ERO1a Expression is Required for Secretion of Extracellular Matrix and Promotes Lung Cancer Progression

Maria Voronkova,1 Brennan D. Johnson,2 Wei-Chih Chen,2 Gangqing Hu,2 Lori Hazlehurst,1 and John Koomen3

1West Virginia Univ; 2West Virginia University; and 3H. Lee Moffitt Cancer Center and Research Institute

Lung cancer is responsible for more deaths every year than breast, prostate, and colon cancers combined, yet patient treatment options are still limited. We aimed to characterize a role of a new potential therapeutic target, ERO1a, in non-small cell lung cancer (NSCLC). Physiologically, endoplasmic reticulum oxidoreductase 1 alpha (ERO1a) participates in formation of disulfide bonds crucial to protein folding in the endoplasmic reticulum (ER). However, high ERO1a expression was shown to be a poor prognostic indicator in multiple cancer types. While the contribution of high ERO1a levels to increased tumor burden and metastatic potential has been demonstrated, the downstream mechanisms are poorly understood. In this study, CRISPR strategies were utilized to knockout ERO1a in two lung cancer cell lines, PC-9 and HCC4006. The knockout variants demonstrated a significant reduction in colony and tumor sphere formation while no changes in cell proliferation and anoikis were observed. This finding correlated with increased survival and decreased tumor burden in SCID-Beige mice injected with ERO1a knockout cells compared to control cells (p<0.0045 Log Rank Test). As ERO1a is an ER resident protein, we hypothesized that ERO1a may alter the secretome produced by NSCLC. In support of our hypothesis, the reduction in colony formation of ERO1a knockout clones can be rescued by media conditioned by control cells. Moreover, this effect is lost when conditioned media is subjected to heat denaturation. Together these data suggest that an ERO1a-dependent secreted protein is responsible for colony formation in lung cancer cells. Analysis of publicly available lung cancer proteomic data set (CPTAC) shows that high ERO1a expression correlates with enrichment in hallmarks of cancer, such as inflammatory response and epithelial-to-mesenchymal transition (EMT). Moreover, high ERO1a expression correlates with increased levels of multiple matrix proteins including Laminin 332, PLOD2, and LOXL2. These findings in primary patient specimens strongly agreed with the knockout cell line models showing that ERO1a expression is required for secretion of matrix associated proteins in both 2D and 3D systems. We are currently exploring whether the reduced expression of LOXL2 or LAMC2 is responsible for decreased colony formation observed in ERO1a-depleted cells. Taken together, our data indicate that ERO1a modifies the local tumor microenvironment and is an attractive target for therapeutic intervention in non-small cell lung cancer.