

## Previews

# Macrophage-tumor cell intertwiner drives the transition into a mesenchymal-like cellular state of glioblastoma

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Macrophages are the major non-neoplastic infiltrates in the glioblastoma microenvironment. In this issue of *Cancer Cell*, Hara et al. (2021) demonstrate that macrophages induce a transition of glioblastoma cells into the mesenchymal-like cellular state associated with an increased mesenchymal program in macrophages themselves and enhanced cytotoxicity of T cells.

Remarkable progress has been made over the last decade in elucidating the origin, genomic landscape, and gene expression profile of adult glioblastoma (GBM). Using multiple high-dimensional approaches, including single-cell RNA sequencing (scRNA-seq), high degrees of both inter- and intra-tumor heterogeneity were identified. Despite these advances and aggressive therapeutic regimens, GBM continues to be an incurable malignancy. An additional critical reason that current targeted anti-neoplastic therapies fail to provide a durable response in GBM is the adaptive nature of the tumor microenvironment (TME). The majority of non-neoplastic cells in the GBM TME, both in humans and in murine GBM models, are innate immune cells called macrophages. Macrophages are comprised of cells from hematopoietic origin, including bone-marrow-derived macrophages, as well as a brain-intrinsic myeloid population called microglia. In addition to demonstrating that macrophages promote tumor progression, we have also learned that therapeutically targeting macrophages has proven to be remarkably difficult. A classic example is CSF1R inhibition, which eliminated ~95% of microglia in naive mice (Elmore et al., 2014). While initially proving effective in targeting macrophages in PDGFB-driven adult glioma mouse models (Pyonteck et al., 2013), CSF1R inhibition failed to demonstrate effectiveness in a clinical trial with unselected adult recurrent GBM patients (Butowski et al., 2016). This failure is largely

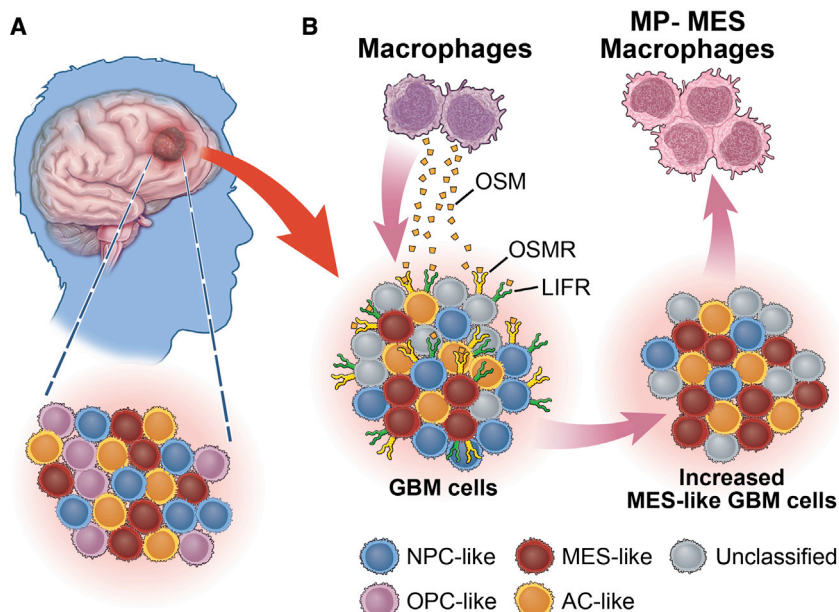
attributed to our incomplete understanding of the cellular heterogeneity of the macrophage compartment in GBM. In this regard, Hara et al. (Hara et al., 2021) leveraged a scRNA-seq platform that offers an unprecedented higher-resolution view of the tumor and immune landscape of GBM. The authors evaluated GBM cell-TME cell interactions using a combination of human tumors, mouse models, and *in vitro* validation techniques.

Previously, The Cancer Genome Atlas (TCGA) initiative using bulk RNA sequencing provided robust gene-expression-based identification of GBM subtypes, including proneural (TCGA-PN), mesenchymal (TCGA-MES), and classical (TCGA-CL) (Wang et al., 2018). These subtypes were established based on the dominant transcriptional patterns at the time and location of tumor resection and are therefore not mutually exclusive (i.e., multiple subtypes can co-exist within a single tumor, both at the regional [Sottoriva et al., 2013] and single-cell levels [Patel et al., 2014]). Recently, using scRNA-seq, malignant cells in GBM were cataloged into four potentially plastic cellular states: neural progenitor-like (NPC-like), oligodendrocyte progenitor-like (OPC-like), astrocyte-like (AC-like), and mesenchymal-like (MES-like) (Nefitel et al., 2019) (Figure 1A). From the aforementioned four states, the MES-like state is very distinct and shows limited association to cell types detected in a healthy human brain. In this current manuscript, the authors demonstrate that the MES-like state shows striking similarities to

the TCGA-MES subtype; both are enriched with macrophages and demonstrate increased T cell presence. To determine whether there is a causal link between increased macrophage presence and the MES-like state in GBM, the authors use mouse models of GBM driven by the expression of *Hras*<sup>G12V</sup> in combination with silencing of *Trp53*. First, they establish that with minor differences compared to human GBM, the four cellular states are recapitulated in mouse models, and macrophages are enriched in the environment of MES-like GBM cells. By treating tumor-bearing mice with clodronate liposomes to eliminate macrophages, they show there are both decreased numbers of macrophages and MES-like cells in tumors. Based on these results, the authors conclude that macrophages induce the MES-like state in GBM. Further, they identify macrophage-derived Oncostatin M (OSM) as a critical driver of the MES-like state. Macrophage-derived OSM, via binding to its receptors OSMR and LIFR in complex with GP130 on GBM cells, induces the activation of STAT3 signaling, which leads to transition into a MES-like state, which in turn induces mesenchymal programs in macrophages themselves (MP-MES macrophages), suggesting the presence of a strong reciprocal loop (Figure 1B). In addition, the GBM MES-like state is also associated with an increased abundance and cytotoxicity of T cells.

This paper highlights the essential role of the TME, especially macrophages, in





**Figure 1. Macrophage-tumor cell reciprocal communication drives the GBM MES-like cellular state**

(A) Schematic illustration of scRNA-seq-based division of malignant GBM cells from fresh patient samples into four highly plastic cellular states: neural-progenitor-like (NPC-like), oligodendrocyte-progenitor-like (OPC-like), astrocyte-like (AC-like), and mesenchymal-like (MES-like).

(B) Macrophage-derived Oncostatin M (OSM) via binding to its receptors (OSMR and LIFR) on GBM cells induces a MES-like state in GBM cells and, interestingly, in macrophages themselves (MP-MES macrophages) *in vitro*.

shaping the MES-like cell state, and will stir active discussions in the field. The manuscript provides additional evidence to the already existing picture in the literature that, although genetically stable, macrophages can change their expression profiles based on the signals they receive from tumor cells; therefore, heterogeneity in GBM creates heterogeneity in macrophages. The results of this manuscript take it one step further and show that macrophages can shape the expression profile of tumor cells; therefore, this interaction between macrophages and tumor cells is reciprocal, at least for driving the MES-like GBM state.

Specific details in the study should be kept in mind. Interestingly, the mouse model used across the study is *Hras*<sup>G12V</sup> driven, a rare genetic alteration in adult human GBM. Yet it closely recapitulates the four states seen in human GBM cells. This suggests that states are remarkably broad. The myeloid compartment is primarily affected by dexamethasone treatment (Herting et al., 2019); how this would impact immune-related scRNA sequencing data interpretation

from fresh GBM patient samples remains to be determined, as nearly all patients receive dexamethasone after diagnosis. It is also interesting that MES-like cells are also observed in tumor spheroid *in vitro* models in the absence of macrophages (Hara et al., 2021). This raises the question of whether multiple mechanisms contribute to the MES-like state, such as driver mutations and TME-derived factors. In that regard, it is documented that *NF1* gene deletion in tumor cells can increase macrophage infiltration in both human and murine GBM models (Chen et al., 2020; Wang et al., 2018). It would be interesting for future complementary studies to provide evidence demonstrating the pre-clinical significance of the authors' findings, by showing whether targeting the OSM/OSMR interaction affects survival time of tumor-bearing mice. The same notion goes for clodronate liposome depletion experiments, where the authors show decreased macrophages and MES-like cell presence in tumors; the effects of these observations on survival of tumor-bearing mice and different myeloid cell

subsets, including neutrophil recruitment, would be informative. The authors raise an important observation from their data that MP-macrophages show a decrease of *CSF1R* and *CSF3R* expression, suggesting that targeting this pathway may not be optimal for MP-MES macrophages. This is an essential observation in light of a recent failed clinical trial with an anti-CSFR1 inhibitor (Butowski et al., 2016). The study raises several intriguing questions: Do we target MP-MES macrophage interactions with tumor cells? And if so, what would happen to the increased number of cytotoxic T cells that are desired to have, and what would be the outcome on survival? Do macrophages have a similar role in other cellular states? Most importantly, it leaves us wondering what to do next: target macrophages or their signals associated with certain cellular states or search for pan-macrophage therapeutic targets that will work in all GBM-cell states?

In summary, the manuscript by Hara et al. significantly contributes to our knowledge of macrophage heterogeneity in GBM and opens an avenue for new studies that we should further investigate, including the following: (1) define the molecular and functional diversity of the myeloid compartment of GBM; (2) determine how distinct myeloid subsets and myeloid-specific genes influence tumor growth, various GBM cells, and TME states; and (3) provide insight into how myeloid subsets influence, and are influenced by, adult GBM-specific driver mutations. Moving forward, our focus should now be on creating better pre-clinical platforms to validate novel identified targets using mouse models driven by human GBM-specific mutations. These pre-clinical models will allow us to develop better treatment strategies aimed at providing meaningful clinical outcomes that are informed by studies like this.

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## Molecular subtypes of upper tract urothelial cancer: Setting the stage for precision therapy

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A comprehensive genomic characterization of a large, high-quality cohort of upper tract urothelial carcinomas (UTUCs) in this issue of *Cancer Cell* reveals that UTUCs can be divided into five DNA-based molecular subtypes. Feasibility data establish that molecular subtyping can be performed non-invasively by sequencing tumor DNA in urine.

Upper tract urothelial carcinomas (UTUCs) are cancers that arise from the epithelial lining of the renal pelvis and ureter. They are less common than urothelial cancers of the bladder (representing 5%–10% of total cases), but they are considered more clinically aggressive—60% of patients present with invasive disease as compared to 15%–25% of patients with bladder cancer. There are also important differences in the etiologies of bladder and upper tract urothelial cancers. Up to 10% of UTUCs are associated with the germline inactivating mutations in mismatch repair genes that characterize Lynch syndrome, and the causal environmental exposures may also be different. For example, the natural product aristolochic acid, which is the causative agent in Balkan endemic nephropathy and is a constituent of some Chinese herbal

supplements, causes UTUCs but not bladder cancers (Chen et al., 2012). The paper by Ogawa and colleagues in this issue of *Cancer Cell* sheds important new light on the molecular heterogeneity and etiology of this under-studied malignancy (Fujii et al., 2021).

Genomic profiling studies have shed light on the molecular heterogeneity of UTUC, but past studies employed focused DNA panels (i.e., MSK IMPACT) (Audenet et al., 2019; Kim et al., 2020) or multi-omics approaches on relatively small cohorts of tumors (Robinson et al., 2019), and as a consequence, no consensus UTUC molecular subtypes have been identified to date. Here, a team of investigators from three Japanese institutions performed comprehensive genomic profiling on a collection of n = 199 flash-frozen UTUCs

consisting of approximately equal numbers of low- and high-grade tumors. They conclude that UTUCs can be grouped into five DNA-based molecular subtypes—FGFR3, RAS, TP53/MDM2, hypermutated, and “triple negative” (i.e., lacking obvious *FGFR3*, *RAS*, or *TP53* pathway defects)—with significant prognostic implications, and they validated their findings in an independent UTUC cohort from Memorial Sloan Kettering Cancer Center (MSKCC) (Figure 1). Cluster of clusters analyses (COCA) based on whole-exome sequencing, copy number analyses, whole-transcriptome RNaseq, and array-based DNA methylation analyses reinforce the significance of the mutation-based subtypes, and the feasibility of identifying them by sequencing tumor DNA from urine sediment is also established. Overall, on

