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 studied novel regulatory signaling mechanisms that cells utilize to metastasize. Here, we focus on how outer mitochondrial membrane protein—Mitochondrial Rho GTPase 2 (MIR2O)—promotes tumor cell invasion and metastasis through a negative regulation of RhoA. Our previous work demonstrated that MIR2O was critical for prostate cancer cell growth and survival in vitro and in vivo. However, it remains unknown if MIR2O only affects primary tumor growth or if this protein is important throughout tumor progression. Using shRNA mediated knockdown (KD) of MIR2O we find MIR2O 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 MIR2O shRNA cells have significantly lower metastatic burden compared to mice injected with control shRNA cells. Mechanistically, we have identified a novel binding partner of MIR2O, atypical myosin IXB (MYO9B), and propose that this interaction is a main mechanism by which MIR2O potentiates metastasis. MYO9B has a well-defined role in controlling cell motility via inactivation of RhoA. Excitingly, we have found 1) MIR2O KD results in increased active RhoA, phosphorycning MYO9B KD and 2) dual ablation of MIR2O and RhoA fully rescues tumor cell invasion. Taken together, we propose a novel signaling mechanism by which MIR2O broadly promotes invasion and metastasis through MYO9B dependent inactivation of RhoA.

Hypothesis
MIR2O promotes tumor cell invasion and metastasis through regulation of downstream effectors.

Results
1. MIR2O is critical for metastasis of 4T1 cells
   - Loss of MIR2O reduces invasive capacity across multiple tumor types
   - Of the top hits, MYO9B reduces invasive capacity to the greatest extent
2. MIR2O is critical for metastasis of PC3 cells
   - Loss of MYO9B reduces invasive capacity in multiple tumor types

Model
- MYO9B requires MIR2O to drive tumor cell invasion

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