Targeting the MARCKS/AXL axis to combat pro-tumor macrophage polarization in cancer progression

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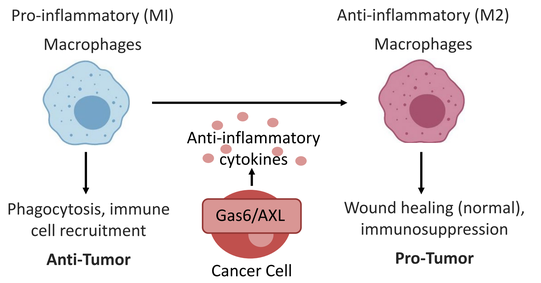
Background

- The **tumor microenvironment (TME)** plays an important role in tumor survival, growth, metastasis, and immunosuppression.
- **Tumor Associated Macrophages (TAMs)** in the TME are converted to M2 phenotype:



- **AXL** is a receptor tyrosine kinase highly expressed on cancer cells that drives cell survival, proliferation, invasion, and M2 macrophage polarization.
- Many drugs targeting AXL have failed clinical trials→more research is needed to understand how AXL is regulated and whether this can be targeted

- **MARCKS** (Myristoylated Alanine-Rich C Kinase Substrate) is a membrane associated protein overexpressed in many types of cancer. General functions include PIP2 sequestration, vesicle transport, and cell cycle regulation.
- MARCKS is homologous across humans and dogs.
- MARCKS has been observed to bind to AXL in pulmonary fibroblasts, but their interaction and whether they interact in cancer cells is unknown.

Objectives

1. Does MARCKS affect M2 macrophage polarization?
2. How does MARCKS interact with AXL?

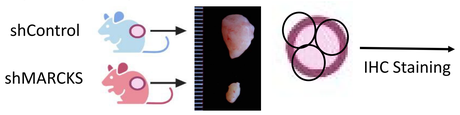
MARCKS → ? → AXL → M2 polarization

Hypothesis: MARCKS increases M2 macrophage polarization through promoting AXL activity

Methods

1. Does MARCKS affect M2 macrophage polarization in vivo?

- Mouse Xenograft model: A549 lung cancer cells (transfected with MARCKS-specific short hairpin RNA) transplanted into nude mice



1. Count 68+ cells (Macrophages)
2. Count 163+ cells (M2 macrophages)

2. How does MARCKS interact with AXL?

Does MARCKS up/downregulate AXL phosphorylation?

- SiRNA transfection to reduce MARCKS and AXL expression in A549 lung cancer cells in vitro → Western blot imaging to visualize AXL expression

Where is AXL localized when MARCKS is silenced?

- Immunofluorescence and confocal imaging to visualize MARCKS and AXL distribution

Statistical Analysis

- Data presented as the mean of 3-4 independent experiments with a standard deviation
- Data analyzed using the Students t-test, unpaired, two-tailed

Results

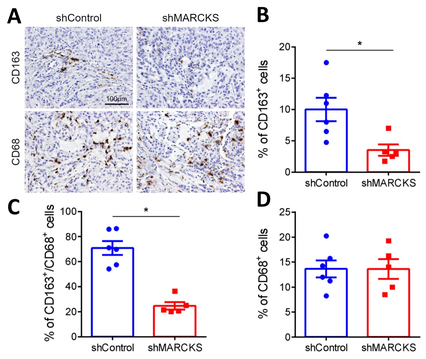


Figure 1. MARCKS silencing decreases M2 macrophage polarization. (A) IHC staining of macrophage markers in lung tumor tissues. Data are expressed as mean with standard deviation. (B,C) The proportion of M2 macrophages were significantly reduced in the MARCKS-silenced tumors ($p<0.05$, unpaired Student's t test). (D) There was a relative equal number of macrophages within each tumor.

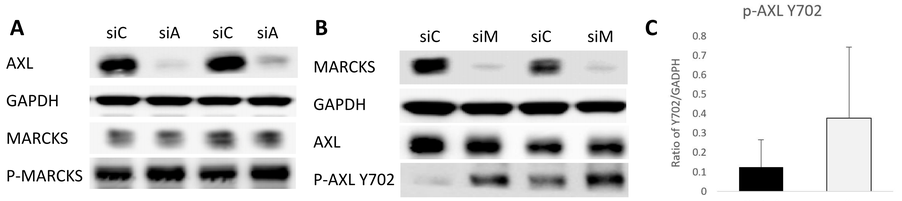


Figure 2. MARCKS and AXL does not affect AXL and MARCKS expression or phosphorylation/activation, respectively. siRNA transfection was used to silence AXL (A) and MARCKS (B) in A549 and CL1-5 lung cancer cells. (C) Quantification of Western blot imaging revealed that MARKS does not affect the expression or phosphorylation of AXL ($p=0.246$).

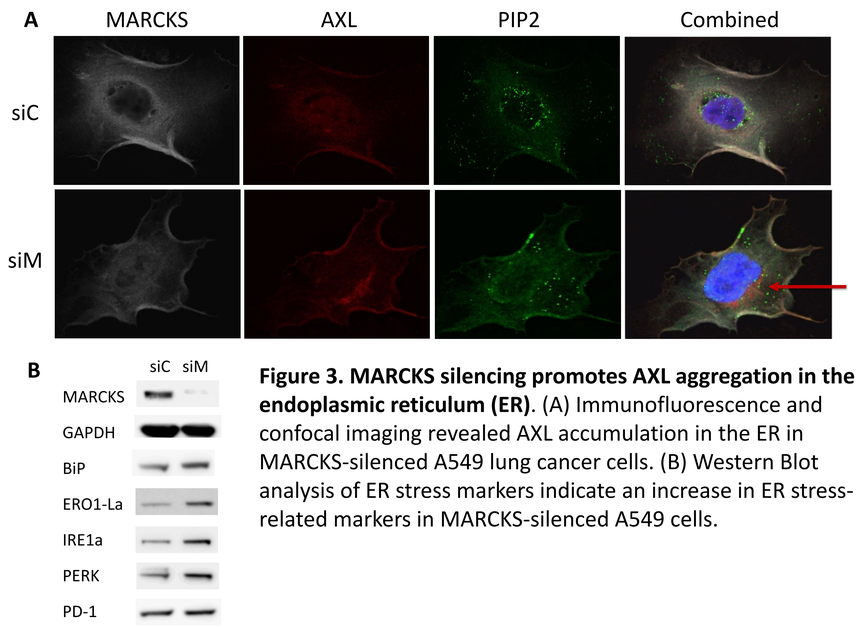


Figure 3. MARCKS silencing promotes AXL aggregation in the endoplasmic reticulum (ER). (A) Immunofluorescence and confocal imaging revealed AXL accumulation in the ER in MARCKS-silenced A549 lung cancer cells. (B) Western Blot analysis of ER stress markers indicate an increase in ER stress-related markers in MARCKS-silenced A549 cells.

Conclusion

1. Silencing MARCKS decreases the proportion of the M2 macrophages within the TME.

- This result showcases a novel strategy of reducing M2 macrophage polarization through targeting MARCKS

2. MARCKS does not affect AXL phosphorylation and thus AXL activation

- Contrary to our hypothesis, MARCKS does not interact with AXL via promoting its phosphorylation and activation.
- Given that MARCKS promotes M2 macrophage polarization and AXL is a key factor in inducing this change in macrophage phenotype, MARCKS likely interacts with AXL in a different way.

3. Silencing MARCKS promotes AXL aggregation in the ER

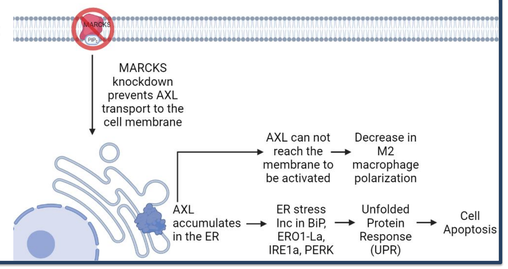
- Confocal imaging indicates that in MARCKS-silenced A549 lung cancer cells, AXL accumulates in the ER.

Current hypothesis: MARCKS is involved in transporting AXL from the ER to the membrane.

- Silencing MARCKS leads to AXL accumulating in the ER, initiating the unfolded protein response and causing ER stress.
- Preliminary Western Blot results supports this hypothesis.

Novel therapeutic strategy

- Target AXL activity through manipulation of MARCKS expression



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