Targeted Treatment In Osteosarcoma; Wnt Antagonists and 2-ME

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Disclosures:

Introduction: Osteosarcoma (OS) is a clinically challenging primary malignant bone tumor affecting mostly children and young adults. Current treatment includes neoadjuvant systemic chemotherapy and surgical resection of the primary tumor for local control. The overall 5 year disease free survival rate for non-metastatic disease reaches 75% while for metastatic disease is only 25%. Wnt signaling is known to play a role in the pathogenesis of several cancers, including breast, colon, and osteosarcoma. Therefore it is a promising target for therapeutic intervention in OS. Secreted Frizzled Related Proteins (sFRPs) are Wnt inhibitor proteins secreted by native osteocytes, which regulate Wnt signaling in bone and have been found in low levels in osteosarcoma tissue. 2-Methoxyestradiol (2-ME) is a naturally occurring estrogen metabolite which has been shown to reduce cell proliferation, induce cell cycle arrest, apoptosis, and autophagy in osteosarcoma cells through various pathways, including the interferon pathway. To further understand the molecular actions of 2-ME on osteosarcoma, we have studied the effect of 2-ME on Wnt signaling pathways using human osteosarcoma cells in culture.

Methods: To evaluate the effect of 2-ME on Beta-catenin/Tcf gene promoter activity, MG63 OS cells were transiently transfected with the Tcf reporter plasmid (TOPflash) and then treated with a negative vehicle control (ethanol), 2-ME, and a positive control (lithium chloride, LiCl). The Luciferase assay was carried out 48 hours after transfection per manufacturer’s protocol (Promega, Madison, WI). To determine if 2-ME induces increased sFRP3 protein levels, cytoplasmic extracts from the negative vehicle control and 2-ME treated cells were analyzed by western blot hybridization using anti-sFRP3 (Santa Cruz, Santa Cruz, CA) and anti-actin (Sigma, St. Louis, MO) antibodies. Quantitation was performed by densitometry and analyzed using Quantity One software (Bio-Rad, Hercules, CA). The data are representative of three independent experiments. Significant differences between groups were determined by Fisher’s post hoc test for multiple-group comparisons, following detection of significance by a global F-test in a one-way analysis of variance (ANOVA). To quantify the sFRP3 in human serum (n=142 patients; 71 OS patients, 71 aged matched non-diseased controls), ELISA analysis was performed as described (Biomedica Gruppe, Austria and Aviscera Bioscience). Clinical data from the medical records was correlated with experimental results. Using a pearsons chi square test the difference in proportions was analyzed. P<0.05 was considered statistically for all tests.

Results: We have examined the effect of 2-ME treatment on the Wnt pathway in osteosarcoma cells. Our results show that 2-ME treatment decreased TOPflash activity by one-fourth compared to the negative control and by four fold compared to LiCl treatment. Protein analysis revealed increased sFRP3 protein levels following 2-ME treatment in various human osteosarcoma cell lines (MG63, 143B and KHOS). The sFRP3 levels were not increased by other estrogenic compounds, nor were they increased following 2-ME treatment in normal osteoblasts. When analyzing human serum down-regulation of sFRP3 was observed in 51%, up-regulated in 23% and no difference observed in 27% (p=0.007) of Osteosarcoma patients and a similar trend was observed in protein analysis. When correlating to clinical data similar trends were observed in both metastatic and local disease.

Discussion: The Wnt pathway is active in osteosarcoma for a variety of reasons, including mutations in beta-catenin or decreased transcription of Wnt antagonists. Our findings suggest down-regulation of Wnt antagonists, particularly sFRP3 is prevalent in patient serum and tissue. Our results also show that Osteosarcoma cell death induced by 2-ME treatment is in part mediated by increased levels of Wnt antagonist and could be independent of Beta-catenin mutations.

Significance: 2-ME presents an attractive molecule for further study as a potential treatment for Osteosarcoma.

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