Polarization of Myeloid Cells By Cancer Associated Fibroblasts

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Background

- Immunosuppression mediated by the tumor stroma remains a major barrier in refractory tumors
- Cancer-associated fibroblasts (CAFs) are a dominant component of the pancreatic tumor microenvironment
- We sought to identify CAF-associated factor(s) responsible for the suppressive polarization of tumor infiltrating myeloid cells, which can directly inhibit antitumor CD8 T cell function
- A CAF-associated soluble factor would provide a target to reverse immunosuppression

Hypothesis

CAFs are associated with recruitment and polarization of suppressive myeloid cells, and this interaction is mediated by a CAF-associated soluble factor

Methods

CAF polarization of myeloid cells

- Bone marrow (BM) progenitors were cultured for 96 hours in normal fibroblast or CAF conditioned media
- Flow cytometry was used to assess the suppressive capacity of fibroblast conditioned myeloid cells

Suppression of T cells by CAF conditioned myeloid cells

- BM progenitors were cultured for 96 hours in normal fibroblast or CAF conditioned media
- Myeloid cells were co-cultured with CD8 T cells for 72 hours
- Flow cytometry was performed

CAF-induced myeloid polarization *in vivo*

- Mice were challenged with MT4-LA tumor cells
- On day 5, mice were injected intratumorally with DMEM, normal fibroblast CM, CAF CM, or left untreated

Heat inactivation of CAF conditioned media

- BM progenitors were cultured for 96 hours in CAF conditioned media that was left at 56C for 2 hours
- Flow cytometry was performed

Results

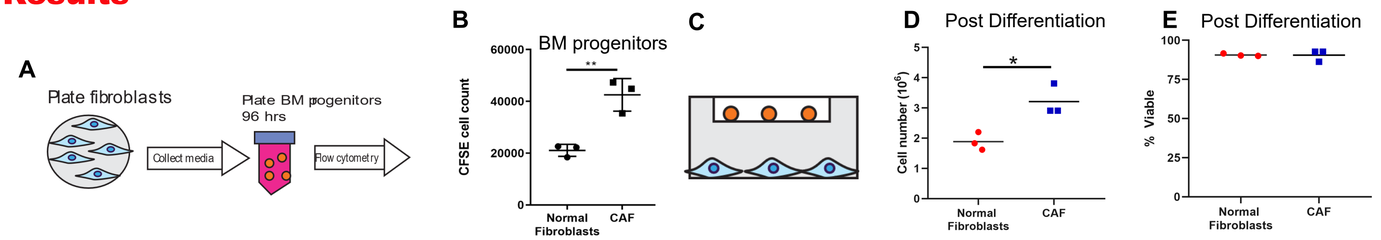

Figure 1. (A) Normal or cancer-associated fibroblasts were plated in serum free media. BM progenitors were then seeded in a transwell, and **(B)** flow cytometry was performed to assess migration of CFSE labeled bone marrow progenitors cell numbers. **(C)** BM progenitors were cultured in normal fibroblast or CAF conditioned media and their **(D)** cell count and **(E)** viability were assessed.

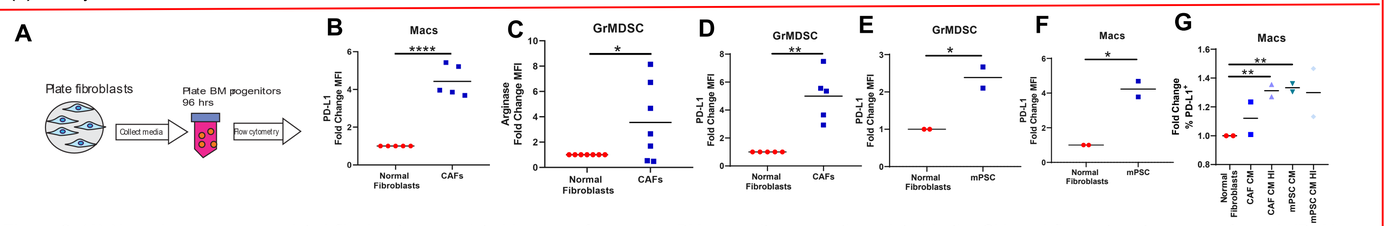

Figure 2. (A) Normal or cancer-associated fibroblasts were plated in serum free media. BM progenitors were then plated in the CM, and flow cytometry was performed. **(B)** Macs showed higher PD-L1 expression in CAF CM compared to normal fibroblast CM. **(C and D)** GrMDSCs showed higher arginase and PD-L1 expression in CAF CM than in normal fibroblast CM. **(E and F)** GrMDSCs and Macs differentiated from BM progenitors in CAF CM showed higher PD-L1 MFI expression. **(G)** Macs maintain PD-L1 MFI expression in heat inactivated mPSC CM.

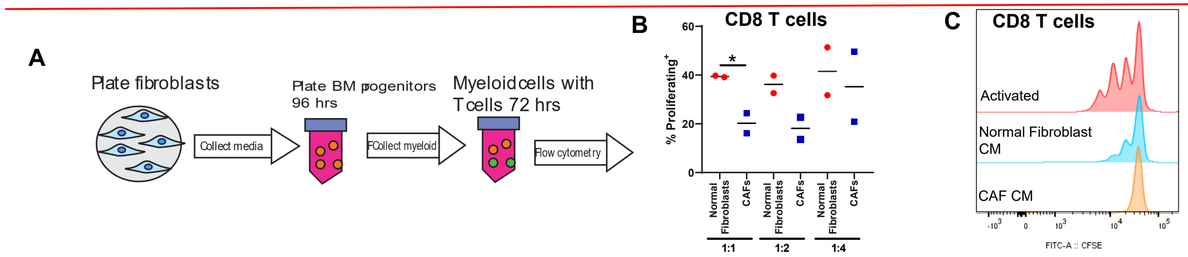

Figure 3. (A) Normal or cancer-associated fibroblasts were plated in serum free media. BM progenitors were then plated in the CM, and flow cytometry was performed. **(B)** CAF CM was associated with decreased proliferation of CD8 T cells, compared to normal fibroblast CM. **(C)** Representative histograms of CD8 T cells alone or cocultured with normal fibroblast conditioned or CAF conditioned myeloid cells.

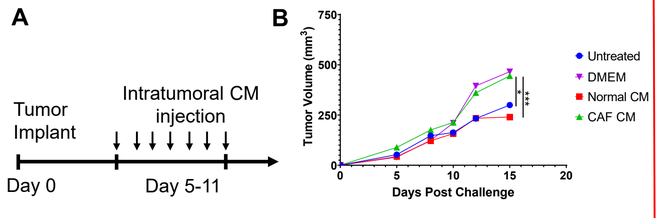

Figure 4. (A) Mice were implanted with MT4-LA tumor cells at day 0, then left untreated or treated with DMEM, normal CM, or CAF CM at day 5. **(B)** Mice showed the greatest tumor volumes in the DMEM and CAF CM groups.

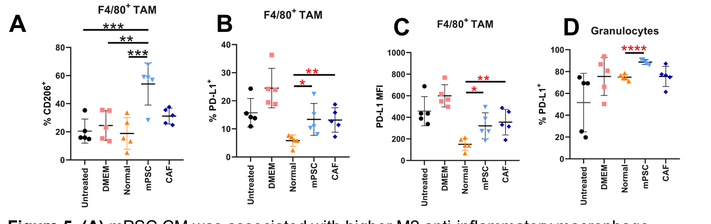

Figure 5. (A) mPSC CM was associated with higher M2 anti-inflammatory macrophage recruitment. **(B and C)** mPSC CM and CAF CM were associated with higher PD-L1 expression in TAMs. **(D)** mPSC CM was associated with higher PD-L1 expression in granulocytes.

Results and Discussion

- CAFs can recruit myeloid cells and influence their suppressive function by promoting PD-L1 and arginase expression
- mPSC CM increased PD-L1 expression
- CAF conditioned myeloid cells reduce T cell proliferation and promotes tumor growth
- Intratumoral treatment with mPSC CM led to increased PD-L1 expression on macrophages and granulocytes
- Heat inactivation did not reduce mPSC-induced expression of PD-L1 on myeloid cells
- Our results suggest that CAFs recruit and polarize myeloid cells.
- Eventual goals include inactivating previously identified CAF-associated soluble factors with the hope of reducing local tumor immune suppression.

References

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