A novel MIRO2/MYO9B/RhoA signaling axis controls tumor cell invasion and metastasis. Boulton, DP. Hughes, CJ. Vaira, V. Raza, M. Purdy, SC. Ford, HL. and Caino, MC. Department of Pharmacology, University of Colorado | AMC.

Metastasis of cancer cells to vital organs remains the leading cause of cancer related deaths, emphasizing a strong need for actionable targets in advanced stage cancer. To address this, we study novel dysregulated mitochondrial signaling mechanisms that cells utilize to metastasize. Here, we focus on how outer mitochondrial membrane protein—Mitochondrial Rho GTPase 2 (**MIRO2**)—promotes tumor cell invasion and metastasis through a negative regulation of RhoA. Our previous work identified higher MIRO2 mRNA expression in cancer vs. normal patient samples in a multitude of cancer types, which correlated with worse patient outcomes. Furthermore, we demonstrated that MIRO2 was critical for prostate cancer cell growth and survival *in vitro* and *in vivo*. However, it remains unknown if MIRO2 only affects primary tumor growth or if this protein is important throughout tumor progression. Using siRNA mediated knockdown (KD) of MIRO2 we find MIRO2 KD ubiquitously reduces tumor cell invasion in breast, melanoma, pancreatic, and prostate cancer cells. Utilizing metastatic prostate and breast cancer models, we demonstrate that mice injected with MIRO2 shRNA cells have significantly lower metastatic burden compared to mice injected with control shRNA cells. Mechanistically, we have identified a novel binding partner of MIRO2, atypical myosin IXB (**MYO9B**), and propose that this interaction is a main mechanism by which MIRO2 potentiates metastasis. MYO9B has a well-defined role in controlling cell motility via inactivation of RhoA. Excitingly, we have found 1) MIRO2 KD results in increased active RhoA, phenocopying MYO9B KD, 2) dual ablation of MIRO2 and RhoA fully rescues tumor cell invasion, and 3) MIRO2 is required for MYO9B driven invasion. Taken together, we propose a novel signaling mechanism by which MIRO2 broadly promotes invasion and metastasis through MYO9B dependent inactivation of RhoA.