Identification of specific Tumor Associated Macrophage targets in human breast cancer

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Introduction
The tumor microenvironment is a complex ecosystem consisting of tumor, stroma and infiltrating immune cells, among which macrophages are the major components.
In mouse, tumor-associated macrophages (TAMs) promote angiogenesis, cell invasion and intravasation, and they mediate tumor cell extravasation, persistent growth and cytolytic T cell responses suppression at the metastatic site. TAMs pro-tumoral behavior in mouse has made them attractive therapeutic targets. Targeting strategies include inhibiting monocyte recruitment, TAMs depletion, and their functional/phenotypic reprogramming. However, the lack of TAM-specific markers and the poor understanding of their functions in human cancers limit these strategies.
The aim of this study is to characterize the transcriptional landscape of human breast TAMs for the identification of specific TAM targets.

Methods/patients
We recruited breast cancer patients (ER+ invasive tumors, naïve to therapy) and age/sex matched healthy controls from three independent cohorts (Albert Einstein Medical College and Duke University, USA; University of Edinburgh, UK). Human TAMs and resident macrophages were isolated by FACS sorting from fresh breast tumor needle biopsies and reduction mammoplasty, respectively. Following RNA extraction and amplification, samples were sequenced using bulk deep RNA sequencing.

Results
TAMs show a distinct transcriptional profile compared to resident macrophages. Bioinformatic analysis identified a TAM-specific 37-gene signature which is significantly associated with poor prognosis in human breast cancer annotated datasets (i.e. METABRIC). Among these 37 genes, we identified SIGLEC1 and CCL8 as top breast TAM targets. SIGLEC1 and CCL8 were confirmed as novel breast TAM targets by extensive validation on independent cancer patient cohorts through flow cytometry, multiplex immunohistochemistry, machine learning assisted multicolor immunofluorescence and fluorescent in situ hybridization.
In vitro experiments using immortalized Pluripotent Stem cells (iPS) derived macrophages defined the mechanism of regulation of the two targets on TAMs and identified a novel crosstalk between cancer cells and human TAMs which leads to increased tumor invasion.

Conclusion
The results of this study reinforce the concept of TAMs as promoter of human malignancy, and underline the importance of identifying uniquely expressed genes in human TAMs to develop new therapeutic strategies and discover novel prognostic markers.

Keywords : Macrophages, Breast Cancer, CCL8, SIGLEC1, Tumor Microenvironment
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