Silencing Metastasis Tumor-associated Protein 2 Inhibited Osteosarcoma Migration and Invasion Through uPA via ERK1/2 Pathway *in vitro*

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INTRODUCTION: Osteosarcoma is a solid malignant bone tumor, and lung metastasis is a major cause of mortality prior to the introduction of polychemotherapy. However, the prognosis remains poor with a low 5-year survival rate. Metastasis tumor-associated protein 2 (MTA2) is a member of the MTA family closely associated with tumor progression and metastasis. It was previously known to be required for maintaining malignant potential in several human cancers. Urokinase plasminogen activator (uPA) is a serine protease associated with the progression of osteosarcoma. However, the roles of MTA2 and uPA in the progression of osteosarcoma remain unclear. We investigated the possible mechanisms of MTA2 regulation by analyzing osteosarcoma cell lines.

METHODS: The expression of MTA2 was assessed in human osteosarcoma cell lines through immunohistochemistry and western blotting assays. Protein levels of MTA2, p-ERK1/2, t-ERK1/2, and uPA were quantified using western blotting. mRNA expression of MTA2 and uPA was detected via RT-qPCR. To reduce the expression of MTA2 and ERK1/2, shRNA and siRNA-ERK were employed.The viability of shLuc and shMTA2 cells was determined using the MTT assay. Migration and invasion assays were conducted using a Boyden chamber with and without Matrigel coating. Protein expression was analyzed using the Proteome Profiler Human Protease Array Kit. All statistical analyses were performed using GraphPad Prism 6, employing Student's t-test or one-way analysis of variance to assess significant differences between groups.

RESULTS SECTION: The osteosarcoma cell lines displayed relatively high levels of MTA2 protein and mRNA. Western blot analysis and RT-qPCR confirmed that shMTA2 effectively reduced MTA2 expression when compared to shLuc cells. Furthermore, the migration and invasion capabilities were reduced following MTA2 knockdown(A). The expression of uPA protein also decreased after MTA2 knockdown(B). Moreover, when recombinant uPA protein was used to overexpress uPA, the migration ability increased in the MTA2 knockdown cell lines(C). Induced ERK1/2 phosphorylation was observed after MTA2 knockdown(D). Silencing ERK1/2 increased the expression of uPA protein(E) and reversed the inhibition of cell migration and invasion caused by MTA2 depletion(F).

DISCUSSION: The functional study suggests that MTA2 knockdown can decrease the migration and invasion abilities of osteosarcoma cells. Additionally, the expression of uPA protein was significantly downregulated. Overexpressing uPA can reverse the reduced migration and invasion abilities observed in MTA2 knockdown cells. These results imply that uPA could serve as a predictive or prognostic biomarker in osteosarcoma progression. MTA2 knockdown in osteosarcoma cells induced ERK1/2 phosphorylation, and silencing ERK1/2 promoted uPA expression. Our results indicate that MTA2 may act as a regulatory factor in the metastasis process of osteosarcoma, particularly through the activation of ERK1/2-inhibited uPA signaling in vitro. However, further studies are needed to investigate the precise mechanisms involved.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study suggests that MTA2 suppresses osteosarcoma cell metastasis through uPA via ERK signaling, offering a new and potential strategy for targeting MTA2 in the fight against osteosarcoma metastasis.

REFERENCES: Include references here. (References are Optional)

IMAGES AND TABLES: