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miR-200c and phospho-AKT as prognostic factors and mediators of osteosarcoma progression and lung metastasis

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ABSTRACT

Lung metastasis is the major cause of death in osteosarcoma patients. However, molecular mechanisms underlying this metastasis remain poorly understood. To identify key molecules related with pulmonary metastasis of pediatric osteosarcomas, we analyzed high-throughput miRNA expression in a cohort of 11 primary tumors and 15 lung metastases. Results were further validated with an independent cohort of 10 primary tumors and 6 metastases. In parallel, we performed immunohistochemical analysis of activated signaling pathways in 36 primary osteosarcomas. Only phospho-AKT associated with lower overall survival in primary tumors, supporting its role in osteosarcoma progression. CTNNB1 expression also associated with lower overall survival but was not strong enough to be considered an independent variable. Interestingly, miR-200c was overexpressed in lung metastases, implicating an inhibitory feed-back loop to PI3K-AKT. Moreover, transfection of miR200c-mimic in U2-OS cells reduced phospho-AKT levels but increased cellular migration and proliferation. Notably, miR-200c expression strongly correlated with miR-141 and with the osteogenic inhibitor miR-375, all implicated in epithelial to mesenchymal transition. These findings contrast epithelial tumors where reduced miR-200c expression promotes metastasis. Indeed, we noted that osteosarcoma cells in the lung also expressed the epithelial marker CDH1, revealing a change in their mesenchymal phenotype. We propose that miR-200c upregulation occurs late in osteosarcoma progression to provide cells with an epithelial phenotype that facilitates their integration in the metastatic lung niche. Thus, our findings identify phospho-AKT in the primary tumor and miR-200c later during

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tumor progression as prognostic molecules and potential therapeutic targets to prevent progression and metastasis of pediatric osteosarcomas.

1. Introduction

Osteosarcoma is the most common primary high grade bone malignancy with highest incidence in children and adolescents (Arndt et al., 2012). Current survival rates vary by age, disease stage, anatomic site and histologic response to presurgical chemotherapy, ranging from 55 to 75% for children and adolescents in most countries (Savage and Mirabello, 2011). However, survival rate is reduced to 10–30% if there is metastatic disease at diagnosis (Kager et al., 2003). After recurrence, mainly to the lungs, there are limited treatment options that are frequently unsuccessful. Thus, there is an urgent need to identify new druggable targets for precision medicine treatments.

MicroRNAs (miRNAs) are important epigenetic regulators in cellular physiology as well as in the pathogenesis of human cancers, including osteosarcomas. Several miRNAs have already been reported to regulate signaling networks in osteosarcoma cells (reviewed in Chang et al., 2015). Among them, those targeting the insulin-like growth factor-1 IGF-I signaling pathway are of particular interest since this pathway regulates pleiotropic responses that vary from cellular metabolism, proliferation, differentiation and apoptosis. Regulation of the IGF-I signaling pathway and mutations in PI3K, one of its downstream targets, have been described to be of relevance in osteosarcoma, correlating with poor prognosis (Choy et al., 2012; Su et al., 2011). In addition, the monoclonal antibody R1507 targeting IGF-IR was reported to be effective alone or in combination with rapamycin in inhibiting growth of osteosarcoma xenografts (Kolb et al., 2010). Moreover, increased expression of IGF-IR has been correlated with tumor metastasis and prognosis in patients with osteosarcoma (Wang et al., 2012). Notably, an orthotopic study in mice with phospho-specific antibodies and kinase inhibitors revealed that only IGF-IR/MEK pathway, but not IGF-IR/AKT pathway, remain active in lung metastasis, suggesting that Ras/Raf/MEK/ERK signaling may play an important role in osteosarcoma lung metastasis (Yu et al., 2011). In addition, c-Myc overexpression enhanced MG-63 and SAOS-2 osteosarcoma cells invasion through the activation of MEK-ERK pathway whereas inhibited the activity of PI3K-AKT pathway (Han et al., 2012). Further downstream in IGF-I signaling is the Nuclear Receptor Coactivator 3 (NCOA3), which was reported to be overexpressed in osteosarcomas driving its progression (Geng et al., 2014). Strikingly, activation of PI3K/AKT upregulates NCOA3 levels by inhibiting its proteasomal degradation (Ferrero et al., 2008), which may result in a feedback loop by increasing IGF-I levels that act in an auto or paracrine manner to activate both, Ras and PI3K pathways. Together, these results support IGF-I signaling as an important growth and survival pathway in osteosarcoma cells.

Different miRNAs in osteosarcoma have been reported to target IGF-I signal transducers and transcription factors responsible for cellular invasion, epithelial to mesenchymal transition (EMT), stemness and chemoresistance. The IGF-IR itself is targeted by miR-133b, frequently downregulated in osteosarcomas (Zhao et al., 2013). PTEN is regulated by miR-93 and miR-23a, resulting in increased proliferation and migration (Kawano et al., 2015; Tian et al., 2015). PTEN is also the target of miR-144 in nasopharyngeal carcinomas (Zhang et al., 2013). However, this tumor suppressor-like behavior described in epithelial carcinomas has a reversed effect in osteosarcoma cells, where its downregulation is associated with cell proliferation and invasion by downregulating its target gene, TAGLN (Zhao et al., 2014). Besides, miR-144 may also regulate migration and invasion of osteosarcoma cells by targeting the cell adhesion protein Ezrin (Cui and Wang, 2015). FOXO1 is a transcriptional factor downstream of the PI3K pathway and a positive regulator of bone formation in osteoblasts (Rached et al., 2010). Interestingly, FOXO1 has recently been reported to be the target of miR-135b and miR-374, thus promoting proliferation and invasion in osteosarcomas (He et al., 2015; Pei et al., 2015). miR-101 and miR-155 have been shown to favor and to inhibit chemosensitivity by respectively blocking and inducing autophagy (Chang et al., 2014; Chen et al., 2014). These counteracting effects are somewhat controversial since both miRNAs target the same pathway, but at different levels: miR-101 downregulates mTOR expression levels (Merkel et al., 2010), a major regulator of autophagy together with AMPK, whereas miR-155 targets the alpha regulatory subunit of PI3K (p85), an upstream activator of mTOR (Huang et al., 2012). One plausible explanation could rely on the promiscuity of miRNAs so that the final outcome depends on other targets regulated by miR-101 and miR-155. In this regard, miR23a was also shown to target molecules other than PTEN that may enhance its oncogenic potential. Overexpression of miR-23a inhibited osteosarcoma HOS cells differentiation by downregulating connexin-43 (Cx43/GJA1), a mediator of intercellular signaling critical to osteoblast development (Gindin et al., 2015). In the same cluster as miR-23a, miR-27a promotes pulmonary osteosarcoma metastasis by downregulating the transcriptional regulator CBFA2T3 (Salah et al., 2015).

Here we demonstrate that miR-200c is upregulated in lung metastasis and ectopic expression of miR-200c in U2-OS cells increased cellular proliferation and migration but reduced basal phospho-AKT levels. In addition, multivariate analysis of immunohistochemical staining for signaling pathways showed a significant association of Akt activation with poorer prognosis. Based on the inhibitory role of miR200 family on Akt signaling, these results suggest that Akt and miR-200c contribute to osteosarcoma progression through converging mechanisms.