1536 EGFL6 INDUCES IL-10 AND CXCL2 IN TUMOR ASSOCIATED MYELOID CELLS: LINK TO ANTI-TUMOR IMMUNOSUPPRESSION AND RESISTANCE TO ANTI-PD-L1 THERAPY IN OVARIAN CANCER

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Background Myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) are critical negative regulators of immunity in cancer.[1 2] Understanding factors which regulate these cells could result in the identification of new approaches to enhance immunotherapy. One such factor is epidermal growth factor-like 6 (EGFL6), a secreted factor known to promote cancer stem like cell migration and regulate cancer cell differentiation.[3] We found that mice which overexpress *Egfl6* have an increased numbers of myeloid cells in both the bone marrow and spleen. The goal of this study is to evaluate the impact of tumor Egfl6 on myeloid cell phenotype in the ovarian cancer (OvCa) tumor microenvironment (TME).

Methods Ex-vivo rEgfl6 and two syngeneic mouse models of OvCa were used to study the impact of tumor Egfl6 on the immune TME. RNA sequencing was employed to identify Egfl6-mediated gene expression in tumor infiltrating myeloid cells. Egfl6+ tumors were treated with anti-Egfl6, or anti-PD-L1 antibodies, as single agents or in combination, and tumors were harvested for immune profiling, gene and protein expression evaluation and e*x vivo* co-culture studies. Immunofluorescence and spatial transcriptomic were used to determine the localization of EGFL6 and myeloid cells in human OvCa tissue samples.

Results In vitro and ex-vivo analysis indicated that Egfl6, via binding with beta integrins and activation of p38 and SYK signaling, (i) acts as a chemotactic factor for myeloid cells and (ii) promotes their differentiation toward a suppressive state. Suggesting an important role in promoting an immunosuppressive TME, we found that expression of Egfl6 on tumor cells increased tumor growth and shortened animal survival. This was associated with an increased accumulation of intra-tumoral MDSCs and TAMs and their expression of immunosuppressive factors, including CXCL2, IL-10 and PD-L1. Both CXCL2 and IL-10 were found to play a key role in the Egfl6-induced anti-tumor immunosuppression. In an immune 'hot' tumor model,[4 5] Egfl6 completely inhibited response to a-PD-L-1 therapy. Finally, we found that Egfl6 antibody decreased the accumulation of tumor-infiltrating CD206+ TAMs and PMN-MDSCs, and their secreted factors, IL-10 and CXCL2, and thereby restored the efficacy of a-PD-L1 therapy. Importantly, in human OvCa tissue samples, EGFL6+ areas were highly infiltrated by myeloid cells.

Conclusions Combined our data show that EGFL6 induces the recruitment of myeloid cells into the ovarian TME and subsequently promotes their immunosuppressive functions. This suggest EGFL6 is a potential novel therapeutic target to enhance response to immune therapy in OvCa patients.

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Ethics Approval Biopsies of patients with high-grade serous ovarian cancer were selected and collected at the Department of Obstetrics, Gynecology, and Reproductive Science, University of Pittsburgh.

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