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MUC18 Contributes to the Metastatic Phenotype of Melanoma via Regulation of Id-1 Expression

The incidence of melanoma has been steadily increasing over the last three decades and has been associated with high rates of patient mortality. Melanoma progresses as a sequential process initiating from melanocytes that subsequently develop into a more invasive and metastatic phenotype.

The molecular changes associated with the transition from non-metastatic to metastatic melanoma are not well understood. Our lab has identified several molecular and genetic changes associated with this transition which contribute to melanoma progression including the Melanoma Cell Adhesion Molecule MCAM/MUC18.

MUC18 is highly expressed in advanced and metastatic melanoma tumors, while in benign lesions it is seldom detected. We have previously demonstrated that inhibition of MUC18, utilizing fully human antibodies, correlated with a decrease in tumor growth and metastasis in immunodeficient mice (nude mice).

The precise mechanism of how MUC18 contributes to the progression of melanoma has not been elucidated. We hypothesized that MUC18 regulates differential expression of genes which contribute to the acquisition of malignant melanoma.

In this study, we stably silenced the expression of MUC18 in two metastatic melanoma cell lines utilizing shRNA which is a more specific tool than antibodies. Silencing MUC18 significantly decreased tumor growth and lung metastasis formation *in-vivo*. To further elucidate the molecular mechanism by which MUC18 contributes to melanoma metastasis, we conducted cDNA microarray analysis (used to identify differential expression of genes in cells) comparing gene expression patterns of MUC18-silenced cells to control cells. We found that MUC18 silencing resulted in decreased expression of the Inhibitor of differentiation/DNA binding 1 (Id-1) gene. Id-1 is part of a family of transcription factors regulating gene expression. Id-1 has been shown to be overexpressed in several cancers including melanoma and has been associated with decreased survival in melanoma. Interestingly, we found that the transcription factor, ATF-3, which has been previously shown to inhibit Id-1 expression, was upregulated in our cDNA microarray analysis after MUC18 silencing. Furthermore, we demonstrated that silencing MUC18 in melanoma cell lines resulted in increased binding of ATF-3 to the Id-1 regulatory gene sequence, therefore decreasing Id-1 expression.

We further aim to understand how MUC18 contributes to the malignant phenotype of melanoma through regulation of Id-1. We will study if overexpression of Id-1 in MUC18-silenced cells, restores the aggressive phenotype of cells. Previous studies suggested that the expression of an enzyme that promotes cells invasiveness, MT1-MMP is dependent on Id-1 expression. We demonstrated that MT1-MMP expression decreased in MUC18-silenced cells and this process is Id-1 dependent. Additionally, we will study how Id-1 regulates MT1-MMP expression by

overexpressing Id-1 in a non-metastatic melanoma cell line and study the effects of Id-1 on the expression of MT1-MMP.

This study illustrates how MUC18 contributes to the metastatic melanoma phenotype as it functions not only as an adhesion molecule, but is also involved in intracellular signaling regulating differential expression of downstream target genes. Revealing the molecular mechanisms will enable the identification of novel molecular pathways which can be further utilized for the development of new treatment modalities for metastatic melanoma.