CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

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Project Number

S1295

Project Title

A Novel Strategy to Inhibit Metastasis of Alveolar Rhabdomyosarcoma through the PAX3-FOXO1 Fusion Gene, HSP70, and PERK

Abstract

Objectives/Goals

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma, and 5-year survival rates for children have remained at 20% despite efforts to uncover chemotherapeutic agents. Two variants of this disease are alveolar and embryonal RMS (aRMS and eRMS), the former associated with an aggressive malignant phenotype. Previous research has identified the PAX3-FOXO1 fusion gene in aRMS but not eRMS, which is responsible for increased cell proliferation. This research aims to inhibit the development of aRMS by exploiting PAX3-FOXO1 and cooperating pathways including HSP70 and PERK.

Methods/Materials

To target PAX3-FOXO1, shRNA sequences were determined through modeling and research input. Following the infection of aRMS cells with shRNA, PAX3-FOXO1 and downstream target protein expression were determined through western blotting. Viability assays of shRNA-infected aRMS cells were also conducted. To analyze the effects of the inhibition of the protein chaperone HSP70, a cell viability assay was conducted on aRMS and eRMS after treatment with HSP70 inhibitor MAL3-101. Finally, changes in the expression of PERK and downstream proteins after treatment with MAL3-101 were tested with a western blot, and qPCR determined the expression of the CHOP over a 12 hour MAL3-101 timecourse, the apoptotic protein downstream of PERK.

Results

PAX3-FOXO1 expression, as well as targets ALK, FGFR4, Met, and IGF1R, decreased after the application of PFhp-210 (PAX3-FOXO1 hairpin 210), but not the individual FOXO1 and PAX3 proteins. Viability assays of PFhp-infected aRMS cells demonstrate significantly decreased but continued survival rates. MAL3-101, a HSP70 inhibitor, decreases cell viability in four aRMS cell lines, but not the eRMS line. Finally, for eRMS, CHOP transcript levels did not increase over the 12 hour timecourse of MAL3-101 treatment. However, CHOP in aRMS line grew to 34.9, 28.2, and 9 times the original level after 4, 8, and 12 hours, respectively.

Conclusions/Discussion

This research elucidates the pathways responsible for aRMS survival and development. The PAX3-FOXO1 protein may act through PERK by activating a phenomenon with IGFR1 known as the unfolded protein response, which causes stress in the endoplasmic reticulum and stimulates PERK. HSP70, a chaperone protein, may be involved in the unfolded protein response through PERK since chaperones aid in the folding of proteins.

Summary Statement

My research identifies novel cellular pathways in alveolar rhabdomyosarcoma, which eventually may lead to new therapeutic solutions for this cancer.

Help Received

Dr. Amit Sabnis, pediatric oncologist from UC San Francisco for his guidance, supervision and mentorship; Dr. Trever Bivona from UC San Francisco, Mr. Rod Wong from Bellarmine College Prep, and my parents for their support.