989 CANCER SCULPTS GRANULOPOIESIS TO GENERATE TUMOR-SUPPORTIVE NEUTROPHILS

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Background Neutrophils dominate the immunological landscape of multiple solid tumors and strongly associate with cancer progression. Because neutrophils are short-lived cells, granulopoiesis is increased to meet the demands of cancers not only in cell number but also by rewiring the neutrophils' functional properties to be produced. Whether these functions are acquired in the tumor microenvironment or are imprinted early during their development in the bone marrow remains unknown.

Methods We built a comprehensive atlas based on the single-cell transcriptomes of mouse neutrophils in health and disease, which we refer to as NeuMap. The NeuMap encompasses over 50 different physiological and pathological conditions, including multiple cancer-associated neutrophils from pancreatic, breast, and lung cancer mouse models. Additional epigenetic and proteomic multiparametric assays were performed to have a comprehensive landscape of granulopoiesis in cancer at single-cell resolution.

Results NeuMap allowed us to identify three unique developmental trajectories of neutrophils from the bone marrow into tissues in health, inflammation, and cancer. Beyond promoting granulopoiesis, we observed that cancer skews the developmental trajectory to favor the generation of tumor-supportive neutrophils. Neutrophils from the bone marrow of tumor-bearing mice express early immunosuppression and angiogenesis markers and notably blocked interferon and antigen presentation programs. Combined inspection of chromatin accessibility and gene expression at the single cell level (DOGMA-seq) of neutrophils from naïve, cancer-bearing, and endotoxin-exposed (inflamed) mice revealed that, compared with inflammatory neutrophils which display type I Interferon (IFN) signaling, those associated with cancer had programs driven by the *JunB* and *Bhlhe40* transcription factors. To functionally validate these observations, we generated mice with depleted expression of *Ifnar*, *JunB* or *Bhlhe40* in neutrophils using the $MRP8^{CRE}$ driver (IFNARdN, JunBdN, and Bhlhe40dN mice, respectively). Functional *in vivo* assays in matrigel implants and *ex vivo* T-cell suppression assays of bone marrow progenitors exposed to multiple cytokines and conditioned media revealed that both JunB and Bhlhe40 are needed for full activation of the immunosuppressive program. In contrast, IFNAR repressed these immunosuppressive properties, and consequently, IFNARdn mice showed accelerated growth of lung tumors.

Conclusions These results, based on a holistic analysis of the granulocytic compartment, suggest that the generation of tumor-associated neutrophils occurs through systemic alteration of early granulopoietic trajectories in the bone marrow. Identifying the drivers that favor cancer-associated immune trajectories should enable rational targeting of pro-tumoral granulopoiesis to curb tumor onset or growth, especially relevant for neutrophil-rich types of cancers in which other immunotherapeutic strategies have proved ineffective.

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