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

Madison Luker, Chih-Wei Chu, Ching-Hsien Chen
UC Davis School of Veterinary Medicine, Department of Internal Medicine

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

Phagocytosis, immune cell recruitment
Anti-inflammatory (M2)

Macrophages

Anti-Tumor

Cancer Cell

Pro-inflammatory (M1)

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

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 transplanted with MARCKS-specific short hairpin RNA (shRNA) transferred into nude mice
   - Count 68+ cells (M2 macrophages)

2. How does MARCKS interact with AXL?
   - MARCKS does not affect AXL phosphorylation and thus AXL activity
   - MARCKS is homologous across humans and dogs.

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).

Figure 2. MARCKS and AXL do 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 MARCKS 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.

Conclusions
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

References

Acknowledgements

Financial support was provided by the UC Davis SVM Students Training in Advanced Research (STAR) Program and NIH grant: ST35DD01056-22.