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Spotlight

Mononuclear myeloid cells can shape neutrophils in brain tumors

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In most human solid cancer types, a high frequency of intratumoral neutrophils is associated with poor prognosis. In a recent study, Maas *et al.* identified an intratumoral niche in which mononuclear myeloid cells drive proinflammatory neutrophil activation in brain tumors. This study sheds new light on the intratumoral modulation of neutrophils.

Compelling evidence from clinical biomarker studies suggests that both an increased neutrophil:lymphocyte ratio in the blood and a high intratumoral density of tumor-associated neutrophils (TANs) are associated with poor survival in most cancer entities. However, compared with dendritic cells (DC), macrophages, and T cells, TANs have received less attention in the early years of modern immuno-oncology and immunotherapy. TAN research was initially stimulated with the advent of the N1–N2 dichotomy and the concept of myeloid-derived suppressor cells (MDSCs). While the N1–N2 concept described the antitumor (N1) versus protumor (N2) function of neutrophils, the MDSC concept established pathologically expanded neutrophils as being T cell-suppressive or more broadly immunosuppressive polymorphonuclear (PMN)-MDSCs [1]. The N1–N2 paradigm was initially based on the blockade of protumorigenic TGF-β activity, but a rapidly increasing number of studies defined multiple scenarios in which neutrophils could acquire antitumor activity. In most of those scenarios, the antitumor activity of neutrophils was in fact induced by therapeutic interventions rather than being intrinsic to the tumor host immune system [2]. Recently, neutrophils became accessible to RNA-sequencing (RNA-seq) technology and multiple transcriptomic states were described in various tumor models. Single cell technologies have facilitated the definition of diverse phenotypes and functional states within a given environment. Along this line of research, studies that were mainly based on murine models performed under homeostatic conditions have demonstrated that the tissue environment can substantially imprint transcriptomic signatures [3]. A major future challenge in the field will be to link an increasing number of reported transcriptional states with their corresponding cell biological function.

In a recent study published in *Cell* [4], Maas *et al.* profiled and characterized peripheral blood and tumor-infiltrating neutrophils in murine and human brain tumors. Microenvironmental co-cultures (MECs), defined as conditioned medium generated from single cell suspensions of tissues, were then co-cultured with purified target neutrophils and used in functional assays.

In the first part of the study, the authors showed that neutrophils infiltrating brain tumors displayed an inflammatory gene signature and an activated phenotype. Previous work in murine models had shown that tissue-specific reprogramming can occur when neutrophils enter different tissues under homeostatic conditions [3]. In line with these findings and extending the concept to the human brain, Maas and colleagues [4] found that the nontumor brain and the tumor microenvironment (TME) from gliomas or lung and breast cancer brain metastases substantially imprinted TAN transcriptional programs. When focusing on molecules and pathways with potential direct functional impact, the authors discovered upregulation of TNF-α signaling in brain metastasis TANs, and reduced expression of reactive oxygen species (ROS) and upregulation of PD-L1 in TANs compared with peripheral blood neutrophils. Further spatial analysis demonstrated PD-L1-positive TANs residing in close proximity to PD-1-positive $CD8^+$ T cells. As hypothesized by the authors, this spatial arrangement might be indicative of a potential immunosuppressive function of those brain TANs. However, PD-L1 upregulation in tumor-infiltrating myeloid cells is classically associated with an IFN-dependent response signature, which was not reported in this study. In addition, neutrophils use a plethora of different mechanisms to execute immunosuppressive and protumorigenic activity: PD-L1 is one candidate, but multiple other mechanisms exist [5] and were not investigated in this study. Along these lines, on the one hand, neutrophil ROS production also represents a potential T cell inhibitory mechanism. On the other hand, ROS produced by neutrophils can kill tumor cells, a hypothesis that is favored by the authors in the context of the investigated human brain tumors [5]. Unfortunately, this functional dichotomy of ROS is still poorly understood and additional spatial single cell image analyses and/or *ex vivo* functional assays will be needed to obtain a full understanding of the *in vivo* function of PD-L1-positive/ROS-low TANs in these human brain tumors.

In the second part of the study, Maas and coworkers sought to identify mechanisms that activate neutrophils in the brain TME [4]. To this end, they screened 1000 proteins in the so-called MEC, comprising single cell suspensions from human brain tumor tissues. They identified 51 proteins that were enriched in MEC-conditioned medium over controls. This set of proteins included well-established modulators of neutrophil activity, such as TNF-α, IL-6, and CXCL8, but also several less studied candidates, such as ceruloplasmin (CP). The authors focused on TNF-α and CP.

They found that TNF-α induced classical phenotypic neutrophil activation (upregulation of CD11b and CD66b, and downregulation of CD62L), while CP induced partial neutrophil activation and reduced ROS in healthy donor circulating neutrophils compared with untreated cells. Interrogation of the human TME RNA-seq data showed that expression of *TNFA* by mononuclear myeloid cells and spatial analysis of tissues were indicative of TAN clusters, but also revealed frequent interactions of TANs with tumor-associated macrophages/microglia (TAMs). In conjunction, these data revealed frequent TAN–TAM interactions in the brain TME, which makes it plausible that TAM TNF-α-mediated activation of TAN might also occur *in vivo*, which remains to be further investigated (Figure 1).

This study from the Joyce laboratory adds several aspects to the 'neutrophils and cancer' research space [4]. Importantly, and taking into account the considerable biological differences between human and murine neutrophils [6], the work provides a robust transcriptomic characterization of brain tumor neutrophils from patients with different types of brain tumor and brain metastases [4]. The authors reported an activated state as a hallmark of those neutrophils and provide evidence that this activation might be triggered by TNF-α release from adjacent TAMs. Although no direct interconnection of cell biological mechanisms and clinical follow-up is presented, the study reported induction of potentially T cell-suppressive PD-L1 and downregulation of putative tumor cytotoxic ROS in brain TANs. This is relevant because these two mediators are part of a larger arsenal of mechanisms utilized by TAN to either inhibit or promote tumor growth and thus influence disease outcome [1,5].

To translate such scientifically important findings into clinical benefit, future work should focus on truly comparative studies to directly distinguish the effects of different protumorigenic mechanisms in a given tumor entity or model and identify the most effective therapeutic approaches for each tumor scenario. Since mouse models that recapitulate all elements of human disease are rare, a major challenge in this regard is the sensitivity of human neutrophils to technical processing procedures [7]. Alternatively, modern image analysis tools (e.g., 'digital pathology') combining cell identity markers and functional markers might provide 'close to *in vivo* evidence' for different human TAN functional subpopulations and states [8]. Ultimately, a broad coordinated and comparative approach utilizing murine models, robust patient profiling, and optimized *ex vivo* functional assays is needed to move neutrophil targeting closer to human cancer putative therapies. Such a strategy is also needed to demystify the term 'context dependent', which is often used when a given mechanism, such as ROS production in this study, exhibits both anti- and protumor activities.

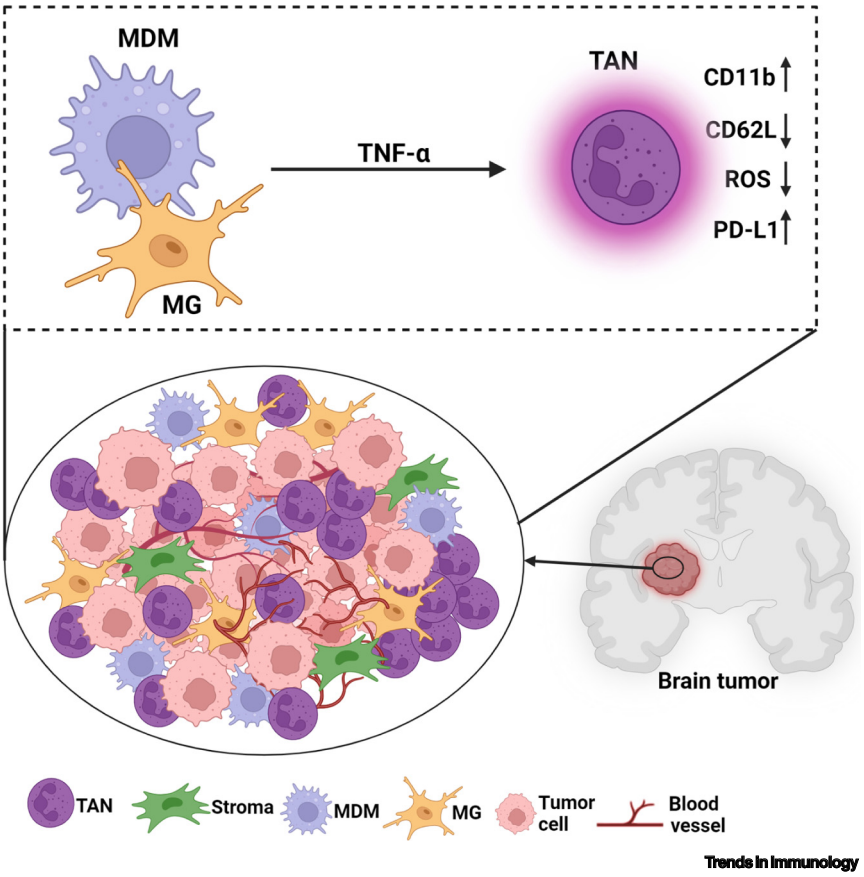

Figure 1. Tumor-associated myeloid cells can induce proinflammatory activation of neutrophils in human brain tumors. The schematic shows the frequency and spatial arrangement of tumor cells, macrophages, microglia, and neutrophils in human brain tumors (lower panel). The interactions of monocyte-derived macrophages (MDMs) and microglia (MG) with tumor-associated neutrophils (TANs) can lead to the induction of an activated TAN phenotype via secretion of TNF-α. The frequency of cells in this diagram is based on Figure 7 from [4]. Figure created with BioRender (www.biorender.com).

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Declaration of interests

The author declares no conflict of interest.

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