The tumor microenvironment

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Contributor: Maximilian Haist, Stephan Grabbe

The tumor microenvironment (TME) is a complex and continuously evolving milieu composed of a heterogeneous assemblage of distinct cancer cells and host cells, including cancer-associated fibroblasts (CAF), endothelial cells, pericytes, immune cells, and extracellular matrix components that constitute the tumor parenchyma and tumor stroma (Beury et al., 2014; Hanahan and Weinberg, 2011). These various cell types exhibit an extensive and reciprocal crosstalk that dynamically regulates the phenotype and function of the individual cells within the TME (Haist et al., 2021). Tumor growth and mechanisms of tumor resistance are profoundly influenced by this relationship of cancer cells with their surrounding environment, making the TME an active promotor of cancer progression.

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1. Introduction

The composition of the TME varies between tumor entities, however, hallmark components include immune cells, stromal cells, blood vessels, and extracellular matrix (Anderson and Simon, 2020). These pivotal host components show a strong and reciprocal relationship, which contributes to the unique features of the TME. The significant molecular, cellular, and metabolic changes induced by cancer cells are critically reshaping the network between host components and cancer cells, which thus favors cancer cell survival, local invasion, and metastatic dissemination. In this regard it has been shown that tumor hypoxia, nutrient deprivation, and the accumulation of metabolic waste products (i.e., lactate) essentially affect the tumor network via the initiation of angiogenesis, inducing a chronic inflammatory state and reshaping host cell metabolism (Horsman and Vaupel, 2016).

2. Stromal cells and extracellular matrix

Stromal cells such as endothelial cells, fibroblasts, or adipocytes are recruited to the TME from nearby endogenous tissues in order to promote critical steps in cancer development: Once the proliferating tumor mass outgrows a volume of $2mm^3$ and diffusion-mediated oxygen and nutrient supply may no longer sufficiently supply the growing tumor mass, a hypoxic and acidic TME develops. Tumor hypoxia results in the activation of hypoxia-inducible factors, which initiate the secretion of proangiogenic factors such as platelet-derived growth factor (PDGF) or vascular endothelial growth factor (VEGF) by endothelial cells, thus resulting in the sprouting of new, but often immature and leaky blood vessels (Horsman and Vaupel, 2016). On the one hand, the immature blood vasculature may ensure nutrient and oxygen supply for the growing tumor mass, while on the other hand providing for the intravasation of cancer cells into the bloodstream due to the lack of proper cell-cell-connections. The process of tumor cell intravasation is supported by a process also called *endothelial-mesenchymal transition*, favoring the conversion of endothelial cells towards CAF being characterized by a loss of endothelial properties and cell-cell contacts, enhanced migratory activity, and cell detachment (Anderson and Simon, 2020).

Next to endothelial cells, CAF are major players within the tumor stroma facilitating the crosstalk between cancer cells and the TME. Being diverse in origin, CAF mostly arise from tissue-resident fibroblasts, but also endothelial cells or pericytes. Like myofibroblasts, which are activated by transforming-growth-factor-b (TGF-ß) in the course of wound healing, fibroblasts may convert into CAF via the secretion of TGF-ß, PDGF, or fibroblast-growth-factor 2 (FGF2) by cancer or host cells within the TME (Bent et al., 2018). CAF are important producers of extracellular matrix components, including growth factors or cytokines, and show a contractile, and secretory phenotype. Their ability to produce large amounts of TGF-ß allows for the control of the so-called *epithelial-mesenchymal transition*, which facilitates the loss of cell polarity and cell-cell adhesions in cancer cells resulting in an enhanced migratory and invasive activity. In concert with IL-1ß, CAF-derived TGF-ß may additionally promote an immunosuppressive phenotype within the TME and enhance neoangiogenesis. Thus, CAF crucially contribute to tumor progression, cancer cell proliferation, and metastasis via remodeling of the extracellular matrix (ECM), promoting neoangiogenesis and enhancing immunosuppression within the TME. Although the concerted

build-up of CAF and extracellular matrix molecules are often associated with poor prognosis (i.e., colorectal cancer or melanoma), a dense fibrous tissue may also serve as a positive prognostic marker in other tumor entities (i.e., lung cancer). The negative prognostic effect has been attributed either to the immunosuppressive and invasive phenotype of CAF, the function of the ECM as a deposit for proangiogenic factors such as VEGF, TGF-ß, or PDGF, or the role of the ECM as a physical barrier for infiltrating immune cells (Anderson and Simon, 2020).

3. Immune cells

Immune cells, being another critical component of the TME, can be distinguished into adaptive, and innate immune cells. The relationship between immune cells, cancer cells, and stromal cells is strongly affected by the surrounding TME. Hence, immune cells may either suppress tumor growth or promote tumorigenesis depending on the presence of growth factors, cytokines, chemokines, oxygen, or nutrients In the context of immunotherapy, the mutual interactions of tumor-infiltrating immune cells have become an increasingly important area of research, as these cells shape the unique properties of the TME (Harder et al., 2019).

The tumor-infiltrating immune cells include both tumor-promoting, as well as tumor-killing subclasses (Hanahan and Weinberg, 2011). Here, it has been shown that tumor infiltration by T cells (mainly CTL) and NK-cells correlates with overall prognosis and with the response to ICI treatment (Lanitis et al., 2017). However, in the course of tumor development, a chronic inflammatory state is frequently being induced, which includes the elevation of proinflammatory mediators, the infiltration of regulatory immune cells, and the recruitment of endothelial cells and fibroblasts (Noy and Pollard, 2014; Wang and DuBois, 2015). The accumulation of both pro-inflammatory mediators, including cytokines (e.g. interleukin; IL-1, IL-6, tumor-necrosis-factor-alpha; TNF-a), chemokines (CC-chemokine ligand 2 [CCL2], and C-X-C motif ligand 2; [CXCL-2]), prostaglandins (prostaglandin E2 [PGE2]) and growth factors (e.g. TGF-ß, granulocyte-macrophage colony-stimulating factor [GM-CSF]), orchestrate the crosstalk between the various cells within the TME. In concert with these soluble mediators, cell-cell-based interactions such as the programmed death protein (PD)-1/ PD-ligand (L)-1-axis contribute to the intense crosstalk between the immunosuppressive cell populations, subsequently enhancing the tumor supporting-capacity of the TME, which tips the scale towards immunosuppression and tumor angiogenesis (Wang and DuBois, 2015). Altogether, these mechanisms antagonize the cancer-directed immune responses and effectively impair the lytic machinery of TIL in the TME (Lindau et al., 2013; Umansky et al., 2014).

Notably, MDSC, TAM, and Treg are the major cellular components of the immunosuppressive TME. It has been demonstrated that the release of pro-inflammatory cytokines within the TME promotes the immunosuppressive potential of regulatory myeloid cells, such as tumor-associated neutrophils (TAN) (Rosales, 2018), TAM (Beury et al., 2014), MDSC (Ostrand-Rosenberg and Fenselau, 2018) and regulatory dendritic cells (DC) (DeVito et al., 2019; Enk et al., 1997; Liu et al., 2009). [52,53]Consequently, a strong tumor infiltration by myeloid cells - being the most abundant cell types within the TME (Schupp et al., 2019) – correlates with rapid tumor growth and a poor prognosis (Lanitis et al., 2017). Here, TAM primarily serve to promote tumor growth and progression via the generation of angiogenetic factors such as VEGF, and the secretion of immunomodulatory cytokines (e.g. IL-6, IL8, and IL-10) (Lin et al., 2019). These cytokines generated by TAM and tumor cells promote an aberrant activation of myelopoiesis resulting in a defective differentiation of myeloid progenitor cells towards MDSC, which exert a strong-pro-tumor activity (Murdoch et al., 2008; Schupp et al., 2019). In particular, it has been shown, that MDSC suppress both CTL and NK-cell activity via immunomodulatory mediators, including IL-1, IL-6, reactive-oxygen-species (ROS), and nitric oxide (NO) (Gabrilovich and Nagaraj, 2009; Gabrilovich et al., 2012; Hanahan and Weinberg, 2011; Qian and Pollard, 2010). Hence, proliferation, activation, and retention of highly immunosuppressive MDSC is not only induced by the chronic inflammatory state within the TME but further enhances these conditions, thus creating a positive feedback loop (Meyer et al., 2011; Umansky et al., 2014). In this context, recent studies revealed that MDSC can modulate the *de novo* induction, development, and activation of Treg, thus further amplifying the immunosuppressive character in the TME (Lindau et al., 2013). $CD4^{+}CD25^{hi}$ Forkhead-Box-Protein P3 **(**$FoxP3)^{+}$ Treg cells are frequently found in the course of tumor progression and counteract APC activity, T cell activation, and anti-tumor functions of effector T cells (Teff) (Lindau et al., 2013; Najafi et al., 2019). Therefore, similar to MDSC, clinical reports confirmed a negative correlation between the frequency of Treg, the patient´s individual prognosis, and the response to ICI treatment (Lindau et al., 2013). Next to their direct immunosuppressive effects, MDSC and Treg implicitly contribute to the establishment of a TME being characterized by hypoxia, the accumulation of lactic acid, and adenosine (ADO). These factors prevent APC maturation, impair Teff functions, and thus counteract tumoricidal functions of activated immune effector cells (Corzo et al., 2010; Hanahan and Weinberg, 2011; Lindau et al., 2013; Schupp et al., 2019).

In summary, the TME reflects the highly heterogeneous character of solid tumors, which are not only a group of cancer cells, but rather a complex and diverse assemblage of infiltrating and resident host cells coordinated by secreted factors, cell-cell-contacts, and extracellular matrix components. Whether the individual TME exhibits an anti-tumor immune

phenotype favoring an efficient anti-tumor immune response or rather shows an immunosuppressive character depends on the exact composition of cells infiltrating the TME, the presence and context of cell-cell contacts, and soluble mediators such as cytokines, chemokines or growth factors, as well as the crosstalk between these various members of the TME. Assessing these unique characteristics of the TME may help to identify new therapeutic targets and strategies suited to the individual mechanisms of tumor resistance and thus overcome current limitations in cancer therapy.
