–94)amide, which is generated along with several other PTHrP species through post-translational processing, appears to interact with a novel receptor that is distinct from the cloned PTH/PTHrP receptor and that mediates its actions through changes in intracellular free calcium, rather than through the second messenger cAMP [14, 15]. It is plausible that this receptor also mediates the actions of PTHrP(38–94)amide and of PTHrP(67–86)amide that were both shown to stimulate the transplacental transfer of calcium in fetal lambs and in PTHrP-ablated murine fetuses, respectively [15, 16]. These recent findings confirmed earlier studies that had provided first evidence for the importance of PTHrP in the regulation of fetal calcium homeostasis [17]. Other secreted fragments, i.e., PTHrP(107–139), which were shown to inhibit osteoclastic bone resorption [18, 19], are also generated through post-translational cleavage of intact PTHrP [15]. These and possibly other PTHrP fragments are likely to mediate their actions through additional, still uncharacterized receptors.

**Ablation and transgenic expression of the PTHrP**

The importance of PTHrP as the PTH-like factor responsible for most cases of the humoral hypercalcemia of malignancy syndrome is now well established [20–22]. However, PTHrP is also expressed in a remarkable variety of normal fetal and adult tissues, which suggested, relatively soon after its discovery, that it has additional biological role(s) and that these yet undefined role(s) are distinct from those mediated by PTH. The most-revealing new insights into these role(s) were obtained from mice, in which the PTHrP gene had been ablated through homologous recombination [23]. Ablation of only one copy of the PTHrP gene resulted in mice that are normal at birth, show normal behavior, and fertility, but develop osteopenia later in life, despite an apparently normal regulation of calcium and phosphorus homeostasis [24]. In contrast, animals that lack both alleles of the PTHrP gene die, despite their grossly normal size and appearance, at birth or shortly thereafter [23]. Further examination revealed that these mice have striking skeletal changes, but no obvious effects on the morphological development of other major organs, providing compelling evidence for an important role of PTHrP in chondrocyte differentiation and bone elongation.

From these and subsequent studies, it is now apparent that PTHrP facilitates the continuous proliferation of chondrocytes in the growth plate, and that it postpones their programmed differentiation into hypertrophic chondrocytes. Consistent with this presumed role of PTHrP in endochondral bone formation, it has been long known that PTH (which was used in all earlier studies instead of PTHrP, which had not yet been discovered) affects chondrocyte maturation and activity in vitro [25, 26]. Recent studies confirmed these previous findings by showing that PTH and PTHrP stimulate, presumably through cAMP-dependent mechanisms [27], the proliferation of fetal growth plate chondrocytes, inhibit the differentiation of these cells into hypertrophic chondrocytes, and furthermore stimulate the accumulation of cartilage-specific proteoglycans that are thought to act as inhibitors of mineralization [28–30]. Due to the lack of these cartilage-specific functions of PTHrP, growth plates of homozygous PTHrP gene-ablated mice have a thinner layer of proliferating chondrocytes, while the layer of hypertrophic chondrocytes is relatively normal in thickness, but somewhat disorganized. These findings suggested that the lack of PTHrP accelerates the normal differentiation process of growth plate chondrocytes, i.e., resting and

![Fig. 1 Biological functions of parathyroid hormone (PTH) and PTH-related protein (PTHrP) that are mediated through the PTH/PTHrP receptor (PTH1R)](image-url)