FAP alpha-CXCL12-CXCR4 Pathway in Glioblastoma: An Underappreciated Therapeutic Target?

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Introduction

- Immunosuppressive factors within Glioblastoma multiforme (GBM) play a significant role in their pathogenesis and treatment resistance\(^1\).

- Carcinoma-associated fibroblasts (CAFs), positive for fibroblast activation protein alpha (FAP\(\alpha\)) and downstream CXCR4/CXCL12 are linked to immune evasion in non-glial malignancies\(^2,3\), but their role in GBM remains incompletely understood.

Methods

• Archival human high grade glioma tissue was obtained from 16 patients via tissue bank of the Surgical Neurology Branch at the National Institutes of Health.

• Immunocompetent C57BL/6 mice underwent intracranial injection of GL261 murine glioma cell line.

• Immunohistochemistry and RNA scope were performed on 5µm paraffin fixed murine and human tumor sections using commercially available antibodies and RNA in-situ hybridization probes for FAPα and CXCL12.

• GlioVis data portal was used for visualization and analysis of FAPα expression in the Tumor Cancer Genome Atlas.
Results

• In human tumors, FAPα protein was noted in extracellular matrix, cytoplasm, and perivascular spaces.

• Of the human gliomas, overall 15/16 (93.8%) were FAPα positive and 13/16 (81.3%) were CXCL12 positive, while 12/16 (74%) were co-positive and 1/16 (6.3%) were co-negative. FAPα mRNA was only intracellular, whereas CXCL12 mRNA was also extracellular.

• FAPα was high in human tumors with large perivascular CD3+ T cell populations. In analysis of the Tumor Cancer Genome Atlas, FAPα expression greater than 7.37 conferred worse prognosis.
Figure 1. GlioVis data portal using The Cancer Genome Atlas dataset demonstrating improved overall survival in patients with low tumor FAP alpha expression as compared to high expression tumors.
Figure 2. RNA ISH targeting FAPa and CXCL12 mRNA in human glioblastoma samples demonstrated intracellular predominance of FAPa RNA transcripts (arrows) with, conversely, mixed intracellular and extracellular localization of CXCL12.
Figure 3. Immunohistochemistry of different distribution patterns of FAP alpha and CXCL12 in human glioblastoma samples. There was no clear correlation between predominant pattern of FAP alpha with that of CXCL12.
Future Directions

• Fearon et al. demonstrated the efficacy of combined checkpoint inhibition via blockade of multiple immunosuppressive pathways in murine preclinical model of pancreatic cancer.\(^2\)

• The combination of anti-PD-L1 antibody and AMD3100 (CXCR4 antagonist) has not been reported for use in preclinical models for high grade gliomas.

Upcoming Survival Study: PD-L1 and CXCR4 Blockade

Day 1:
IC injections, Osmotic pump Implantation

Day 1-14: 4 groups
1. Control
2. IP injections of anti-PD-L1
3. Anti-CXCR4 via osmotic pump
4. Combination anti-PD-L1/anti-CXCR4

Day 21:
MRI to evaluate tumor burden

Day 42:
MRI to evaluate tumor burden

Endpoint:
Symptomatic tumor burden, sacrifice, immune phenotyping tumors
Summary

• FAP alpha and CXCL12 are expressed in up to 93.8% of primary tumor samples surveyed, significantly higher than previously reported\textsuperscript{4,5}.

• Consistent expression patterns of FAP alpha and CXCL12 in extracellular matrix, perivascular, and cytoplasmic distributions in positive tumors.

• High levels of FAP alpha expression in human tumors associated with a significantly decreased overall survival compared to low expressing tumors and with high perivascular CD3+ cells, suggesting sequestration.

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