The Effects of a Glutamine-Free Diet on Tumor Progression and the Immune Landscape of the Ovarian Tumor Microenvironment

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Background

Epithelial ovarian cancer, the most common type of ovarian cancer, is a leading cause of cancer deaths in women. Additional research on factors that may impact ovarian cancer proliferation and aggressiveness is needed to develop more effective therapies. Research on the tumor microenvironment (TME) can provide insight into the behavior of ovarian cancer as it includes fibroblasts, endothelial cells, lymphocytic infiltrates, and extracellular matrix proteins, which can directly affect cancer cell initiation, growth, migration, and differentiation[1].

Targeting glutamine metabolism in ovarian cancer has been shown to reduce tumor growth. Cancer cells have an upregulated glutamine metabolism due to increased energy needs for cell division. In ovarian cancer, glutamine metabolism is dysregulated in cancer-associated fibroblasts (CAFs) and ovarian cancer cells. Increased CAF-derived glutamine can also affect the activity of immune cells, modulating HGSC cell growth[2]. A preliminary study found that co-targeting glutamine synthetase (GLUL) in stroma and glutaminase in cancer cells reduces tumor growth and metastasis[2]. In addition to tumor cells, targeting glutamine metabolism has been shown to modulate the activities of various immune cell types, which subsequently suppress tumor cell growth[3]. We therefore hypothesize that lowering glutamine levels by adopting a glutamine-free diet can suppress ovarian cancer progression by suppressing the malignant phenotype of ovarian cancer cells directly or modulating the activities of various immune cell types.

Methods

In vivo Mouse Experiment

- The effects of a glutamine-free diet on tumor growth were determined using a novel mouse model in which syngeneic fallopian epithelial cell-derived cancer cells PPNM were injected IP into C57BL/6 mice[4]. Mice were either fed the normal diet or the glutamine-free diet 2 weeks before tumor cells were injected (Figure 1).

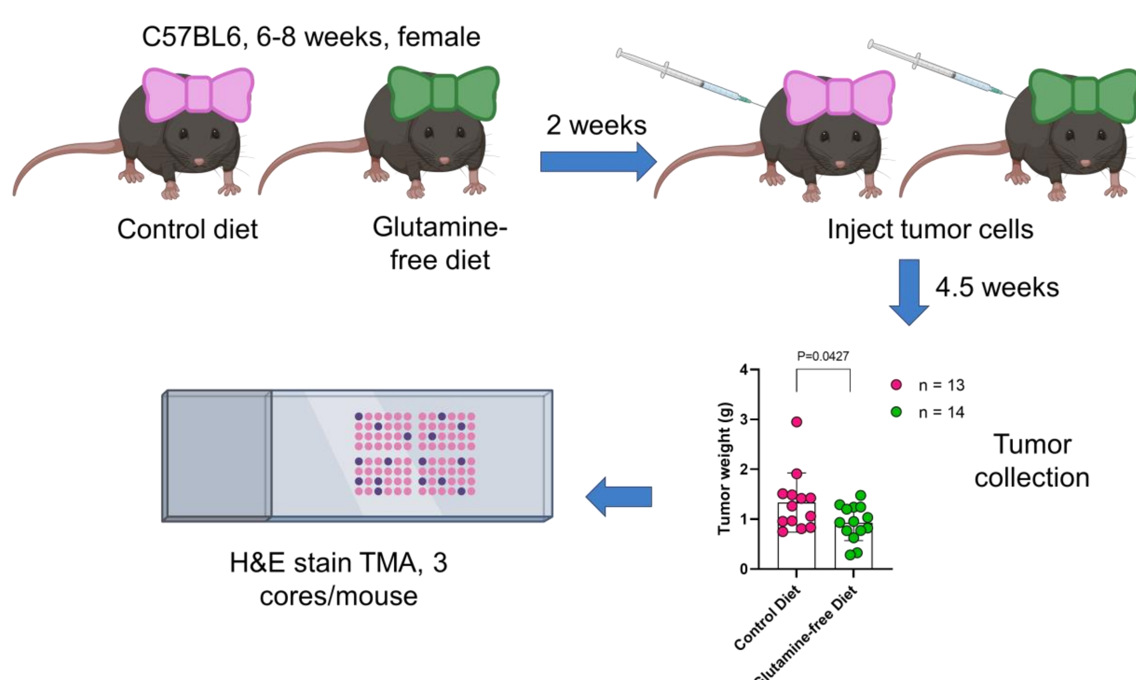

Figure 1. PPNM cells were injected IP into each C57BL/6 mouse 2 weeks after mice were fed with either normal or glutamine-dree diet. Tumors were collected and measured after 4.5 weeks. Tissue microarrays (TMA) were developed, which consisted of 3 tissue cores per mouse, and H&E stains were performed on these TMAs.

- Imaging mass cytometry (IMC), a technology to evaluate complex phenotypes and immune spatial interactions in the tissue microenvironment, was used to identify spatially resolved key immune cell types in their states and explore EMT-related proteins in tissues from mice fed with either the control or glutamine-free diet.

IMC Pipeline

Isotope	Mass Channel	Marker	Cell types/Pathways
141Pr	141	aSMA	Fibroblasts
144Nd	144	P-tyrosine	Tumor cells
147Sm	147	Epcam	Tumor cells
149Sm	149	Vimentin	Fibroblasts
150Nd	150	Ki67	Replicating cells
151Eu	151	CD45	Immune cells
152Sm	152	Fibronectin	Fibroblasts
153Eu	153	CD44	Stem cells
154Sm	154	b-ACTIN	Tumor cells, endothelial cells
155Gd	155	Granzyme B	Cytotoxic T cells
156Gd	156	F4/80	Macrophages
158Gd	158	E-cadherin	Tumor cells
159Tb	159	CD4	T cells
161Dy	161	MHC class II	Antigen presenting cells (APCs)
162Dy	162	CD8	T cells
164Dy	164	Arginase-1	Activated macrophages, MDSC, neutrophils
165Ho	165	FOXP3	Treg Cells
166Er	166	Ly-6G	Macrophages, MDSC, and neutrophils
171Yb	171	CD31	Endothelial cells
173Yb	173	COL1A1	Fibroblasts
174Yb	174	PAN-CK	Tumor cells
176Yb	176	B220	B cells
191Ir	191	Iridium	DNA-intercalator
193Ir	193	Iridium	DNA-intercalator

Panel of 22 metal-conjugated antibodies designed

TMA stained with the antibodies following an immunohistochemistry protocol

Tissue ablation with Fluidigm Helios CyTOF instrument equipped with a Hyperion System laser ablation module

Ions separated by mass and time-of-flight (CyTOF)

High dimensional imaging

Image analysis

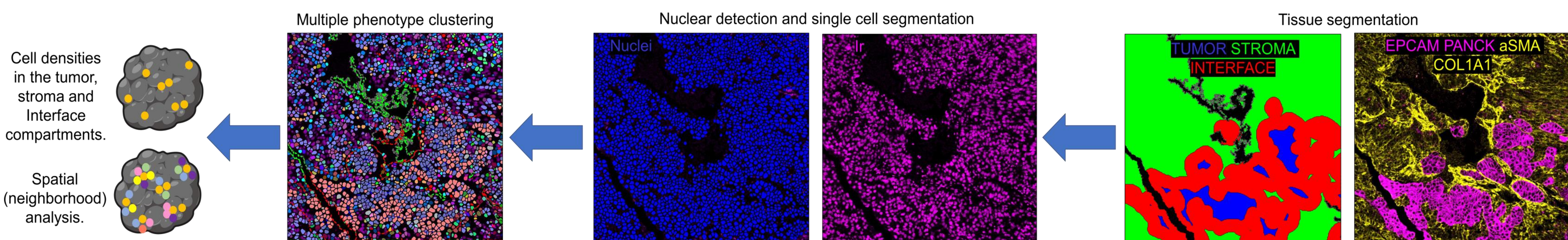

Figure 2. Imaging mass cytometry (IMC) was used to analyze the TME and to identify spatially resolved key immune cell types in their states in oviduct samples. Three representative areas in each sample were chosen to develop a tissue microarray (TMA). TMAs were stained with a panel of 22 metal-conjugated antibodies as previously described[5,6]. Antibodies were metal conjugated at the Flow Cytometry and Cellular Imaging Core Facility at MD Anderson. IMC data was acquired by a Standard BioTools Helios CyTOF instrument equipped with a Hyperion System laser ablation module (Standard BioTools) in the Flow Cytometry and Cellular Imaging Core Facility at MD Anderson. With the Standard BioTools Helios CyTOF instrument equipped with a Hyperion System laser ablation module, a UV laser ablated the tissue, creating plumes of metal isotopes. These isotopes were separated by mass and time of flight (TOF). Image analysis including tissue detection, tissue segmentation, nuclei detection, and phenotyping were performed using Visiopharm Phenomap software, an AI-driven precision pathology software. Automatic single cell segmentation and neighborhood analysis was performed, and cell densities in the stromal and epithelial compartments and expression levels of various biomarkers were quantified by Visiopharm.

Results

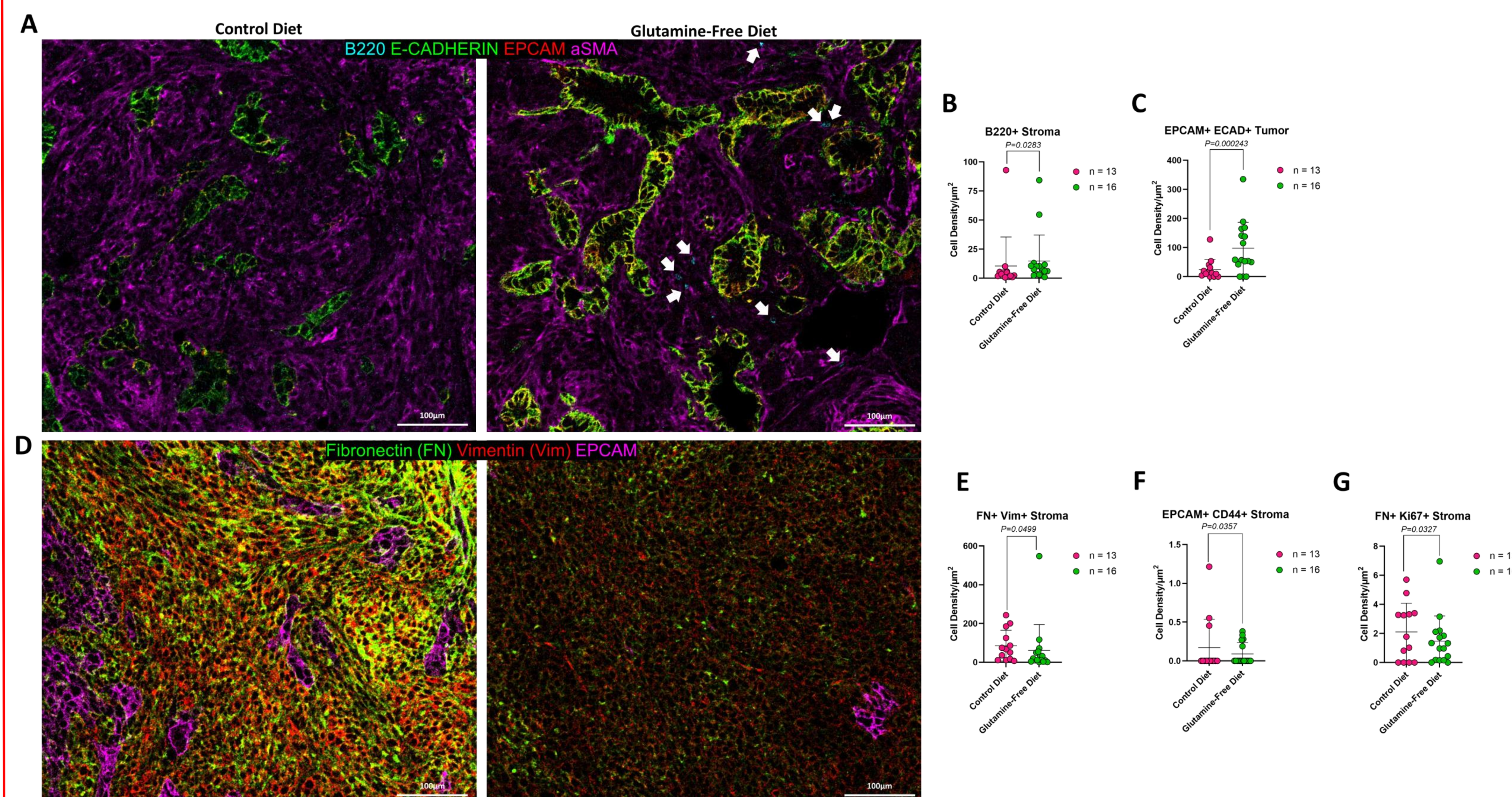

Figure 3. A. Representative images of B220 (B cells), E-cadherin, EPCAM, and aSMA in control and glutamine-free diet samples. **B.** Cell density of B220+ cells in the stroma of mice fed with the control and the glutamine-free diet (*P=0.0283*). Greater cell density of B220+ cells in the stroma of mice fed with glutamine-free diet. **C.** Cell density of EPCAM+ ECAD+ cells in the tumor (*P=0.000243*). Greater cell density of EPCAM+ ECAD+ cells in the tumor of mice fed with glutamine-free diet. **D.** Representative images of Fibronectin (FN), Vimentin (Vim), and EPCAM in control and glutamine-free diet samples. **E.** Cell density of FN+ Vim+ cells in the stroma (*P=0.0499*). Greater cell density of FN+ Vim+ cells in the stroma of mice fed with control diet. **F.** Cell density of EPCAM+ CD44+ cells in the stroma (*P=0.0357*). Greater cell density of EPCAM+ CD44+ cells in the stroma of mice fed with control diet. **G.** Cell density of FN+ Ki67+ cells in the stroma (*P=0.0327*). Greater cell density of FN+ Ki67+ cells in the stroma of mice fed with control diet.

Conclusions

- Mice fed with a glutamine-free diet have significantly lower ovarian cancer burden.
- Mice fed with a glutamine-free diet have greater B-cell-related immune response.
- Tumor tissues from mice fed with a glutamine-free diet have reduced stemness and EMT of ovarian cancer cells in the TME.
- Tumor tissues from mice fed with the control diet have more activated CAFs, which may result in increased stiffness of the ECM and may enhance the malignant phenotype of ovarian cancer cells.
- We plan to perform spatial analysis to further understand the implications of a glutamine-free diet. We will also further classify the subtypes of immune cells by performing more IMC using different metal-conjugated antibodies.

References

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