Title: **Autotaxin impedes anti-tumor immunity by suppressing chemotaxis and tumor infiltration of CD8+ T cells**

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Introduction

To improve the efficacy of immunotherapy, it is essential to better understand how cytotoxic CD8+ T cells infiltrate into tumors. Here, we examine a role for autotaxin (ATX) in this process. ATX (encoded by ENPP2) is a secreted phospholipase that produces the lipid mediator lysophosphatidic acid (LPA) to regulate multiple biological functions via specific G protein-coupled receptors, termed LPAR1-6. ATX/LPA promotes tumor cell migration via LPAR1 and T-cell motility via LPAR2, yet its actions in the tumor microenvironment remain unclear. Here, we show that tumor-intrinsic ATX suppresses T-cell infiltration to impede anti-tumor immunity, and identify LPAR6 as a T-cell migration inhibitory receptor. Hence, ATX inhibition may show clinical benefit for patients with cancer.

Material and Methods

We apply a variety of molecular, cell biological and biochemically techniques for in vitro and ex vivo studies, as well as single-cell RNA-seq analysis of human tumors. In addition, we use a murine anti-cancer vaccination model to monitor ATX-dependent T-cell responses and tumor infiltration, and apply single-cell RNAseq analysis of human tumors.

Results and Discussions

We find that ATX secreted by melanoma cells is a chemo-repellent for ex vivo expanded tumor-infiltrating lymphocytes (TILs) and peripheral CD8+ T cells, overruling chemokine activity. Mechanistically, T-cell repulsion is mediated by G12/13-coupled LPAR6, which is highly expressed in immune cells. Contrary to prevailing notions, secreted ATX is bioactive at

physiologically insignificant steady-state LPA levels, revealing its secondary function as an LPA carrier or chaperone. Upon anti-cancer vaccination of tumor-bearing mice, tumor-intrinsic ATX does not affect the induction of systemic T-cell responses but, importantly, suppresses tumor infiltration of cytotoxic CD8+ T cells and thereby impairs tumor immune control. Moreover, ENPP2 expression in melanoma tumors – in both malignant and stromal cells – is associated with reduced T-cell infiltration, as inferred from single-cell transcriptomics. These findings highlight an unexpected role for the pro-metastatic ATX-LPAR axis in suppressing CD8+ T cell infiltration and anti-tumor immunity.

Conclusion

By opposing T-cell infiltration while activating tumor cells via different LPA receptors, the ATX/LPA complex exerts dual synergistic actions in the tumor immune microenvironment. ATX inhibition could benefit patients with cancer, depending on their tumor ATX expression profile.