A novel role for Mitochondrial Rho GTPase 2 in tumor cell invasion and metastasis

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Background

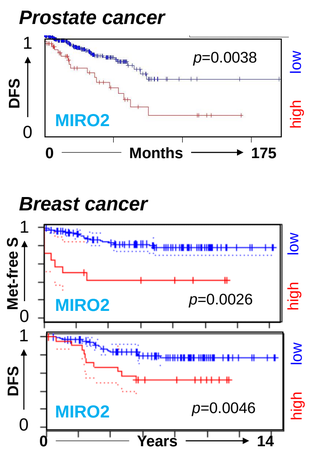
Metastasis of cancer cells to secondary sites remains the leading cause of cancer related deaths, emphasizing a strong need for actionable targets in advanced stage cancer. To address this, we study novel dysregulated signaling mechanisms that cells utilize to metastasize to distal sites. Here, we focus 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, which correlated with worse patient outcomes. Furthermore, in the context of prostate cancer we demonstrated that MIRO2 was critical for 2D cell growth, anchorage-dependent and -independent colony formation, and tumor growth *in vivo*. However, it remains completely unknown if MIRO2 affects primary tumor growth or if this protein is important throughout tumor progression. Using siRNA mediated knockdown (KD) of MIRO2 we clearly show that MIRO2 KD ubiquitously reduces tumor cell invasion in breast, melanoma, pancreatic, and prostate cancer cell lines. Preliminary experiments modeling late-stage metastasis showed that tail vein injection of PC3 cells stably expressing MIRO2 targeting shRNA had reduced kidney and liver metastatic burden in comparison to PC3 cells with control shRNA. In further mechanistic studies, we identified novel MIRO2 binding partners and used siRNA to KD the top hits and observe changes in invasive capacity. We found that atypical myosin IX B (MYO9B) reduced the invasion of cells to the largest extent. MYO9B is a plus-end direct atypical myosin known to control cell motility through spatially inactivating RhoA at the leading edge of migrating cells. Excitingly, MIRO2 KD showed an increase in active RhoA phenocopying MYO9B KD. Lastly, in pilot experiments we found that dual KD of MIRO2 and RhoA rescued invasive capacity in comparison to MIRO2 KD alone. Taken together, we propose a novel signaling mechanism by which MIRO2 broadly promotes invasion and metastasis through MYO9B dependent inactivation of RhoA.

Preliminary Data

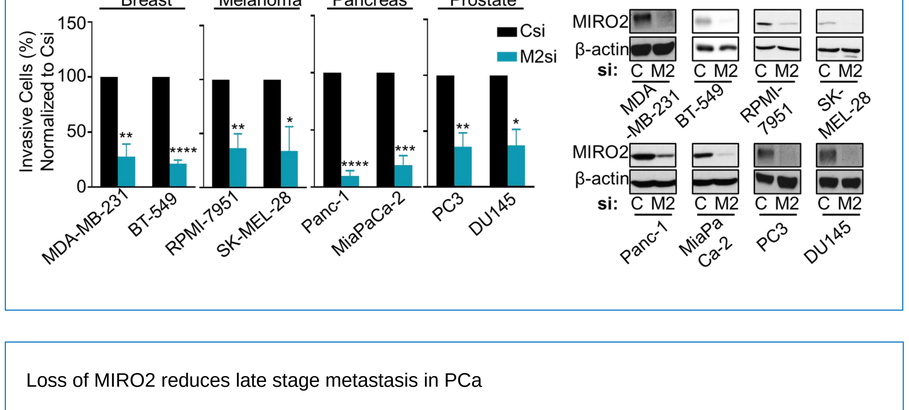
Differential Expression of MIROs in cancer

Cancer	MIRO1 N sets		MIRO1 Best Ratios C/N fold	MIRO1 Best Ratios Surv HR	MIRO2 N sets		MIRO2 Best Ratios C/N fold	MIRO2 Best Ratios Surv HR
Bladder	2	1	-1.7		1		1.6	
Brain		10	-2.0	0.6				1.3
Breast		2	-1.5	0.4	9	1	1.8	2.7
Cervical							1.7	
Colorectal	7		1.8				1.1	
Esophageal	1	4	-1.8					
Gastric					3		1.6	
Head & Neck	2		1.7				1.3	
Kidney	2	3	-4.9		5		1.9	1.8
Leukemia	1	4	-2.7		1	3	1.7	
Liver								
Lung		1	-1.6	0.1	5		1.5	2.4
Lymphoma	3	3	-2.0	0.5	1		1.7	5.5
Melanoma		2	-2.0	0.4	2		2.5	6.8
Myeloma	2		2.0		1		1.7	
Other	3	3	-2.3		2	1	2.5	
Ovarian		2	-2.2	0.7	1		1.6	
Pancreatic	1		1.5				1.3	
Prostate		8	-1.3		9		1.4	2.4
Sarcoma	4		2.3		1	2	1.5	

C/N = Cancer/Normal mRNA expression

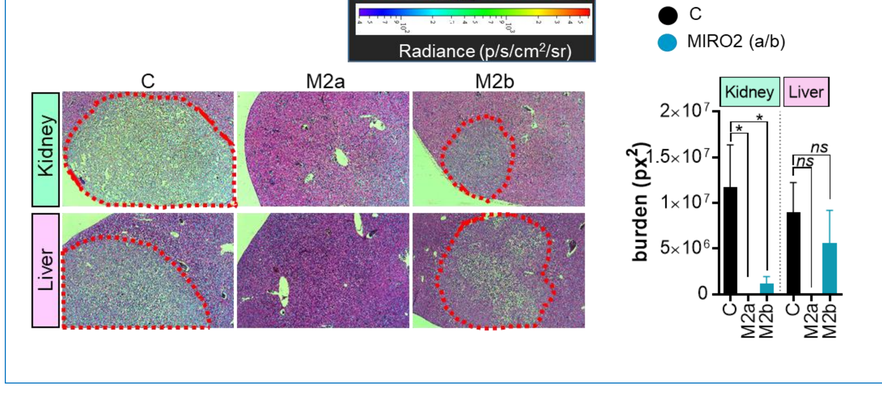


Loss of MIRO2 reduces PCa colony formation, 2D cell growth, and tumor growth *in vivo*



C: control
M2a/b: MIRO2 sh sequence a/b

Loss of MIRO2 reduces invasive capacity across multiple tumor types



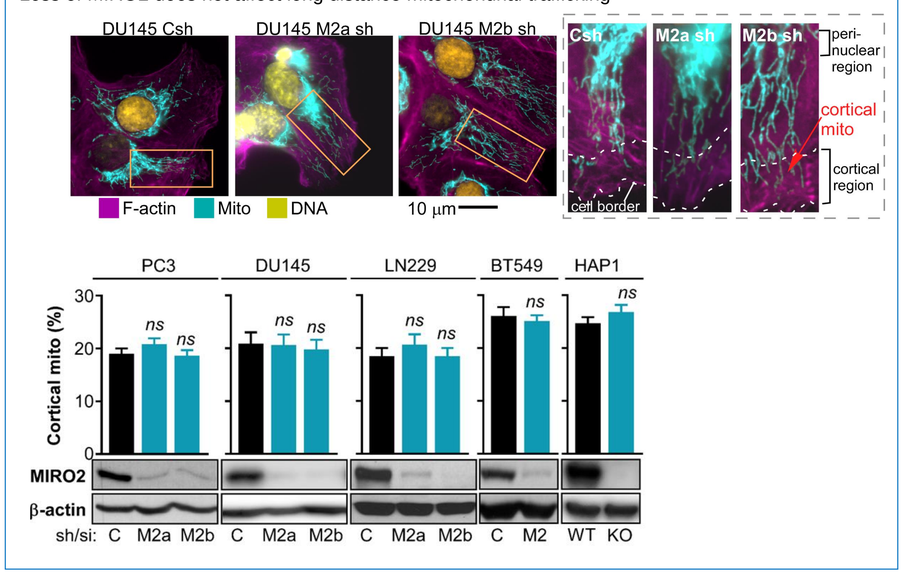
Loss of MIRO2 reduces late stage metastasis in PCa

Hypothesis

MIRO2 universally promotes tumor invasion and metastasis through modulation of downstream signaling.

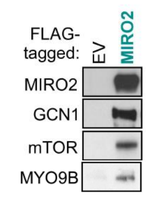
Results

Loss of MIRO2 does not affect long distance mitochondrial trafficking

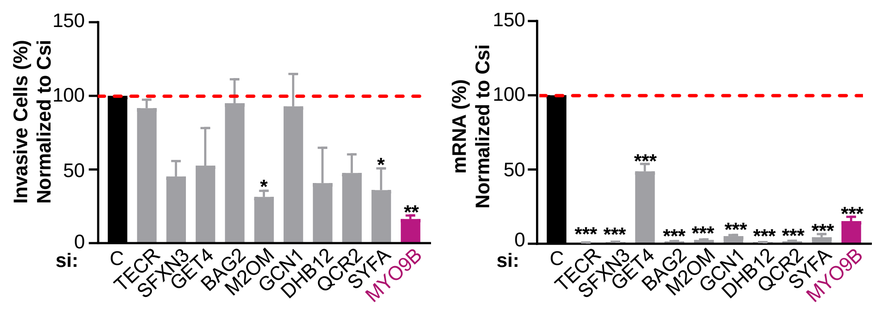


Identification of novel MIRO2 binding partners

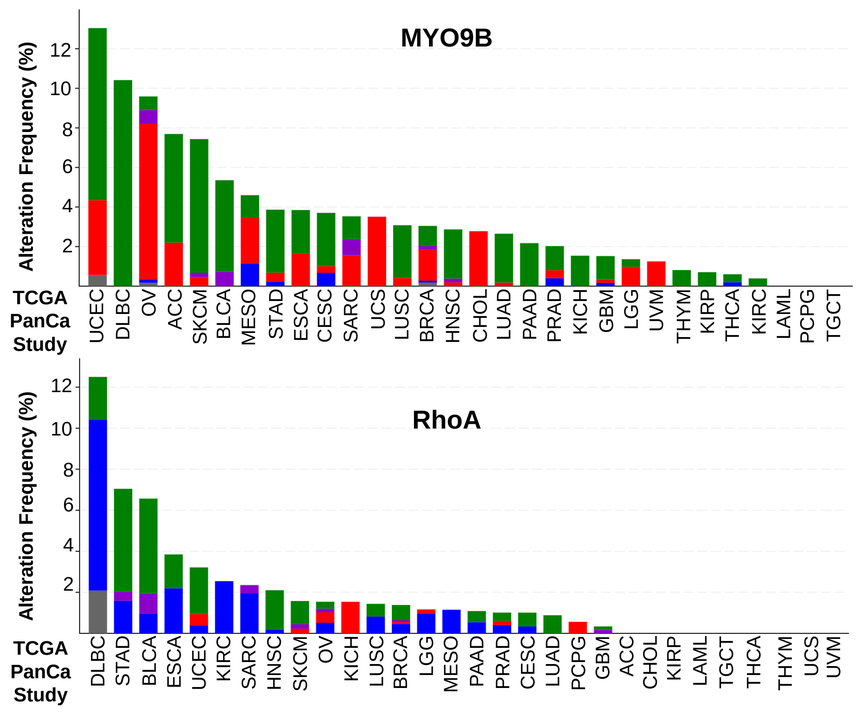
Prot ID	MW	BAP	MIRO2	Counts/kDa	
RHOT2	68 kDa	0	615	9.04	← MIRO2
TECR	36 kDa	0	51	1.42	
SFXN3	36 kDa	0	42	1.16	
GET4	37 kDa	0	35	0.95	
SYFA	58 kDa	0	53	0.91	
BAG2	24 kDa	0	21	0.89	
M2OM	34 kDa	0	29	0.86	
GCN1	240 kDa	0	240	0.82	
DHB12	34 kDa	0	28	0.81	
QCR2	48 kDa	0	39	0.81	
MYO9B	243 kDa	0	26	0.11	



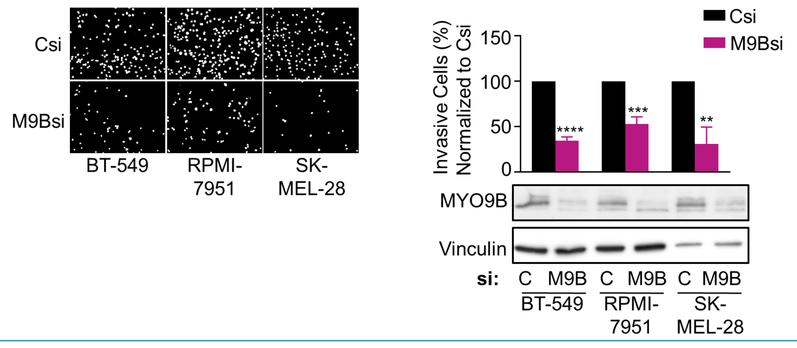
Of the top hits, MYO9B reduces invasive capacity to the greatest extent in MDA-MB-231 cells



MYO9B and RhoA alterations from TCGA PanCancer Atlas

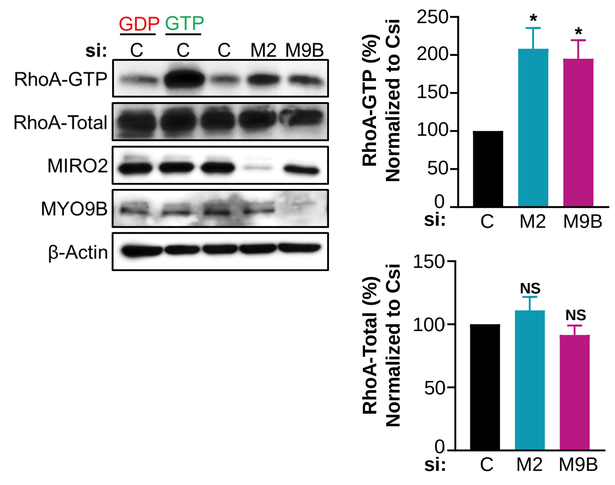


Loss of MYO9B reduces invasive capacity in multiple tumor types



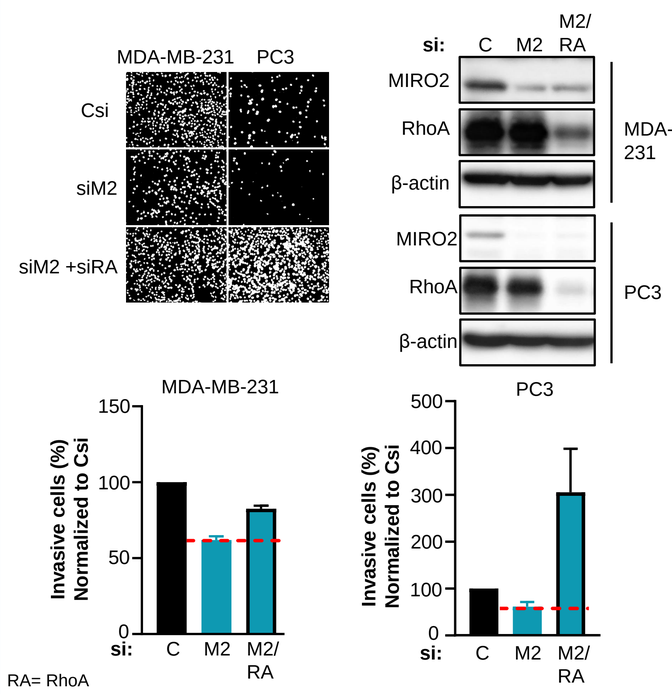
Results

Loss of MIRO2 results in increased active RhoA in MDA-MB-231 cells



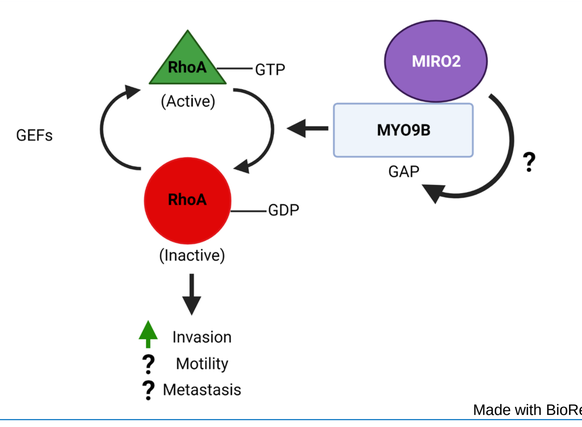
Dual ablation of MIRO2 and RhoA rescues invasive capacity of cells

N=2



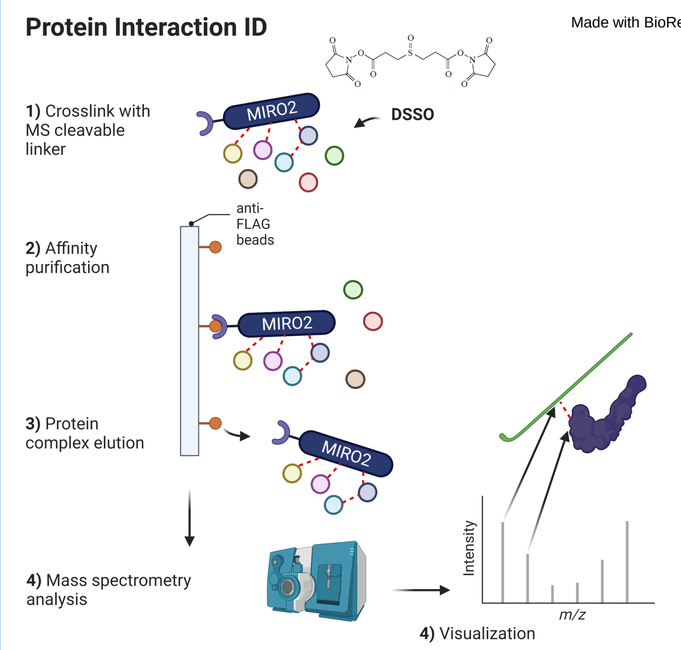
RA= RhoA

Model



Made with BioRender

Future Directions



- Solidify MIRO2 in metastasis using models that metastasize from the orthotopic site
- Determine if increases in active RhoA after MIRO2 ablation is through MYO9B
  - Rescue experiments with exogenous expression of $MYO9B^{WT}$ or $MYO9B^{GAPmut.}$
- Establish mutants that selectively ablate interaction between MIRO2 and MYO9B
  - Is loss of invasive capacity and metastatic burden through MIRO2 and MYO9B interaction?

Acknowledgements

This work was supported by: the American Cancer Society IRG-16-184-56; Boettcher Foundation AWD-193249; Department of Defense W81XWH-21-1-0408; National Institutes of Health R35 GM142774 and T32 GM007635; The Cancer League of Colorado AWD-193451-JT; and Cancer Center Support Grant (CSSG) P30 CA046934.
Pharmacology T32 Training Grant GM007635-41 and GM007635-42; NIH/NCI F-31 (F31 CA271652-01)
Thesis Committee: Dr. Chandra Tucker (chair), Dr. Heide Ford, Dr. David Jones, Dr. Arianne Theiss, Dr. Andrew Thorburn