Role of Vascular Endothelial Glutaminase in Tumor growth and Metastasis

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BACKGROUND

Tumor Angiogenesis

- Angiogenic switch promotes new vessels supporting growth and survival
- Ligands for RTKs such as VEGFs and angiopoietins are critical mediators of tumor angiogenesis
- Tumors acquire oxygen and nutrient via these vessels
- Tumor blood vessels are abnormal, dysfunctional, chaotic and leaky

EphA2 and Glutaminolysis

- EphA2 RTK overexpression is associated with tumor progression and poor survival in breast cancer patients
- EphA2 overexpression promotes glutamine metabolism and tumor growth by activating YAP and TAZ

Glutaminolysis in Endothelial cells

- Glutamine consumption is achieved by conversion to glutamate by GLS
- Glutamine metabolism is required for proliferation and vessel sprouting in normal ECs
- The role of EC glutaminolysis in tumor progression is not well known

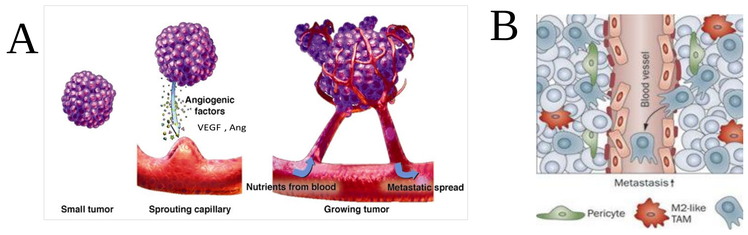

Figure 1: **A)** Tumor cells secret angiogenic factors promoting new vessels development (Adapted from Loizzi 2017). **B)** Tumor blood vessels are abnormal and leaky (Adapted from De Bock 2011) . **C)** EphA2 overexpression promotes glutamine metabolism (Edwards et al, 2017). **D)** Glutaminolysis is important for sprouting angiogenesis in endothelial cells (Huang et al. 2017). **E)** Schematic asking if EphA2 also regulates glutaminolysis in ECs?

GOAL

Investigate the effect of vascular endothelial glutaminase on tumor growth and metastasis

METHODS

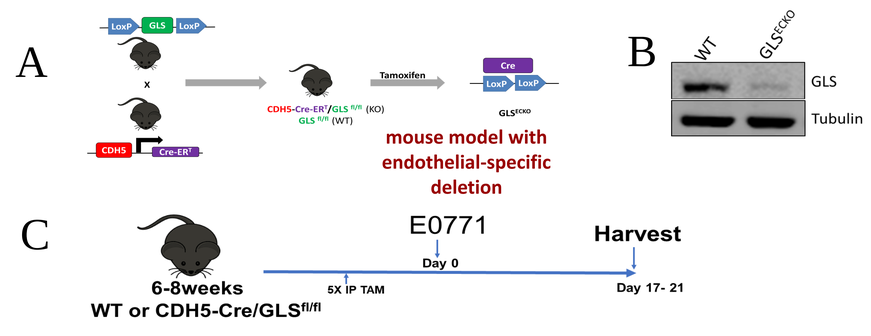

Figure 2: A) Using transgenic C57BL/6 to generate loss of GLS in the endothelium upon tamoxifen treatment. B) Western blot analysis showing loss of GLS in endothelial cells. C) Schematic representation of tumor cells implantation for tumor growth measure.

RESULTS

➢ EphA2 overexpression promotes glutamine metabolism

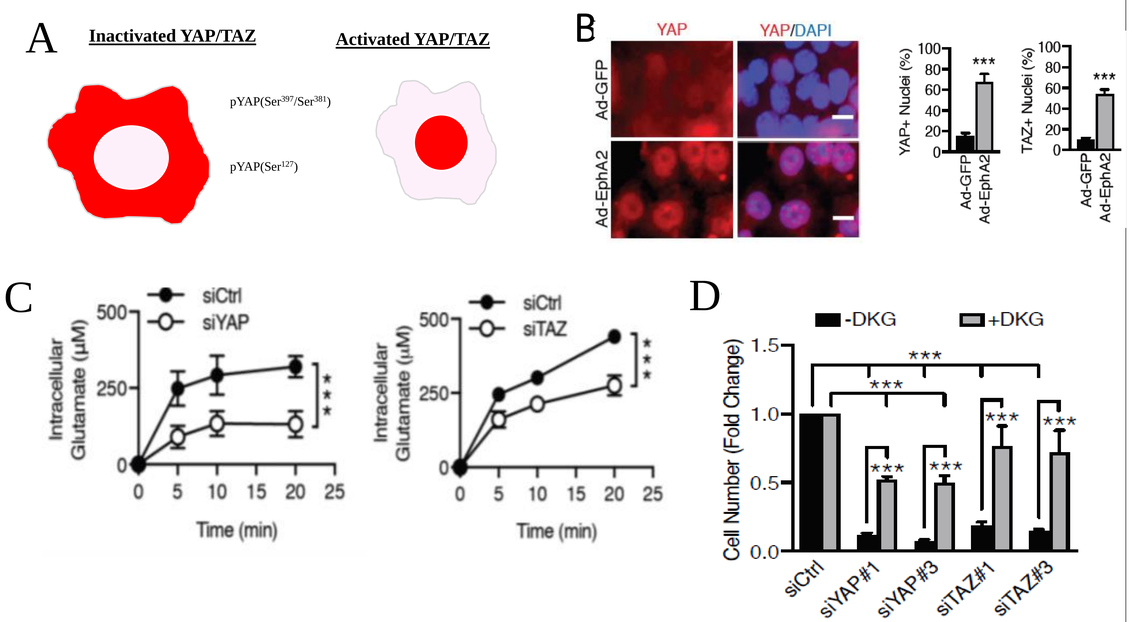

Figure 3: **A)** YAP/TAZ subcellular localization dynamics . **B)** Immunofluorescence of YAP or TAZ in *MMTV-Neu* cells infected with Ad-GFP or Ad-EphA2. **C)** Intracellular glutamate concentration was determined in MCF10A-HER2 cells with *YAP* or *TAZ* knockdown.***p<0.005 **D)** Growth assay of *YAP* or *TAZ* knockdown MCF10A-HER2-EphA2 cells treated with vehicle (-DKG) or dimethyl α-ketoglutarate (+DKG) for 3 days. Fold change in cell number was calculated based on controls. ***p<0.005

➢ Loss of GLS in the endothelium slows breast cancer growth in murine model

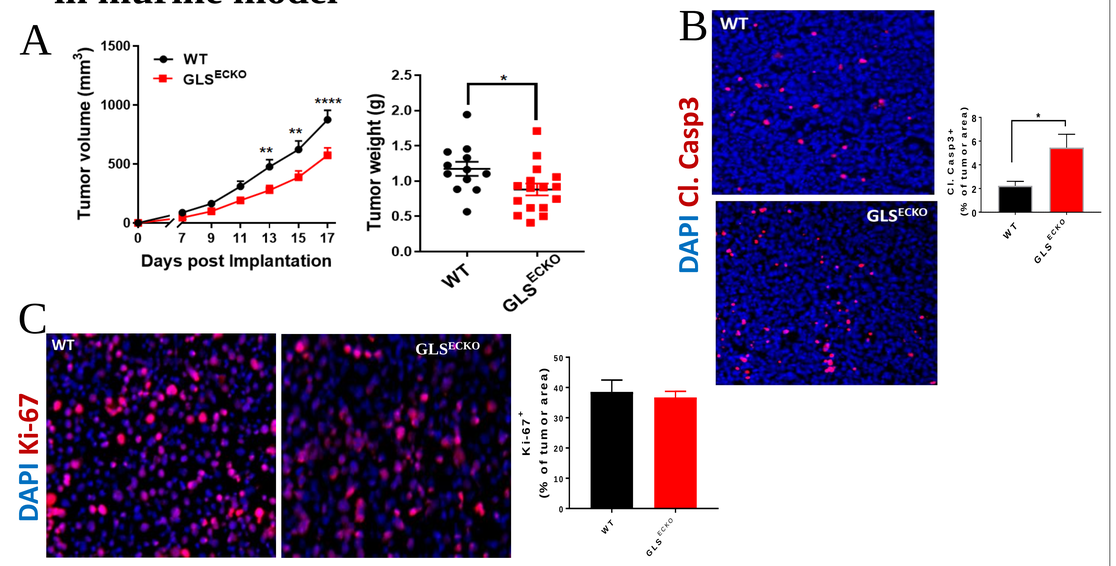

Figure 4: EC-specific GLS loss deficiency decreases tumor growth, increases apoptosis, but no effect on proliferation. **A)** E0771 growth curves on WT and KO mice. Tumor volume was calculated as: volume= length × width^2 ×0.5 N=12-16 per group. *p<0.05, **p<0.009, ****p<0.0001. **B)** Apoptosis as measured by cleaved caspase 3 decreases with GLS^{ECKO} mice compare to WT. *p<0.05. **C)** No in proliferation (Ki-67$^+$) in GLS^{ECKO} compare to WT.

➢ ECs-specific loss of GLS affect tumor vasculature

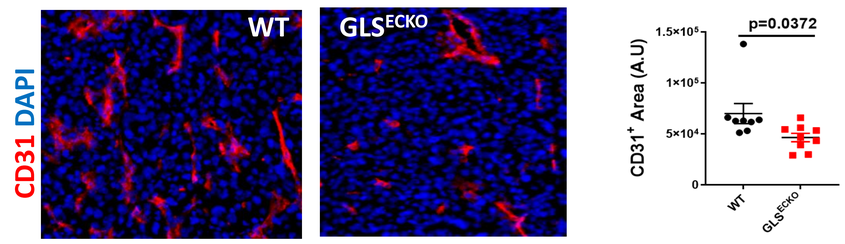

Figure 5 : Loss of GLS in host vasculature decrease tumor vessel formation. Representative and quantification of blood vessels numbers in E0771 tumors stained by endothelial cell marker CD31 (Red.).

SUMMARY

- EphA2 overexpression promotes YAP/TAZ nuclear accumulation and activation in breast cancer cells
- YAP/TAZ regulate glutamine metabolism.
- YAP/TAZ knockdown reduces intracellular glutamate hence affecting tumor cell viability.
- Endothelial cells require glutamine for proliferation
- Endothelial cell-specific GLS deletion decreases E0771 tumor cell growth, tumor weight increases apoptosis (cl.caspase 3), but no effect on proliferation
- GLS loss in host vasculature decreases tumor vessel numbers (CD31$^+$)

FUTURE STUDIES

- Investigate how the EphA2-YAP/TAZ signaling axis impacts the tumor microenvironment.
- Examine if loss of GLS in the endothelium is affecting metastasis.
- Determine the molecular mechanism of the observed phenotype in GLS^{ECKO} tumors.

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