Abstract. Osteosarcoma (OS) is a primary bone tumor of mesenchymal origin mostly affecting children and adolescents. The OS extracellular matrix (ECM) is extensively altered as compared to physiological bone tissue. Indeed, the main characteristic of the most common osteoblastic subtype of OS is non-mineralized osteoid production. Parathyroid hormone (PTH) is a polypeptide hormone secreted by the chief cells of the parathyroid glands. The PTH-related peptide (PTHrP) may be comprised of 139, 141 or 173 amino acids and exhibits considerable N-terminal amino acid sequence homology with PTH. The function of PTH/PTHrP is executed through the activation of the PTH receptor 1 (PTHR1) and respective downstream intracellular pathways which regulate skeletal development, bone turnover and mineral ion homeostasis. Both PTHR1 and its PTH/PTHrP ligands have been shown to be expressed in OS and to affect the functions of these tumor cells. This review aims to highlight the less well known aspects of PTH/PTHrP functions in the progression of OS by focusing on ECM-dependent signaling.

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1. Introduction

Osteosarcoma (OS) is the most common primary bone tumor. It affects both children and adolescents (with a second peak incidence in the middle aged population (1). Of note, a higher frequency in males as compared to females has been reported (2). The most common presenting site for OS is the metaphysis of long bone (3) The lesion is defined as a mass composed of transformed osteoblasts secreting various mineralized components of the extracellular matrix (ECM) and is associated with local bone and soft tissue destruction. Conventional OS exhibits 7 distinct pathological subtypes (4). The most commonly diagnosed subtype of OS (approximately 60% of cases) is the osteoblastic subtype with osteoid matrix as its dominant feature (5). The outcomes for patients do not vary significantly among the subtypes, with the cohort size being a limiting factor, as the numbers of patients with the more rare subtypes are not many in number, and thus patients with different subtypes cannot be effectively compared (6). In the past, the

Parathyroid hormone/parathyroid hormone-related peptide regulate osteosarcoma cell functions:
Focus on the extracellular matrix (Review)

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Abbreviations: OS, osteosarcoma; ECM, extracellular matrix; MSCs, mesenchymal stem cells; PTH, parathyroid hormone; PTHR1, parathyroid hormone receptor 1; GPCR, G-protein-coupled receptor; PTHrP, parathyroid hormone-related peptide; CREB, cAMP response element-binding protein; PGs, proteoglycans; GAGs, glycosaminoglycans; FGF, fibroblast growth factor; Runx-2, runt-related transcription factor 2; SLRPs, small leucine-rich repeat proteoglycans; HA, hyaluronic acid; MMPs, matrix metalloproteinases

Key words: parathyroid hormone, parathyroid hormone-related peptide, parathyroid hormone receptor 1, osteosarcoma, extracellular matrix, signaling, hyaluronan, proteoglycans
are excreted by the kidneys in a PTH-regulated manner (13). This process simultaneously mobilizes phosphate ions into the blood. Simultaneously, the mobilized phosphate ions relative abundance of various PTH-derived peptides that is inversely proportional to PTH (13), and specifically modulates the glands and the blood calcium levels regulates the secretion of PTH (13). The feedback between the parathyroid glands and the kidneys (13) is thought to increase blood calcium and decrease blood phosphate levels, which is a cytoplasmic PDZ protein, binds PTHR1 through its PDZ motif to anchor it to the actin skeleton, and thus mediates the downstream signaling of this receptor (36). A feedback loop may be established where the addition of exogenous PTHrP perpetuates without the addition of exogenous PTHr, thus indicating that PTHR1 downstream signaling. Thus, PTHR1 was found to continue to signal through a G-protein-mediated pathway within endosomes, challenging the established ground rule in GPCR biology of transient membrane receptor activation with subsequent rapid deactivation and receptor internalization (19).

Early toxicological studies using rat models suggested that long-term PTH treatment may increase the risk of developing OS (27). Experiments on both Fisher 344 and Sprague-Dawley rats indicated that the occurrence of OS depends on the level of the PTH 1-34 dose and the duration of treatment (27-29). Importantly however, the doses tested in the rat models were a 100-fold higher as compared to the human dose approved by the Food and Drug Administration (30). Up-to-date clinical trials and patient follow-up have not shown a correlation between PTH 1-34 use and the incidence of OS (31-33). Moreover, in the US post-marketing surveillance study of adult OS and teriparatide treatment, it was determined that after 7 years, there were no patients with OS who had a prior history of teriparatide treatment (33).
mechanism is suggested for PTH/PTHrP, as PTH was shown to transcriptionally downregulate PTH/PTHrP receptor gene expression in osteoblast-like cells via a cAMP-dependent, PKA-independent pathway (37).

In an early study on OS cells, it was demonstrated that PTH induces c-fos transcription, which is important to bone metabolism. Indeed, the induction of c-fos transcription by PTH appears to occur principally through the activation of PKA, that then targets CREB and the c-fos calcium/cAMP response element (38). Thus, in PTH-treated SaOS2 OS cells, CREB was shown to be phosphorylated at Ser133, leading to the decreased motility of the CREB:CRE complex. The phosphorylated CREB:CRE complex recruits an adapter protein that enhances c-fos transcription (39).

PTHrP is widely expressed in fetal and adult tissues, and is able to regulate cellular calcium transport, as well as cell proliferation, development and differentiation. The dysregulated expression of PTHrP has shown to correlate with cancer-related pathologies, as the key inducer of malignancy-associated hypercalcemia; however, new developments suggest its crucial participation in the progression of skeletal metastasis (40). In a previous study, it was demonstrated that increased autocrine secretion of, and responsiveness to, PTHrP results in the inhibition of the growth kinetics of the rat osteoblastic OS cell line, UMR 106-01, both in vitro and in vivo (41). Moreover, the osteostatin domain of C-terminal PTHrP induces the activation of Src, extracellular signal-regulated kinase (ERK) and Akt, resulting in the phosphorylation of vascular endothelial growth factor receptor 2 (VEGFR2) in rat osteoblastic OS UMR-106 cells thus, regulating the functions of these cells (42). Two human osteoblast-like OS cell lines show distinct expression and differential regulation of PTHrP expression (43). Furthermore, PTHrP expression possibly mediates the function of bone microenvironment-related growth factors in MG-63 OS cells. It is of clinical relevance that PTHrP and anticancer drugs show opposing interactions on death receptor-triggered, as well as on mitochondrial apoptotic pathways. Thus, stimulation experiments of the CD95-, the TNF-R- and the TRAIL-R-death receptor systems in Saos2 human OS cells revealed that PTHrP can block signaling via each of these death receptors. In addition, PTHrP induces chemoresistance by interference with p53 family-dependent apoptotic signaling pathways, and the p53-mediated transactivation of apoptosis target genes (44).

5. PTH/PTHrP-induced ECM remodeling in OS: Novel insights

The ECM is a network of molecules secreted by cells, with an inherent self-assembly ability, that provides structure and organization to tissues. This extensive network is mainly composed of collagens, elastin, proteoglycans (PGs), glycosaminoglycans (GAGs), fibronectins and laminins (45,46). Similar to the cell membrane, the ECM forms an active interface, which is the basis of both out-in and in-out signaling. The constant interactions between cells and ECM components are mostly perpetrated through cell membrane receptors (47,48), whereupon the binding of a ligand results in the activation of a cascade of signaling pathways which in turn induce several cellular responses (49), including proliferation, adhesion, invasion, cell motility and metastasis (50,51).

The OS ECM is extensively altered as compared to physiological bone tissue. Indeed, the main characteristic of the most common osteoblastic subtype of OS is the non-mineralized osteoid production (52,53). Normally, osteoblasts produce a highly specialized ECM, osteoid, which is a complex of mineralized proteins. The changes in the ECM of OS have been directly correlated to disease progression (54-57). Importantly, ECM is a pool of growth factors, hormones, cytokines and other active mediators, whose bioavailability (45) is altered in OS, and may contribute to disease progression (56). Extensive feedback exists among these mediators and the OS ECM content and organization which ultimately regulate OS biological function (54-57). Importantly, a significant facet of PTH function is the regulation of bone ECM, osteoid (58).

Recently, the anabolic effect of PTH 1-34 on bone metabolism has been shown to correlate with changes in fibroblast growth factor-2 (FGF-2) expression. These FGF fluctuations have been shown to modify the nuclear accumulation and subsequent action of runt-related transcription factor 2 (Runx-2) and CREB transcription factors, key in the regulation of osteoblast growth and differentiation (59). Indeed, in a FGF-2-dependent manner, Runx2 modifies the transcription of genes related to signaling mechanisms perpetrated by PGs, including syndecans, glypicans and versican. Moreover, it has been proposed that Runx2/FGF-2/PG downstream signaling constitutes an ECM-mediated feedback loop that regulates the growth of osteoblastic lineage cells (60). The existence of this signaling axis was recently confirmed in OS cells (61). Thus, PTH 1-34 intermittent treatment induces a significant increase in FGF-2 transcripts. Enhanced FGF-2 signaling decreases the expression of the small leucine-rich repeat proteoglycan (SLRP), biglycan, in a manner positively correlated to MG63 OS cell migration (61). Indeed, Datsis et al speculated that ‘the PTH 1-34-FGF-2 axis, by causing strong downregulation of biglycan expression, dramatically changes the ratio among the SLRP members, resulting in altered bioavailability of growth factors involved in OS cell migration’ (61). Importantly, the family of SLRPs has shown to be associated with the progression of OS (51,56,57). In particular, decorin seems to affect differentiation of OS cells (62), whereas biglycan, is related to the growth, proliferation and migration of OS cells (3,57). The expression of another SLRP, lumican, was found to be ‘positively correlated with the differentiation and negatively with the growth of human OS cells’ (51). The GAG chains bound in to PG cores participate in the fine modulation of osteoblastic cell functions (63).

It has been shown that under estrogen deficiency, resulting from bilateral ovariectomy (OVX), ECM proteins, including biglycan, tenasin and fibronectin have an altered expression and distribution in OVX as compared to control animals (64). The increase in biglycan expression has been shown to correlate with the regulation of bone formation and matrix mineralization by facilitating osteoblast differentiation through Erk activation and increased Runx2 transcriptional activity (65). Periostin, a conserved ECM protein, is crucial for the process of tumorigenesis (66). Notably, the expression of this bone matrix protein is regulated by PTH (67). Importantly, periostin
Figure 1. The effect of parathyroid hormone (PTH)/PTH-related peptide (PTHrP)-dependent extracellular matrix (ECM) signaling on osteosarcoma (OS) cell functions. Schematically depicted are: (A) PTH receptor 1 (PTH1R) activation; (B) receptor respective downstream signaling; (C) transcriptional regulation; (D) modulation of ECM-correlated target genes; (E) regulation of basic OS cell functions. CREB, cAMP response element-binding protein; cAMP, cyclic adenosine monophosphate; Runx2, runt-related transcription factor 2; FGF-2, fibroblast growth factor-2.

was found to be overexpressed in various types of human cancer, where it interacts with multiple cell-surface receptors, most notably integrins, and signals mainly via the PI3-K/Akt to promote cancer cell survival, and metastasis (66).

Another ECM component, the glycosaminoglycan, hyaluronan (HA), was established to regulate cancer cell function (50,68). In an early study, PTH was demonstrated to strongly increase HA synthesis in UMR 106-01 BSP OS cells (69). Moreover, periosteal and endosteal osteoblastic cell populations exhibited metabolic differences in their HA synthesis responses to PTH (70). Interestingly, Berdiaki et al (71) suggest that there is a regulatory effect of PTH 1-34, in an administration mode-dependent manner, on HA metabolism that is essential for OS cell migration. The effects of PTH were shown to correlate with OS cell differentiation and behavior. Specifically, intermittent PTH 1-34 treatment of poorly differentiated and aggressive MG-63 cells increased their HA-synthase-2 expression, which resulted in enhanced high-molecular size HA deposition in the pericellular matrix, in association with the increased ability of these cells to migrate. Interestingly, continuous PTH 1-34 treatment stimulated well-differentiated Saso2 cell HA production and modestly enhanced their migration (71). Recently, PTH intermittent treatment was shown to increase HA deposition in rat long bones (72).

OS pathogenesis is, additionally, characterized by the differential expression of matrix metalloproteinases (MMPs) which results in ECM integrity disruption (73). MMPs are proteolytic enzymes responsible for ECM remodeling under both pathological and physiological conditions (73-78). Importantly, the overexpression of MMPs has been observed in many types of cancer (77), indicating that their expression may be utilized as a possible prognostic marker (78). Furthermore, it has been suggested, that the increased expression of MMP-1 and -9 may, in OS, predict an adverse outcome such as invasion or metastasis (79-81). Indeed, Husmann et al (76) based on in vivo and in vitro experiments, suggested that MMP-1 overexpression in OS plays an important role in tumor burden and pulmonary metastasis. In a recent study, PTH was shown to increase MMP-13 expression in UMR 106-01 OS cells, as well as in normal osteoblasts (82). On the other hand, the daily injection of rhPTH 1-34 has been shown to result in a decrease in serum Runx2 and MMP-13 levels in post-menopausal women (83). The PTH/PTHrP-dependent ECM signaling on OS cell functions is shown in Fig. 1.

6. Conclusions

In conclusion, this review has highlighted the evolving roles that PTH/PTHrP signaling play in the progression of OS. PTH/PTHrP are established to specifically regulate bone remodeling, as well as to participate in the progression of commonly debilitating and degenerative bone diseases. Further progress in discerning the specific signaling pathways of PTH/PTHrP in the pathogenesis of OS is warranted.

References


38. Evans et al., Hipskind RA and Bilbe G: Analysis of signaling pathways used by parathyroid hormone to activate the c-fos gene in human SaOS2 osteoblast-like cells. J Bone Miner Res 11: 1066-1074, 1996.


