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PTHR1, its receptor, and protein kinase A activation in osteosarcoma

Carl R Walkley*, Mannu K Walia, Patricia W Ho, and T John Martin*

St. Vincent’s Institute of Medical Research and Department of Medicine; St. Vincent’s Hospital; University of Melbourne; Fitzroy, VIC, Australia

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In osteosarcoma, knockdown of the parathyroid hormone-related protein (PTHR1) receptor reduces activation through cyclic AMP-dependent protein kinase A (PKA) and substantially decreases tumor differentiation, invasion, and proliferation in vivo. These findings complement other evidence supporting a central role of the PKA pathway in osteosarcoma biology and pathogenesis.

Osteosarcoma (OS) is the fifth most common cancer in children. Although cytotoxic chemotherapy and improved surgical approaches have increased the overall long-term survival rate to 70%, patients with metastatic disease have a survival rate lower than 20%. The biology of OS has become the focus of recent attention, and an improved understanding could lead to new pathways of treatment. The role of parathyroid hormone receptor (PTHR1) signaling in OS has never been associated. Subsequent studies in OS cell lines before the existence of PTHrP was appreciated. From several species have established PTH responsiveness as a common, if not universal, feature of OS. PTHR1, a G-protein coupled receptor linked to adenyl cyclase, is activated by the N-terminal regions of both PTH and PTHrP. PTHrP was discovered as the factor responsible for the humoral hypercalcemia of cancer, and was found to act physiologically as a paracrine/autocrine factor in many tissues, including bone, where it is produced by cells in the osteoblast lineage. There are 3 main subtypes of OS: osteoblastic, fibroblastic, and chondroblastic. Genetically engineered mouse models of OS have been used to generate the fibroblastic subtype by deletion of p53 and pRb from the osteoblast lineage, and the osteoblastic subtype by shRNA-mediated knockdown of p53 within the osteoblast lineage. Primary and metastatic tumors from either subtype express functional PTHR1. OS cultures of both subtypes responded to treatment with either PTH or PTHrP with the expected changes in gene expression and all expressed PTHrP. The functional relevance of PTHR1 to OS biology has not been clearly defined in vivo. Consistent with a role for PTHR1 in OS, higher expression of PTHR1 mRNA was detected in metastatic or relapsed samples of a human OS series than in primary sites, and overexpression of PTHR1 in an OS cell line increased proliferation and invasion. Knockdown of PTHrP in murine fibroblastic OS resulted in reduced tumor cell invasion in vitro and changes in gene expression that reflected loss of activation of PTHR1 and cAMP-dependent protein kinase A (PKA). Notably, PTHR1 knockdown resulted in greatly reduced proliferation and increased mineralization of the tumor in vivo. PTHrP was present as an intracellular protein in OS cells, but its secretion was very low to undetectable. Interestingly, treatment with a neutralizing monoclonal antibody against PTHrP failed to modify OS cell proliferation in vitro or to influence tumor size in vivo. Although the latter findings, together with evidence for its nuclear localization in these and in other cells, suggest that PTHrP might be acting in an intracrine manner in OS, in such a situation the action of PTHrP on PTHR1 would be difficult to explain. It seems more likely that an autocrine/paracrine action of PTHrP is responsible and that the antibody is insufficiently effective in this context.

The role of PKA activation in endocrine tumors is well known and the subject of recent interest in OS. In mice with osteocalcin promoter-driven SV40T/t antigen-induced OS, a subset of OS with low expression of the α regulatory subunit of PKA type I (PRKAR1A) was identified. This PRKAR1A-low OS was highly invasive, leading to the conclusion that Prkar1a is an OS tumor suppressor. The functional consequence of reduced PRKAR1A is enhanced PKA activity. Consistent with a role of elevated PKA...
activity, an OS was identified with amplification of $Prkaca$, which encodes the catalytic component of PKA, and $Prkaca$ RNA was shown to be overexpressed in that tumor. It seems that enhanced PKA signaling in OS might be mediated by later events in the PKA/CREB cascade, rather than strictly being ascribed to $Prkar1a$ as a tumor suppressor. Figure 1 illustrates changes in the PKA pathway induced through these mechanisms and through PTHrP/PTHR1.

Also of interest is the observation that loss of PRKAR1A increased the production of receptor activator of nuclear factor κB ligand (RANKL) in OS,\(^6\) whereas upon PTHR1 knockdown the expression of RANKL expression decreased and that of osteoprotegerin (OPG) increased.\(^6\) Two aspects of RANKL biology are significant in OS. It promotes both osteoclast formation, thereby favoring tumor establishment and proliferation, and the establishment and growth of metastatic cancers in bone. Importantly, however, there are also examples of osteoclastogenesis-independent effects of RANKL: blockade of RANKL/RANK can inhibit metastasis to bone by preventing cell migration, and RANKL can promote breast cancer metastasis to bone by a pro-migratory effect through its receptor, RANK, expressed on the cancer cells.\(^9\) Furthermore, in OS RANKL stimulated both invasion through matrigel and anchorage-independent growth, and each of these effects was prevented by blockade of the RANKL receptor.\(^10\)

Taken together, these findings provide a compelling case for a role of the PTHrP→PTHR1→PKA axis in the maintenance of OS. If the driver through this axis is indeed PTHrP, manipulating this upstream target, for example through small-interfering RNA or neutralizing antibodies, could be used to regulate OS behavior.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Author Contribution Statement
The content of this manuscript was discussed with all authors. The manuscript was written by T.J.M. and C.W. and reviewed by all authors.

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