Semaphorin7a expression in breast cancers promotes susceptibility to immune checkpoint blockade

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Abstract

Five-year survival rates for women diagnosed with stage II breast cancer are >85% on average; however, in women diagnosed with stage II breast cancer (BC) within 10 years of last childbirth, we observe a 5-fold increase in risk for developing distant metastasis when compared with nulliparous women[1]. Major driving factors for stage II diagnosis include increased tumor-associated lymphatic vessel density (LVD), lymphovascular invasion (LVI), and lymph node positivity (LN+); all are associated with poor prognosis for BC patients. The mechanisms underlying development of LN metastasis and how LN metastasis seed distant metastases has remained elusive. LVD, LVI, and LN+ can be driven by tumor-associated macrophages (TAMs), which have been implicated in creating a pro-tumor tumor microenvironment (TME) in many types of cancer. Additionally, podoplanin (PDPN)-expressing macrophages (PoEMs), are a newly characterized type of TAM that specifically contribute to tumor-associated lymphatic vessel formation[2,3]. Our lab identified that semaphorin7A (SEMA7A)—a neuroimmune molecule—is significantly associated with increased metastasis in LN+, but not LN-, BC patients, as well as increased recurrence in cases of BCs diagnosed within 10 years of childbirth, designated postpartum breast cancers (PPBCs). In mouse models, SEMA7A expression is associated with increased LVD and TAM presence, including PoEMs[3]. Furthermore, we and others have shown that PoEMs intercalate into lymphatic vessels to form PoEM-LEC chimeric vessels, where tumor cells can often be found at the PoEM-LEC junctions[2]. We have observed that BC cells, lymphatics, macrophages, and PoEMs exhibit SEMA7A-dependent expression of PD-L1 in vitro, which may make SEMA7A+ tumors sensitive to αPD-1/αPD-L1 therapies. Therefore, we utilized multiple orthotopic, immunocompetent mouse models with E0771 SEMA7A overexpressing (OE) and empty vector (Ctrl) tumors and flow cytometry on harvested tumors to produce the following results.

Objectives

- Establish a link between SEMA7A and immune-suppressive TME.
- Investigate the link between SEMA7A and susceptibility to immune checkpoint inhibition.
- Identify the immune checkpoint blockade-induced alterations to the TME in SEMA7A+ tumors.

SEMA7A exposure induces tumor cell expression of PD-L1

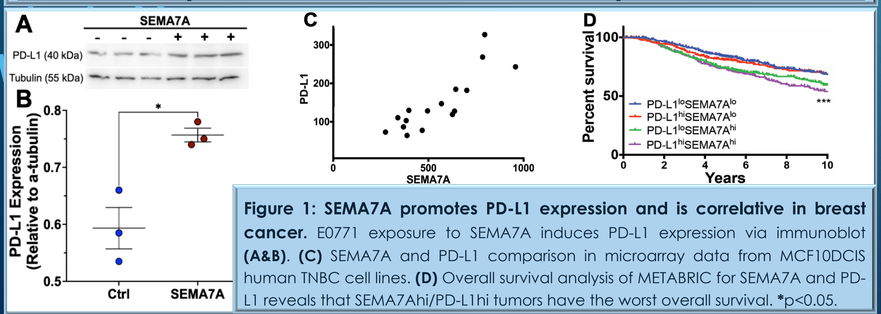

Figure 1: SEMA7A promotes PD-L1 expression and is correlative in breast cancer. E0771 exposure to SEMA7A induces PD-L1 expression via immunoblot **(A&B)**. **(C)** SEMA7A and PD-L1 comparison in microarray data from MCF10DCIS human TNBC cell lines. **(D)** Overall survival analysis of METABRIC for SEMA7A and PD-L1 reveals that SEMA7Ahi/PD-L1hi tumors have the worst overall survival. *p<0.05.

SEMA7A promotes PD-L1 expression on tumor cells and LECs

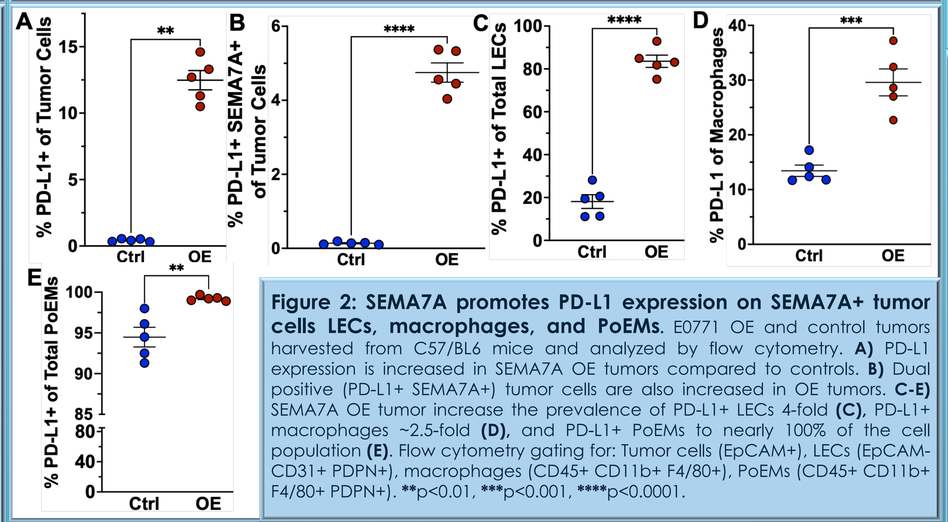

Figure 2: SEMA7A promotes PD-L1 expression on SEMA7A+ tumor cells LECs, macrophages, and PoEMs. E0771 OE and control tumors harvested from C57/BL6 mice and analyzed by flow cytometry. **A)** PD-L1 expression is increased in SEMA7A OE tumors compared to controls. **B)** Dual positive (PD-L1+ SEMA7A+) tumor cells are also increased in OE tumors. **C-E)** SEMA7A OE tumor increase the prevalence of PD-L1+ LECs 4-fold **(C)**, PD-L1+ macrophages ~2.5-fold **(D)**, and PD-L1+ PoEMs to nearly 100% of the cell population **(E)**. Flow cytometry gating for: Tumor cells (EpCAM+), LECs (EpCAM-CD31+ PDPN+), macrophages (CD45+ CD11b+ F4/80+), PoEMs (CD45+ CD11b+ F4/80+ PDPN+). **p<0.01, ***p<0.001, ****p<0.0001.

α-PD-1 and α-PD-L1 therapy inhibit growth of SEMA7A+ tumors

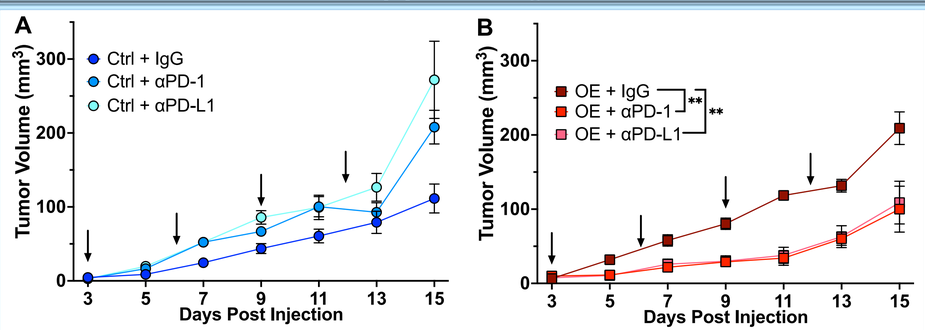

Figure 3: SEMA7A+ tumors are sensitive to both α-PD-1 and α-PD-L1. E0771 OE and control tumors were treated with IgG (vehicle), α-PD-1, or α-PD-L1. 250ug administered IP every third day once tumors were palpable. Control tumor growth was not affected by α-PD-1 or α-PD-L1 **(A)** but SEMA7A+ tumor growth was significantly inhibited **(B)**. **p<0.01.

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α-PD-1 and α-PD-L1 treatment decrease PD-L1+ cells, but increase SEMA7A expression

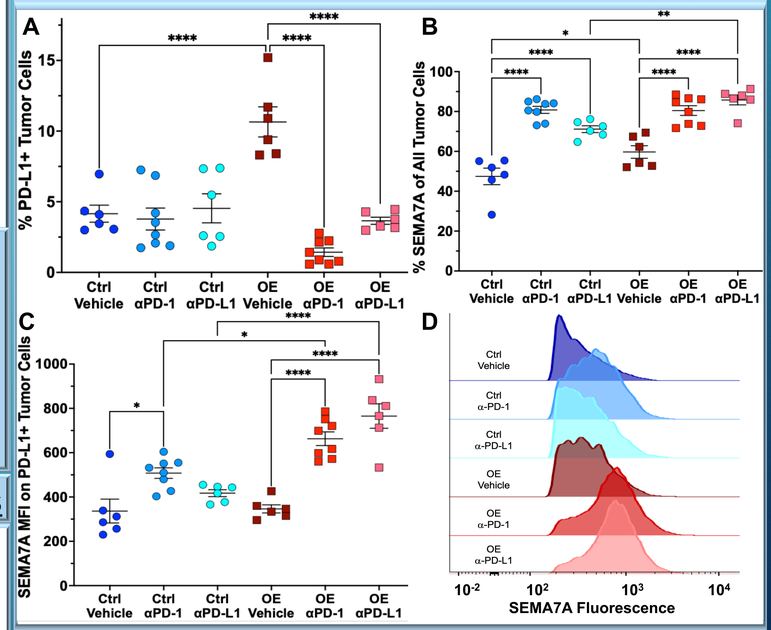

Figure 4: α-PD-1 and α-PD-L1 treatment causes upregulation of SEMA7A expression on control and SEMA7A OE tumor cells. E0771 OE and control tumors were treated **(Figure 3)** with IgG (vehicle), α-PD-1, or α-PD-L1 and analyzed by FC. α-PD-1 and α-PD-L1 eliminated PD-L1+ tumors in SEMA7A OE tumors **(A)** but increased the presence of SEMA7A+ tumor cells **(B)**. Expression of SEMA7A on the remaining tumor cells increased in both control and SEMA7A OE tumors with α-PD-1 and α-PD-L1 treatment **(C and D)**. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Conclusions

- **SEMA7A upregulates PD-L1** expression on tumor cells, LECs, macrophages, and PoEMs.
- **SEMA7A+ tumor** cells are **susceptible** to both **α-PD-1 and α-PD-L1** immunotherapy.
- **α-PD-1 and α-PD-L1** treatment **increases the presence of SEMA7A+** tumor cells in control and OE tumors.
- **α-PD-1 and α-PD-L1 treatment upregulate expression of SEMA7A** on control and OE tumor cells