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Mini-review

The Parathyroid Hormone Family of Ligands and Receptors

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Abstract: The PTH family of ligands and receptors have a wide range of vital functions from calcium homeostasis to tissue and bone development from the embryo to adult. This family has undergone whole genome duplication events predating vertebrate evolution, indicating more primitive and ancient functions other than skeletal development. The N-terminal region of the ligands, have been widely studied by biophysical and functional analysis, resulting in the discovery of key characteristics essential for ligand-receptor activation being elucidated. Multi-substituted amino acid analogs with differential binding affinities and either antagonistic or agonistic signalling potencies have been created based on these findings allowing for improvement on potential therapies affected by the PTH system in skeletal and embryonic development. The PTH family has diversely evolved to cover a wide range of pivotal pathways crucial to growth and development throughout all animal life.

Keywords: Parathyroid hormone (PTH); parathyroid hormone related protein (PTHrP); tuberoinfundibular peptide 39 (TIP39); parathyroid hormone like peptide (PTH-L); G-protein coupled receptors (GPCRs); parathyroid hormone receptors (PTHR1, PTHR2, PTHR3); epithelial-to-mesenchymal transition (EMT); zebrafish, xenopus; elephant shark

1. Introduction

The Parathyroid Hormone (PTH) family of ligands and receptors are a lynchpin of regulatory networks necessary for pivotal processes during embryonic development through to adult physiologies [1–6]. The ligands include PTH, PTH-related peptide (PTHrP), and tuberoinfundibular peptide (TIP39, also known as PTH2) [7–9]. The most recent new peptide addition to the family is PTH-like peptide (PTH-L or TIP38), which is only present in non-mammalian species including Xenopus, teleosts, and chicken [3,10,11]. The mature peptide sequences of these hormones share significant homology within the first 34 amino acids (aa) but are encoded by separate genes [7,9,12].

PTH is the principal regulator of blood calcium (Ca^{2+}) homeostasis and metabolism, modulating osteoclastic bone resorption and calcium reabsorption in the kidneys [1,4,13]. Human PTH is highly expressed and secreted by the parathyroid gland as an 84 aa unmodified polypeptide in response to low blood Ca^{2+} levels [14], and in smaller amounts by the hypothalamus, pituitary and thymus [15–17].

Bone formation by osteoblasts and resorption by osteoclasts is tightly coupled, where the amount of bone formed equals the bone resorbed so that microfractures sustained during normal activity are repaired and bone size, morphology, and mechanical properties are maintained [18–20]. Osteocytes exert positive and negative effects on both osteoblasts and osteoclasts regulating bone remodelling [21]. PTH and PTHrP play crucial roles in this skeletal development and maintenance [22]. PTH has also been demonstrated to have a negative correlation with the glomerular filtration rate (eGFR) associated in patients suffering from chronic kidney disease (CDK), with increases in PTH associated with decreases in eGFR, with implications towards PTH regulation by sufficient Vitamin D minimising the incidence of bone defects as well as kidney disorders in ageing populations [23,24].

PTHrP is a 141 aa peptide first discovered as a hypercalcaemia causing factor of malignancy, activating pathways involved in skeletal metastases, one of the most common life threatening cancer associated disorders [25,26]. Knockout models of *pthrp* and its receptor (PTHR1) helped establish PTHrPs vital role in regulating embryonic development of the skeleton, through the regulation of chondrocyte growth and differentiation in the growth plates of developing long bones [5,27–29]. PTHrp is known to influence epithelial-to-mesenchymal transtition (EMT), a process critically involved in cancer metastasis and invasion [30–32]. PTHrP activates a variety of mitogenic pathways including MAPK, PI3K/Akt, and has multiple autocrine/paracrine functions regulating a diverse range of tissue and organ development (smooth muscle, vascular, intestinal, uterine, bladder, renal, placental, oviduct, mammary gland), from proliferation to differentiation [5,6,33,34]. PTHrP has also been found to play a role in an intracrine pathway, where the protein is translocated to the nucleus via a nuclear localisation sequence acting on the PTHR1 receptor also localised at the nucleus. This evidence suggested roles for the PTH/PTHrP-PTHR1 system in regulating nuclear events either on the nucleoskeleton or directly on gene expression [35–37].

Both PTH and PTHrP act through the same **PTHR1** receptor with equal affinity [38]. PTHR1 is a class B/classII G-protein coupled receptor (GPCR) with seven transmembrane domains mainly expressed in bone and kidney, mimicking expression of its ligands [2]. Co-expression of these peptides and their receptor are strongly linked to metastatic cancers, such as renal, prostate, and skeletal cancers [30]. The receptor is strongly coupled to the adenylyl cyclase (AC)-protein kinase A (PKA), and the phospholipase C (PLC)-protein kinase C (PKC) intracellular Ca^{2+} signalling pathways [1,27,38]. Mutations in the *PTHR1* gene have been linked to primary failure of eruption (PFE) of teeth, characterised by severe posterior open bite caused by problems with tooth movement from its developmental site within the alveolar process toward its function position in the oral cavity [39].

TIP39 is a peptide of 39 residues and shares a relatively lower level of sequence homology with PTH or PTHrP but high resolution NMR studies suggest that it has a similar three-dimensional structure [40,41]. It contains 9 important functionally conserved amino acid residues with PTH and PTHrP [8,42]. A second receptor **PTHR2** was identified sharing more than 50% aa homology with *PTHR1* with PTH binding exerting agonistic effects [43,44]. PTHR2 was found to have a ligand preference to PTH over PTHrP with further investigations revealing robust activation of PTHR2 by TIP39, suggesting TIP39 as its native ligand [8,45,46]. The tissue distribution profile of PTHR2 assessed by mRNA expression in rats was distinct from PTHR1, detected at various loci in the brain, thyroid parafollicular cells, and gastrointestinal cells [47,48].

TIP39 binds exclusively to the PTHR2 receptor however amino acid substitutions can alter its affinity towards the PTHR1 receptor with antagonistic effects [41]. TIP39 and PTHR2 are expressed in highest concentrations in the hypothalamus and spinal cord in mammals and zebrafish, with TIP39s synthesis mainly in the subparafasicular area of the thalamus and the medial paralemniscal nucleus of the pons [8,9,49]. Little is known about the TIP39-PTHR2 signalling system, with suggested roles of TIP39 as a neuroendocrine hormone that modulates several aspects of the stress response, pain perception, blood pressure, and body temperature [8,9,11,45,50]. The TIP39-PTHR2 system does not appear to have a role in calcium regulation nor the patterning of tissues [51]. The system has been implicated in regulating mammalian renal and cardiovascular haemodynamics and osmoregulation with presumably conserved implications in teleosts [11,52].

A third receptor, **PTHR3**, found only in non-mammalian vertebrates was identified through various genome projects sharing closer ligand specificity and structure to PTHR1 over PTHR2 [53–55]. PTHR3 has a stronger affinity for PTHrP over the other PTH receptors in zebrafish, seabream, and chicken, with functions yet to be clarified [53,54,56].

The ***Pth-l*** gene structure is similar to that of the *pth* gene, but the mature peptide of PTH-L shares a higher level of sequene homology to PTHrP [3]. The expression of PTH-L has been investigated in chicken and *Xenopus*. The peptide was found widely but differentially expressed in various tissues of these organisms. Abundant PTH-L transcripts were detected in cartilage in chicken, and in brain, lung, and bone in *Xenopus laevis* [57]. Although the physiological role has not yet been fully established, some reports have suggested that PTH-L in teleosts (Seabream) and *X. Laevis* is the

most potent calciotropic factor among all PTH peptides [57,58]. The PTH system appears more complex in fishes than in mammals providing evidence of continued differential evolution between nontetrapod and tetrapod species [10,22].

2. Evolution of the PTH Family

The presence of the parathyroid hormones (PTH, PTHrP, TIP39, PTH-L) and their receptors (PTHR1, PTHR2, PTHR3) in vertebrates, are proposed to be the result of two rounds of whole genome duplication at the beginning of vertebrate diversification [7,59,60]. While mammals have two PTH receptor genes, *PTHR1* and *PTHR2*, zebrafish have three, *pthr1*, *pthr2*, and *pthr3* [10,12]. Bioinformatics analyses, chromosomal synteny studies and the characterization of the PTH ligands and their receptors from various vertebrate species provide evidence strongly supporting the hypothesis [7,61]. The PTH family of peptides may have existed with more ancient function predating early vertebrate evolution from agnatha [7,60]. However, identification of homologous ligand-receptor pairs in invertebrates and vertebrates is difficult because of the low levels of sequence identity between the orthologs of distant species [62]. In invertebrates, PTHR-like genes have been found in protostomians, cephalochordates, and urochordates, indicating ancestral PTHR evolved before the deuterostome-protostome split [59,61,63]. PTH-like peptides have also been identified in tunicates and amphioxi [64].

PTHrP exhibits more changes in its gene structure than PTH after divergence from their last common ancestor [7]. The *pth* gene structure has been conserved from elephant shark to humans, containing three exons with the pepro-peptide endcoded in the last two exons [10,22,57,65]. The *pthrp* gene structure contains extra (nine in humans) exons upstream and/or downstream of the mature peptide coding regions, along with the presence of splicing variants in humans, rat, mouse, chicken and *Xenopus* [35,57,66]. This allows post-translational processing of mammalian pepro-PTHrP to create three different initial translation products; mature PTHrP, a middle region of PTHrP, as well as osteostatin [6,35,67]. This increase in *pthrp* gene complexity reflects changes in the physiological roles this peptide adapted towards different terrestrial environments, in tetrapods [3,68]. The hypothesis that the PTH system evolved to enable calcium homeostasis with bone as a reservoir for land animals to survive away from their previous readily available and abundant reservoir of marine Ca^{2+}, was extinguished once genome projects revealed the existence of these systems in more ancient sea-dwelling life forms [12,22,55,60,65].

PTH-L is considered an intermediate between PTH and PTHrP in non-mammalian vertebrates, due to its independent phylogenetic position [7,57,58], while TIP39 has 3 exons like PTH but lacks any similar motifs and is distantly positioned phylogenetically [8,9].

The PTH receptors like their ligands are conserved (~70%) from the cartilaginous elephant shark, to the teleost zebrafish, to humans [54,65].

3. Therapies Based on the Ligand-receptor Binding Complex of PTH Family Members

Significant levels of sequence homology are found in the first 34 amino acids of mature PTHrP, PTH and PTH-L peptides [3,57]. The physiological action induced by PTH and PTHrP is dependent on this N-terminal domain of these peptides for ligand binding. The N-terminal residues of PTH 1-34 and PTHrP 1-36 are highly conserved in evolution, with eight of the first 13 amino acids being identical, a large degree of structural homology [69,70], and are essential for receptor binding and hormonal signal transduction [1,2,71]. Cyclic AMP formation was equally stimulated by PTH 1-34 and PTHrP 1-34 in rat bone and kidney cells [72]. Several studies have shown PTH and PTHrP 1-34 stimulated bone remodelling similar to the effect of the mature peptides [4,13,27,73,74].

Synthetic peptides containing only these N-terminal domains of either PTH or PTHrP bind to and activate the receptors with amino acid substitutions affecting the binding affinity, thus potency and subsequent biological activity [1,2,43,75]. The variations in the N-terminal region have thus been targeted for developing antagonistic and agonistic therapies for maladies defined by the many developmental processes the PTH family is involved in, particularly in skeletal development for patients suffering from osteoporosis.

X-ray crystallography of PTH bound to the PTHR1 receptor, identified Val21, Trp23, Leu24, Leu28, Val31 and Phe34 of PTH, forming important hydrophobic interactions with the receptor [76,77]. PTH Arg20 is one of two residues conserved in all peptides known to activate the PTHR1 receptor, and via multiple substitution experiments was found to be critical for full activity forming an important salt-bridge with PTH1Rs Asp-137 [78-80].

Analogs of the ligands such as PTH (3-34), PTH (7-34), [Phe3]and[Phe6]PTH, [D-Trp12,Tyr34]PTH(7-34), PTHrP (7-34), [D-Trp12]PTHrP(7-34), [Asn10,Leu11]PTHrP(7-34), [Asn10,Leu11,D-Trp12]PTHrP(7-34), [Ile5,Trp23]PTHrP(1-36), TIP(7-39), and TIP(9-39) bind to PTHR1 with high affinity yet elicit negligible signal transduction acting as strong antagonists [41,74,81-83].

PTH (1-31) and PTH (1-28) are the shortest native peptides that maintain full receptor affinity and signalling potency [84,85], while PTH (1-14) is the shortest native N-terminal peptide for which some agonist activity exists [86]. Residues at positions 1-9 form a crucial receptor activation domain, and amino acid substitutions at these positions increase potency up to 250 fold, as well as activating the inactive native PTH (1-11) peptide [87].

Both functional and biophysical analysis have elucidated the molecular mechanisms by which the PTH ligands interact with their common GPCRs, with cross-linking of both agonist and antagonist ligands to the PTH receptors laying the groundwork for identifying critical signalling determinants in the ligand binding pocket of the receptor as well as determining signalling selective PTH and PTHrP analogs that can prevent or reduce adverse effects, such as hypercalcemia, hyperparathyroidism, chondrodysplasia, and osteoporosis, among current therapies [1,35,82]. Two peptide-linker-lipid constructs that target the PTHR1 receptor, were designed and prepared, with both showing increased agonistic effects [88]. PTH hyper-secretion induces bone resorption as seen in hyperparathyroidism, whereas intermittent injection of PTH is a potent bone anabolic reagent

increasing bone mass by stimulating osteoblast proliferation and differentiation, useful in the treatment of osteoporosis [43,89,90]. Osteoporosis is currently treated with the osteoclast suppressor's calcitonin, bisphosphonates, or oestrogen, which stop further bone resorption without stimulating new bone growth. Small adenylate cyclase-stimulating fragments of the parathyroid hormone are promising therapeutic agents for osteoporosis that potently stimulate osteoblasts to make mechanically strong or supranormally strong bone [84].

The C-terminal regions of PTH and PTHrP are postulated to interact with receptors and potential functions are still being identified and investigated [91,92].

4. Conclusion

The expansive and diverse findings up to date on the PTH family and their critical roles for the precise and sensitive development of all life forms, demonstrate the intricate complexity involved with growth and developmental pathways. This growing knowledge needs to be combined, mapped and viewed together in synchrony with other regulatory pathways such as the Wnt signalling network, Hedgehog pathways, and other oncogenic pathways, to get a more complete picture of the fractal nature of our universe, connecting the meshwork of life microscopically and macroscopically in its entirety, from ancient genomes to humans. Understanding the cross-talk between these pathways both upstream and downstream is crucial to understanding the delicate effects of these signalling networks. Our understanding of the human genome has benefited greatly from comparative studies with other species and can only expand from here to magnify gene sequences responsible for different phenotypes in closely related genomes, as well as the differentially conserved roles of specific gene families between distantly related species [8−12,51,54,55,60,65,93].

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Conflict of Interest

There are no known conflicts of interest with the publishing of this article.

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