



Role of Various Immune Cells in the Tumor Microenvironment

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Abstract: The tumor microenvironment (TME) has numerous promotive and suppressive effects on tumor growth. The TME harbors adaptive and innate immune cells such as T cells, B cells, natural killer (NK) cells, macrophages, neutrophils, and dendritic cells. The development, survival, and invasion of tumor cells at the tumor site are accompanied by various interactions with several biomolecules and cells. Identification of cells and molecules, differences between their roles in classical immune system and those that operate in the TME enhances the understanding of biological principles governing cancer cell mobility and plasticity to survive in adverse conditions against various defensive immune cells. Understanding the role of cellular and non-cellular components of TME in tumor growth and metastasis (pro and anti-tumor) is essential in designing appropriate molecular targeted therapies. Possibly, the accumulated knowledge would allow us to develop strategies to overcome major therapeutic barriers that operate in the TME such as cancer immune escape, stromal challenges, antigen escape, and invasion regulated by genetic and epigenetic modifications in the tumor cells and immunosuppressive TME. There are still many unknowns about the diversity, plasticity, modulators of adaptive and innate immune cells, and the mechanisms of cancer escape from immune cell surveillance. This review accounts for current understanding of immune cells' function and their cross interaction in the TME.

Keywords: Tumor microenvironment; Immune cells; Solid tumors; Chronic inflammation; Immune suppression; Tumorigenesis.

Introduction

Solid tumors are one of the most common forms of tumors that account for higher rate of mortality and morbidity globally^[1]. The tumor cells are promiscuous cells that invade within organized cell mass and promote their growth and survival through various signaling pathways that cause alterations at cellular and molecular levels^[2]. Primarily, genetic, and epigenetic changes in the cancerous cells and components of TME play a critical role in tumor formation and progression^[3]. Therefore, TME plays an important role in neoplastic progression and immune evasion. The TME consists of different cell types such as tumor cells, stromal cells, vascular endothelial cells, immune cells including T cells, B cells, natural killer (NK) cells, macrophages, neutrophils, myeloid - derived suppressor cells (MDSCs) dendritic cells (DCs) and various molecules secreted by these components^[4]. Multiple factors that are anti- or pro-tumor are found to be associated with tumor progression. At the early phase of tumorigenesis, survival of pro-tumorigenic cells, growth of the tumor, and invasion in nearby tissues emerge within the TME that encourages metastasis fur-

ther. The selective entities such as elevation in cancer-associated fibroblasts (CAFs), degradation of extracellular matrix (ECM), and immune escape cumulatively aid in the cancer progression. In the TME uncontrolled cell proliferation occurs; increases the oxygen demand much more than that of supplied by the blood vessels and makes the TME hypoxic^[5,6]. The hypoxia in TME can lead to the alternation in gene expression, increase in autophagy and inhibition of apoptosis^[7,8]. In the progressive phase, the TME favours angiogenesis to meet the oxygen and nutrient requirements. Moreover, adaptive and innate immune cells can also either inhibit or promote tumor progression. The suppression of immune inflammatory response can provide suitable microenvironment for tumor growth and progression. The role of the immune cells in tumor progression depends principally on the level and type of cytokines (pro or anti-inflammatory) secreted by Th1 and Th2 type immune cells. It is also notable that intense inflammatory immune response can harm the other host immune mediators and can provoke tumorigenesis, however underlying mechanism remain elusive^[9]. In principle there is a complex interaction among tumor cells, immune cells, and their non-cellular components. To this end, various factors such as chronic inflammation, angiogenesis and immune suppression may contribute to the tumor development. As immune cells play an important role in the TME, studying their role will improve our ability to guide immunotherapeutic responsiveness and to find novel therapeutic targets.

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Tumor microenvironment

The TME can be defined as an environment around a tumor that

consists immune cells, fibroblasts, blood vessels, signalling molecules and ECM. The tumors interact with its TME constantly and can influence the microenvironment by releasing extracellular signals, promoting angiogenesis and immune tolerance. Tumor is a well-defined multistage abnormal cellular mass, escalating from a mutated cell to organ level through metastasis into the nearby stroma, parenchyma of a target organ, vessels, and lymph nodes. Each transition involved in tumor progression is governed by the microenvironment of benign tumors. To this end, circulating and disseminating tumor cells can grow, survive, and invade new sites in several organs^[10,11]. These cells may show dormancy leading to a late diagnosis of the primary tumor. Angiogenic dormancy has been also identified as a consequence of micro metastases that hold cancer succession due to balanced apoptosis and homeostasis^[12]. Hence, a lot of alterations are expected in the TME during metastasis.

The process of being turned malignant tumor is instructed by cancer cells to endorse cancerous significance in terms of responding to intrinsic as well as extrinsic factors. The TME serves distinctly at each level of the tumor and in various aspects such as immune cell content, metabolic pathway, pH, oxygen consumption, and mechanistic activity^[13]. Assessing the microenvironment and crosstalk among communicator molecules such as chemokines, cytokines etc. may provide better direction towards the development of newer therapeutic approaches. The cellular and non-cellular components that reside in the TME corresponds to different type of tumors based on the specificity of extracellular matrices, immune cells, signaling pathways, stromal cells, metabolites, and blood vessels. The TME has an operative role in the advancement of cancer^[14]. Initially, an active and complementary association helps tumor growth and establishment of the TME that further aids in the survival of cancer cells, promotes local invasion, and aggressive metastasis. In the course, the TME organizes angiogenesis to conquer the hypoxic and acidic microenvironment and further to restore oxygen, nutrients supply and to remove metabolic waste. Various adaptive and innate immune cells infiltrate into TME and can exert both pro and anti-tumorigenic effects. Therefore, a thorough knowledge on TME might be helpful in therapeutic intervention.

Hypoxic and acidic niche

The increased glycolytic metabolites, lowering of pH and induction of ROS due to altered metabolism upregulate the HIF (hypoxia inducible factor) in majority of tumors and influence nearby cells as well. Usually, HIF-1 and HIF-2 are responsible for acute and chronic hypoxia respectively^[15]. The function and stability of HIF is modulated by P53 and MDM2 protein interactions. The hypoxic tumor microenvironment serves as a chronic hypoxic condition which has tumor suppressing effects and it deactivates CAFs. Moreover, HIF-2 modulation can limit the vascularization in TME^[16]. A wide range of diversity has been observed among hypoxia-associated genes (VEGFA, SLC2A1, PGAM1, ENO1, LDHA, TP11, P4HA1, MRPS1, CDKN3, ADM, NDRG1, TUBB6, ALDOA, MIF, and ACOT7) and can be anticipated both for prognostic stratification of patients and development of novel therapies^[17].

The severity of hypoxia varies with cancer type as lung and

cervical squamous cell carcinoma are most hypoxic while chronic lymphocytic leukaemia and thyroid adenocarcinoma are least hypoxic. Few other types of cancer have been also reported with variable range of hypoxia incident like biliary adenocarcinoma, lymphoid B-cell non-Hodgkin's lymphoma, and lung adenocarcinoma^[18]. Further, the increase in lactate formation ultimately leads to either metabolization or CO₂ hydration^[19] and induction of hypoxia which may directly proportionate to decrease in intracellular pH which induces proliferation and metastasis in TME with restricted apoptosis. In the normal cells, the higher and lower pH ranges between 7.4–7.2 however, cancer cells can have a pH greater than 7.4 or the pH can also drop down to around 6.7. These differences in pH create a perfect storm for metastatic progression and play essential role in evasion of apoptosis^[20]. Further, lactate along with proton (H⁺) is carried through MCT (mono carboxylate transporter) and transferred into cancer cells cause acidosis which in turn promotes metastasis. In 2008, Sonveaux et al. studied lactate-fuelled respiration within tumor cells and suggested lactate-based metabolic symbiosis for the first time^[21]. EMT (epithelial to mesenchymal transition), upregulation of oncogenes (Ras and Myc), downregulation of tumor suppressor gene (p53) and invasion of melanoma cells are evidences of acidic niche as also seen in breast cancer and neuroblastoma. Decrease in pH cause macrophage polarization toward tumor promoting M2 phenotype, stimulate neutrophils or DCs and restrict cytotoxic activity of TILs (Tumor Infiltrating Lymphocyte)^[20,22]. The V-ATPase (Vacuolar-type H⁺-ATPase) may also mediate alkaline intracellular environment as inhibition of p38 MAPK or V-ATPase is reported for metastasis restriction and multidrug resistance^[23].

Immune microenvironment

The tumor formation initiates in a microenvironment that contains both cellular as well as non-cellular components that act both as tumor promoters as well as suppressors. The tumor microenvironment contains immune cells such as T cells, B cells, NK cells, TAMs (tumor-associated macrophages), MDSCs, mast cells, granulocytes, DCs, and tumor-associated neutrophils^[2]. Mesenchymal stem cells derived from EMT reside in the primary TME and are known to exhibit immunosuppressive property, but it can also serve as an APC to present HLA class I restricted peptides with reduced efficiency. The reduced efficiency is the result of defects in several APM (antigen processing machinery) components^[24]. It has been well documented by several studies that chronic inflammation plays a role in tumor progression. Among the various immune mediators for chronic inflammation the macrophages account for 30–50% of the total tumor mass. The macrophages can dominantly home into the TME and can secrete a variety of inflammatory cytokines such as TGF-β, IL-6, IL-10, and TNF-α, which promote EMT and enhance the stemness of cancer cells^[25]. Thus, the EMT happens by the support of factors like cancer-associated macrophage (CAMs) mediated cytokine secretion and metastasis provoked by motile mesenchymal-like carcinoma cells in TME^[26].

Immune cells exert their function directly in TME. Most of the immune cells express both anti and pro-tumorigenic property, for example helper T cells produce cytokines (TNF-α, IL-12, IL-17,

IL-18, IL-21, and IL-27) that acts as anti-tumor whereas the transformation of Th17 lymphocytes into Treg lymphocytes exhibits pro-tumor activity. The IL-2 produced by B cell exhibits anti-tumor properties whereas IL-10 and TGF- β are pro-tumorigenic. Neutrophils are known for producing anti-tumor factors like TNF- α , and IFN- γ but also reported to produce pro-tumor factors like MPO, MMP9, HGF, and VEGF^[27]. So, malignant cells escape from the immune cells as they can acquire potential metastasis after ECM remodelling and develops pre-metastatic niche where several immunosuppressive factors such as CD4 + CD25 + FOXP3 + regulatory T (Treg) cells, Ly6G + neutrophils, MDSCs play a role but at same time Th1 CD4⁺ or CD8⁺ T cells, Ly6G⁺ neutrophils and NK cells encourage anti-cancerous acts^[28]. Furthermore, the reduction of NK cells was also observed in primary lung adenocarcinomas and lung cancer metastases compared to normal lung tissues; suggesting that TME allows decreased survival or homing of NK cells at the tumor site^[29].

Metabolic microenvironment

In cancer, there is an enhanced requirement of nutrient supply, a switch between complete oxidation and incomplete oxidation, an escalation of glucose metabolism and elevated lipid and amino acid metabolism. Lactate and ROS levels are elevated inside malignant and stromal cells. According to the Warburg effect, to meet the increased energy consumption, cancer cell evolve from oxidative phosphorylation or complete oxidation to enhanced glycolysis and lactate production and metabolism, at the cost of oxygen content^[30]. TME contains increased lactate because of anaerobic glycolysis. Later, lactate are used in tricarboxylic (TCA) acid cycle for glucose reformation and transamination which actively synthesizes aspartate and glutamate with α -ketoglutarate. All of these processes provide evidence of the programming of a new metabolic series in the TME^[31]. The effect of lactate enriched microenvironment ultimately supports cancer progression in various ways, such as increasing macrophage polarization, which leads to proinflammatory and tumorigenic effect, and survival of Tregs (Regulatory T cells) through expression of *Foxp3* and suppression of *Myc*^[32]. Similarly, glutamine helps in survival of cancer cell in terms of providing carbon, nitrogen, energy, and protein uptake through macro-pinocytosis activated by RAS signalling. It has been reported that pyruvate mediated collagen hydroxylation (not collagen synthesis), regulated by α -ketoglutarate in breast cancer cells, contributes to ECM remodelling and can reprogram the lung metastatic niche^[33].

As discussed, HIF being upregulated in TME, mitochondrial ROS is also elevated to stabilize the HIF and consequently support tumorigenicity. ROS induces autophagy to maintain homeostasis in the microenvironment, which may allow cancer cell survival under stress by deregulating cell death progression. Although autophagy maintains apoptosis, ROS mediated autophagy promotes tumor survival within the TME. ROS-mediated autophagy induces several mechanisms such as response to hypoxic circumvent, antioxidant enzyme activity, and protein modification of tumor suppressors such as p53 and TGF- β 1. These mechanisms activate Ras-mediated signalling, and promote EMT transition, leading to reduced effectiveness against anti-tumor drugs. To better understand ROS-mediated autophagy, it is im-

portant to consider the redox regulation potential of autophagy, its susceptibility to posttranslational modification and associated proteins^[34]. The accumulation of oxygen radicals in TME due to ROS production affects MDSCs, TAMs, CAFs, and T cells. Immunosuppression and ineffective programmed cell death protein 1 (PD-1) therapy have been reported in mice with ovarian cancer due to oxidative stress. Interestingly, a reduced oxidative state was found to improve T cell survival and potentiate anti-tumor activity^[35,36].

TME regulating cancer progression is also accompanied by lipid metabolism. Cholesterol in TME increases the expression of various immune checkpoints in CD8 + T cells. Say for instance, it increases the expression of PD-1 in T cells. The binding of PD-1 to PD-L1 in cancer cells inhibits the cytotoxic activity of T cells. The increased expression of immune checkpoints leads to exhaustion of T cells in TME^[37]. It has been suggested that cholesterol-reducing therapy may be beneficial against ER (endoplasmic reticulum) stress caused by elevated cholesterol. Moreover, involvement of lipid metabolism in emergence of pre-metastatic niche, lymphatic metastasis, trans-coelomic seeding has been reported earlier^[38].

Mechanical microenvironment

A recently discovered mechanical microenvironment that influences the tumor includes intracellular proteins, extracellular matrices, intercellular signalling, and stromal cells as these components are known for mechanical support of cellular structure. Types of cytoskeletal elements namely microfilament, intermediate filament, and microtubules are highly integrated protein fibres in normal cells known for subcellular organization and stability of cell division and movement in context of maintaining cell shape. Reorganization of protein fibres are possible because of their plasticity which accidentally assists metastasis via distinct projection founding such as lamellipodia, filopodia, and invadopodia^[39].

Numerous factors and proteins interact with microfilaments such as actin and neurofilaments to stimulate multiple signalling cascades to attain tumor cell invasion and metastasis. For example, robust ECM remodelling has been reported for glioblastoma cell progression whereas its degradation/remodelling is promoted through interacting matrix metalloproteinases or activating yes-associated protein^[40]. Integrin signalling promotes tumor-matrix rigidity through HIF mediated oxidase generation. HIF indirectly helps in the crosslinking of collagen which in turn increases the tumor-matrix rigidity^[41]. Also, it has been demonstrated that tumor rigidity enhances metabolism rate for its maintenance and later emerge as heterogeneously specialized TME^[42].

Immunophenotype of tumor microenvironment

Cancer cells escape normal cell cycle and undergo uncontrolled cell division, leading to the formation of benign or malignant tumors. Tumor immune profile may vary based on its interaction with the immune system and can be categorized into three types as follows: (I) tumors, invaded by T lymphocytes and inflammatory factors are called a hot tumors, (II) tumors, invaded by low number of immune cell are called cold tumors and (III) tumors

with immune cells at the periphery but without deeper infiltration are called intermediate tumors^[27]. Inflammatory or hot tumors constitute B lymphocytes, helper T cells (CD4⁺), cytotoxic T cells (CD8⁺), Treg lymphocytes, macrophages, fibroblasts, MDSC, intra-tumor chemokines (CXCL9, CXCL10, and CCL5)^[43]. While, IFN- γ is known for its anti-tumor effect, other factors such as IDO (indole 2, 3-dioxygenase), PD-1 and PD-L1 can induce immunosuppression in the TME. Among various signalling, CD28 interact with CD80 and CD86 present on surface of tumor cells to stimulate T cells whereas interaction of CTLA-4 with CD28 induces T cell depletion. Overall, inflammatory tumors show significant dense PD-1-positive T lymphocyte invasion^[44,45]. Another concept suggests that T lymphocytes are unable to penetrate the tumor parenchyma due to the presence of firm collagen fibres. Hence, no intercellular interactions were found because of compact fibroblast cells^[46,47]. Furthermore, the induction of blood vessel formation by VEGF (vascular endothelial growth factor) reduces cell adhesion. Adhesion molecules like E-selectin, vascular cell adhesion molecule (VCAM) and intercellular adhesion molecule (ICAM) are downregulated by VEGF and FGF, which subsequently initiates the Fas- FasL and restrict T lymphocytes homing in tumor. TME rich in TGF- β (tumor growth factor beta) and PD-L1 (programmed cell death ligand) molecules reduces T lymphocytes survival but activate MDSC cells. The state of impotent adherence and invasion of T lymphocytes in TME is termed as endothelial cell anergy^[48]. In this situation, immune cells can attain different immunophenotypes and heterogeneous functionality.

In a recent study, immune score of the tumor microenvironment was analysed through bioinformatics, where a competitive endogenous RNA (ceRNA) network was generated via differential analysis of the mRNA data based on cold and hot tumors. Overall, 217 samples showed differentially expressed genes (DEGs), along with ceRNA such as mRNA, tRNA, rRNA, long non coding RNA (lncRNA), miRNA, pseudogene RNA and circular RNA^[49]. They also found moderately expressed lncRNAs and miRNAs after screening^[49,50]. The regulatory role of miRNA in cancer is well known. A subset of five miRNAs, particularly hsa-mir-204, hsa-mir-128, hsa-mir-214, hsa-mir-150 and hsa-mir-338 were identified to be involved in various cancer types like melanoma, breast cancer and liver cancer. For instance, findings imply that hsa-mir-128-3p regulates sensitivity elevation in colorectal cancer cells against chemotherapy. In case of papillary thyroid carcinoma, hsa-mir-214 is responsible for proliferation and metastasis. Similarly, hsa-mir-150 and hsa-mir-338 were found to be involved in growth and invasion of colorectal cancer, non-small cell carcinoma and cervical cancer^[50].

Immune cells in the tumor microenvironment

The TME contains various type of inflammatory and immunosuppressive cells such as T cells, B cells, DCs, macrophages, neutrophils, mast cells, NK cells, Tregs and MDSCs. These cells have different role in TME; depicted in Fig.1 and summarized in Table 1.

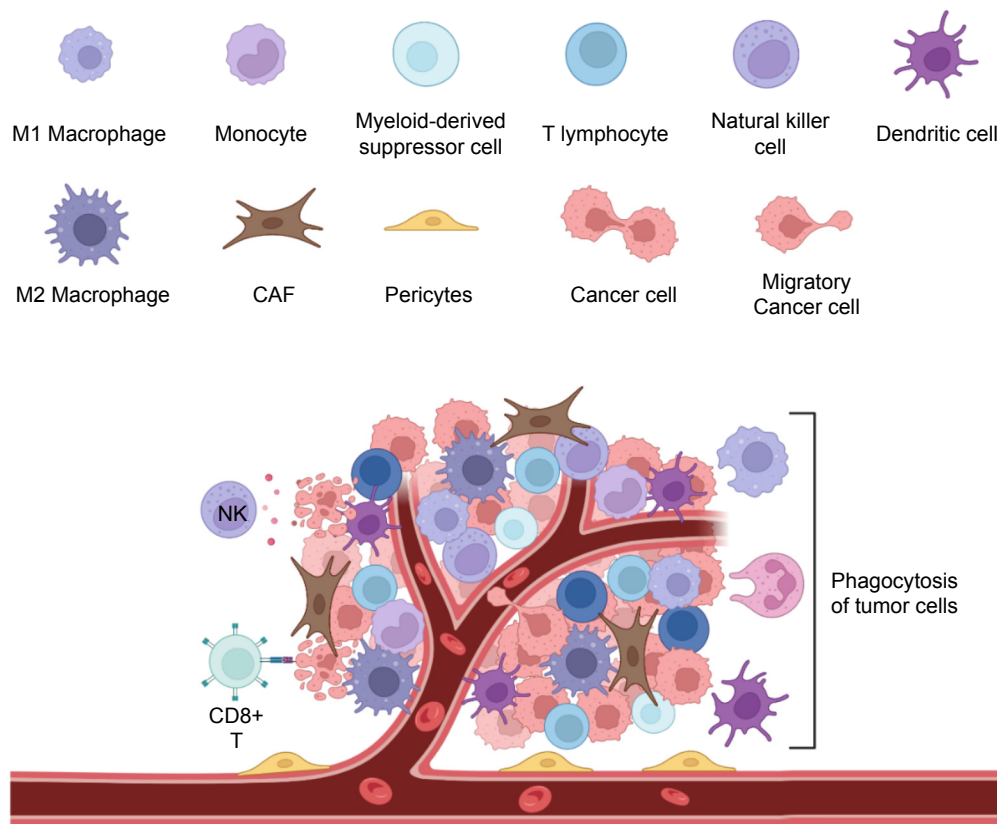


Fig. 1. Diagrammatic overview of a tumor microenvironment.

Table 1. Description on roles of immune cells in tumor microenvironment and associated regulatory products.

No.	Type of Immune Cells	Effect on Tumor		Major anti- and pro-Tumor Role in TME	References
		Anti-tumor	Pro-tumor		
1	Helper T Cells	TNF- α , IL-12, IL-17, IL-18, IL-21, IL-27	IL-10, TGF- β	Th1 express pro-inflammatory effects by secreting IL2, Th2 inhibits Th1 cells, induce M2 phenotype macrophages.	[27,51]
2	Cytotoxic T Cells	Perforins, Granzymes, IL-2, TNF- α , IFN- γ	IL-10, TGF- β	CD8 + T cells transiently express immune-checkpoint molecules to induce an immune-suppression and promotes cancer survival	[51,52]
3	Regulatory T Cells	IL-10, TGF- β , adenosine, PGE2, IL-35	IL-2, IL-10, CCL-18, PGE2, TGF- β , dIL-1R, and Eotaxin-2/CCL24	Favor cancer progression through IL-2 production, remodulate NK cell homeostasis, enhance metastatic circumvents by increasing fibroblast, endothelial and stromal cells interaction.	[9,53]
4	B Cells	IFN- γ	IL-10, TGF- β , CD20 +	Stimulate Tregs and promote angiogenesis,	[54,55]
5	NK Cells	IFN- γ , TNF- α	IL-10, TGF- β	NK cells involve in determining the infiltration and recruited cells influence immunosuppression, NK cell priming due to low cytotoxicity within tumor	[56,57]
6	Macrophages	IFN- γ , IL-12, PD-L GM-CSF	IL-10, CCL-18, PGE2, TGF- β , dIL-1R, and Eotaxin-2/CCL24	TAMs share pro-tumor features by limiting inflammatory response	[9,27]
7	Neutrophils	TNF- α , IFN- γ , CCL3, ICAM-1	TGF- β , MPO, MMP9, HGF, VEGF, CCL2, CCL3, CCL4, CCL8, CCL12, CCL17, CXCL1, CXCL2, IL-8/CXCL8, CXCL16	N1 support apoptosis and phagocytosis whereas N2 promote angiogenesis and inflammatory processes	[27,58]
8	Dendritic Cells	Type I IFN, IL-6	TGF- β 1, TNF- α , IL-10	Limits antigen presentation and its processing	[59]
9	Mast Cells	MCP-3, MCP-4, IFN α , TNF α , LTBA, IL-4, IL-6	IL-10, IL-8 FGF2, VEGF, PDGF and NGF	Enhance vascularization and invasion in tumor through IL-10, IL-8 FGF2, VEGF	[59]
10	MDSC	–	TGF- β , MMP-7/MMP-9/MMP-12	Suppress immune responses by compromising anti tumor role of neutrophils and monocyte	[60]

IL: Interleukin, Th1: helper T lymphocyte 1, Th2: helper T lymphocyte 2, TGF- β : Transforming Growth Factor beta, TNF- α : Tumor Necrosis Factor alpha, PGE2: Prostaglandin E2, PD-L: Programmed Death Ligand 1, TAM: Tumor Associated Macrophages, IFN- γ : Interferon gamma, GM-CSF: Granulocyte Macrophage Colony Stimulating Factor, HGF: Hepatocyte Growth Factor, FGF: Fibroblast Growth Factor, VEGF: Vascular Endothelial Growth factor, MMP: Matrix Metalloproteinase, CCL: Chemokines, ADCC: Antibody Dependent Cell Cytotoxicity, MPO: Myeloperoxidase, IGF: Insulin-like Growth Factor, NGF: Nerve Growth Factor, N1 and N2: subtype of neutrophil population, M1 and M2: subtype of macrophage population, dIL-1R: decoy Interleukin-1 Receptor.

Adaptive immune cells

T lymphocytes

The T cells with distinct T cell receptors reside in the TME and have both positive as well as negative effect on cancer development. Cytotoxic CD8⁺ T cells target tumor antigens of cancer. These cytotoxic cells can induce the killing of tumor cells by secreting IFN- γ ; which is also reported to have a significant role in the reduction of angiogenesis^[52]. Additionally, various subtypes of CD4⁺ T can also secrete pro-inflammatory cytokines such as IL-2 and IFN- γ ^[51]. Further, Tregs (regulatory T cells) are known to downregulate the anti-tumor effect of cytotoxic T cells by producing cytokines like TGF- β , IL-10, and IL-35. These regulatory cells can suppress the antigen presentation by DCs and functionality of CD4⁺ helper T cells, which leads to the reduction in the cytotoxic capability of CD8⁺ T cells against tumor cells. In principle, the Tregs can make the cytotoxic T cells exhausted in TME by the immunosuppressive effect of TGF- β , IL-10, and IL-35^[43]. The population of exhausted T cells featured by low proliferation, limited survival, low cytokine production, upregulated PD-1 expression, and metabolic re-wiring^[47]. Thus, it is evident that immunosuppression in TME is majorly contributed by Tregs. To this end, targeting Tregs might be an approach to cure the disease or monitor the tumor progression. Indeed, when the T cells

in TME get exhausted by the immunosuppressive effect of cytokines secreted by Tregs, they start to express exhaustion markers like PD-1/PD-L1 and CTLA-4; led to the development of immunotherapy based on immune checkpoint blockade (ICB) to achieve prolonged survival of T cells^[44,45]. In the recent past tumor invading T lymphocytes were studied extensively to understand the inflammatory process and their impact on TME of solid tumors such as SCLC (small cell lung cancer) and TNBC (triple negative breast cancer). The studies have shown that SCLC exhibited limited T cell infiltration with increased tumor proliferation^[53]. Therefore, most SCLC immunophenotype is observed as either immune-excluded or immune desert tumor^[61]. However, upregulated VEGF, TILs (tumor-infiltrating lymphocytes), and TAMs were reported in TNBC. Here, a high number of CD8⁺ and CD4⁺ T lymphocytes along with Fox P⁺ were observed in the TME^[61]. Among most solid tumors, PD-1 inhibition along with CTLA-4 blockade upregulate the T- cell priming and invasion within CD8⁺ rich TME but among few patients, the checkpoint blockade was not sufficient to induce T cell infiltration^[62].

B lymphocytes

B lymphocytes are cells of lymphoid origin known to produce antibodies, acts as antigen-presenting cells (APCs) and can secrete

cytokines. B cells can exert multiple functions that may have positive or negative effects on cellular immunity. These cells can positively regulate the cellular immune response by acting as APCs or by providing the necessary co-stimulatory signal to T cells^[63,64]. Furthermore, the B cells have been reported to negatively regulate the inflammation and cellular immune response by producing IL-10^[54,65]. Therefore, the B cells can affect tumor immunity either positively or negatively. In principle these antigen-presenting B cells can also induce tumor-specific cytotoxic T-cell response as well^[66]. It has been well documented that mice depleted of B cells do not develop immunity towards virus-induced tumors^[67,68]. Published reports also suggest that B cells can occasionally infiltrate the tumor site; usually, they remain localized at invasive margins of the tumors or predominantly home into the draining lymph nodes and tertiary lymphoid structures (TLS)^[46]. Murine model-based studies also suggested that B cells can increase T cell functionality. Moreover, CD20⁺ B cells are found to be associated with a good prognosis of human cancers such as ovarian, cervical, and non-small cell lung cancer (NSCLC)^[69-71]. However, published reports also suggest that B cells contribute to the downregulation of anti-tumor immune response in case of squamous carcinogenesis^[55]. Furthermore, immunotherapeutic success in prostate cancer requires the elimination of IL-10 and PD-1 secreting B cells; which contribute to the exhaustion of cytotoxic T cells^[72].

Innate Immune cells

Natural killer cells

NK cells cooperate in cell-mediated pathways and release inflammatory cytokines to kill tumor cells and inhibit metastasis. The mechanism of tumor killing via NK cells includes death receptors (Fas- FasL and TRAIL), perforins, granules, and the release of cytokines such as IFN- γ and TNF- α ^[56].

Across TME, less NK cell invasion has been reported than their corresponding normal or healthy tissue^[73]. For example, in lung cancer^[74], renal cell carcinoma^[75], colorectal carcinoma^[76], and gastrointestinal stromal tumors (GIST), NK cells are often localized throughout the tumor and can move out the tumor site through stroma^[77]. The chemokine production within TME and the expression of corresponding receptors on NK cells are the key determining factors for infiltration. A TGF- β dependent mechanism limits the production of ligands corresponding to CD56^{dim} NK cells' receptors (CXCL1, CXCL2, CX3CL1, and CXCL8)^[78,79] and escalates the ligands corresponding to CD56^{bright} NK cells' (CXCL9, CXCL10, CCL19, and CCL5) in the tumor^[80]. In-situ apoptosis of intratumoral NK cells can be another reason behind fewer NK cell infiltration because apoptosis-associated genes were found to be overexpressed in intra-tumoral NK cells of lung and hepatic cancers^[81,82]. Many studies suggest that the dysfunctionality of intra-tumoral NK cells is influenced by immunosuppressive microenvironment induced by IDO, prostaglandin E2, IL-10, and TGF- β to deregulate the functioning of NK cell receptors^[57]. The NK cells in the TME are deprived of glucose as there is an accelerated glucose uptake by tumor cells. Low glucose level inhibits MTORC1 (a primary regulator for NK cell metabolism) which in turn diminishes the cytotoxic activity of CD3- CD56^{dim} CD16 + cells by inhibition of INF-

γ production. In addition, reduced glucose levels cause an increase in lactate uptake by the cells. Intracellular lactate decreases intracellular pH levels and ATP generation. This leads to ROS accumulation and mitochondrial stress. The increased ROS content into the TME can suppress the anti-tumor activity of NK cells. Based on The Cancer Genome Atlas (TCGA) database analysis, researchers have reported, a higher ROS score as well as favoured IL-15 primed therapy among smokers compared to non-smoking NSCLC patients. through. IL-15 is established as a prognosis marker. It is observed that activated Trx⁺ NK cells resided in the core of solid tumors and induce anti-tumoral effect and target ROS-rich TME. This mechanism involves nuclear TXNIP transfer to the cytoplasm and increased thiol molecule to protect the immune cells in TME as well as the enhanced ability of NK cells to invade solid tumor through IL-15 priming^[83]. Various factors influence immunosuppressive TME negatively through impairment of NK cell priming, metabolism, and antitumor responses. CB-839 has been identified as a possible molecule to stop glutaminolysis without affecting NK cell cytotoxicity. The blocking of adenosine receptor with A2aR antagonists, help NK cells attain maturation, activation, and cytokine production in TME^[84].

Macrophages

Monocyte derived macrophages can adopt either pro-tumorigenic (M2 subtype) or anti-tumorigenic (M1 subtype) phenotypes depending on the signals they receive from the TME. phenotype they acquire. The TME favours the switch of M1 to M2 phenotype under the influence of cytokines and hypoxic conditions. The reverse process can also occur when TAMs interfere with the NF- κ B pathway. The balance between the subtypes determines the macrophage phenotype to be expressed in cancer^[85]. Excess macrophage infiltration into the TME is associated with angiogenesis and poor patient prognosis^[86].

Macrophages that assist in tumor progression are called pro-tumorigenic TAMs and they originate from various sources. F4/80⁺ macrophages come from the yolk sac, low F4/80 macrophages come from the bone marrow^[87,88], Langerhans cells come from fetal liver and few develop through extra medullary haematopoiesis^[89]. However, majority of them are believed to be derived from circulating monocytes. Additionally, tissue-resident macrophages (TRMs) arise from embryonic progenitors as well as from primary circulating monocytes, exhibiting distinct phenotypes. It has been reported that in ovarian cancer metastasis, a subset of CD163⁺ Tim4⁺ TRMs can reduce the spread of ovarian cancer cells^[90]. Similar results were observed in a study of lung carcinoma^[91]. Furthermore, a meta-analysis showed that TAMs load among patients was associated with 1.5-fold increased mortality^[92]. As per clinical-pathology, TAM density is directly proportional to the progressive tumor stage. Large tumor size and vascular invasion has been detected with elevated TAMs density in breast cancer^[93]. Altogether, TAMs are suggested as strong diagnostic as well as prognostic markers for cancer^[94].

TAMs are involved in three major events including tumor angiogenesis, chronic inflammation, and immune suppression. TAMs produce cytokines and growth factors such as VEGF and TGF - β , that promote angiogenesis^[95]. TAMs actively modulate proteases like MMP - 9, which can activate ECM budding, VE-

GF activation, and endothelial cell invasion^[96]. TAMs produce VEGF, PDGF, TGF- β , and FGF, which can induce tissue remodelling in TME for tumor growth. M-CSF (Macrophage colony-stimulating factor) helps promote the M2-like macrophage phenotype and attracts Th2 cells and regulatory T cells, which can suppress T-cells activity in tumor niche via the release of IL-10, CCL-18, and PGE2, TGF- β , dIL-1R, and Eotaxin-2/CCL24. Moreover, TAM-mediated PD-L1 and PD-L2 expression highly participate in the suppression of antitumor activity in the TME^[9]. The M1-like macrophages elevate glycolytic metabolism and ROS generation while M2-like macrophages engage in oxidative metabolism to assist tissue repair. This functional plasticity enables macrophages to maintain homeostasis as well as immune remodelling^[97].

Neutrophils

Neutrophils constitute the maximum number of cells among all circulating white blood cells and are known to respond at the earliest against invading pathogens. These immune cells exhibit both pro-tumor and anti-tumor effects depending on the type and stage of cancer. Based on cytokine stimulation, neutrophils can polarize to anti-tumor (N1) or protumor (N2)^[98-100]. Fridlender et al., 2009, suggested that pro-tumorigenic (N2) neutrophils phenotype polarization is stimulated through immunosuppressive cytokines like TGF- β and IL-10 and their depletion limits tumor growth in mouse models^[99]. On the other hand, the polarization of anti-tumorigenic (N1) neutrophils phenotype causes them to accumulate in TME through TGF- β inhibition and type 1 interferons (IFNs) stimulation. Immune profile of N1 and N2 tumor-associated neutrophils (TANs) express high levels of TNF α , CCL3, ICAM-1 and the chemokines CCL2, CCL3, CCL4, CCL8, CCL12, CCL17, CXCL1, CXCL2, IL-8/CXCL8, CXCL16, respectively^[58]. Also, for tumor progression, neutrophil extracellular traps (NETs) exhibit functional as well as phenotypic exhaustion on T cells through various mechanisms such as PD-L1/PD-1 interaction that limits cytokine production and failure in evading cancer^[101].

The tumor-secreted protease cathepsin C (CTSC) was identified in both neutrophil recruitment and NETs induction at the tumoral niche. It has been reported that metastasis to the lung from breast cancer is inhibited by CTSC inhibition in mice. This protease catalyses neutrophil membrane-bound proteinase 3 (PR3) activation for interleukin-1b (IL-1b) and nuclear factor-kB activity which subsequently upregulate neutrophil recruitment through IL-6 and CCL3. Further, this mechanism involves neutrophil-mediated ROS production through CTSC-PR3-IL-1 β stimulation that develops NETs and degrades thrombospondin-1 to assist metastasis toward the lungs. In a recent study, AZD7986 compound has been proved to be effective against CTSC targeted in mice model which implies that neutrophil-associated inflammatory microenvironment was the major cause behind lung metastasis of breast cancer^[102].

Dendritic cells

Dendritic cells mediate communication among innate and adaptive immune cells and act as Antigen Presenting Cells (APCs) to promote pathogen-specific T-cell response. The DCs are believed to involve in anti-tumor function within the TME but they

may also participate in tumor progression through the stimulus of tumor-supporting cytokines. Myeloid cells generate various subsets of DCs and exhibit unique morphology and immunological features such as antigen presentation and processing, CD8⁺ T cells priming and humoral immunity^[103]. DCs are mainly categorized as conventional DCs (cDCs), plasmacytoid DCs (pDCs), and monocyte-derived DCs (moDC). The cDCs initially activate CD4⁺ T cells and induce the antitumor effect of CD8⁺ T cells but are also reported to exert tolerance against tumor antigens within the TME. Similarly, pDCs also exert tolerance, otherwise they induce antitumor effects by type I IFN production, and moDCs act according to a stimulus received within the microenvironment^[104].

The two subsets of cDCs namely cDC1 and cDC2 cells reside in TME and induce antigen uptake, PD-L1 expression, and IL-12 downregulation mediated through receptor tyrosine kinase AXL pathway and by IL-4 signalling^[105]. Further, DCs differentiation can be induced by IL-6, gangliosides, prostanoids, and lactic acid^[104]. The signal regulatory protein alpha (SIRP α) assists DCs-mediated tumor-derived mitochondrial DNA recognition^[106]. Tumor-derived Liver X receptor alpha (LXR α) limits DCs migration through the CCR7 pathway within TME^[107]. The pDCs subsets have been observed to mediate type I IFN deregulation and IRF7 expression through TGF- β 1 and TNF- α , in cases such as breast tumor cells, murine lung tumor and melanoma models. The moDCs produce comparatively high IL-10 and low IL-12 rather than cDC subsets. These cells can efficiently uptake the tumor antigens but immune suppressive effects result due to insufficient T cell stimulation by regulation of nitric-oxide pathway and PD-L1 expression. Hence, the exhibition of anti-tumor or pro-tumor effects depends on factors present in the TME^[104].

Mast cells

Mast cells are tissue-residing leukocytes derived from multipotent progenitors in the bone marrow. Mast cells produce various cytokines and growth factors that can either promote or inhibit tumor growth and modulate the TEM. These cells enhance vascularization in tumors with numerous angiogenic factors such as VEGFA, VEGFB, VEGFC and VEGFD. Mast cells exhibit protumoral effects due to high expression of IL-10, IL-8, FGF2, VEGF, PDGF (platelet-derived growth factor), and NGF (nerve growth factor). The secretion of MCP-3 and MCP-4 (monocyte chemoattractant protein-3 and -4), histamine, IFN- α (interferon alpha), TGF- β , TNF- α , LTB-4, chymase, IL-2, IL-4, IL-6 and with low expression of IL-10, IL-8 exerts anti-tumoral effect^[59].

As per the pathological aspect, mast cells are involved in both IgE as well as non-IgE mediated allergic responses^[108]. Local cholangiocarcinoma (CCA) progression showed enrichment of MVD (microvascular density) in the clinical sample, promoting angiogenesis mediated through mast cells. Evidence suggests that the c-Kit/SCF pathway encourages tumor growth and invasion and their inhibition by sodium chromolybdate limits the expression of mast cell markers, CCA proliferation, and angiogenesis^[109]. The trafficking inside TME includes interaction among numerous chemokines and receptors such as LTB4 to BLT1 and BLT2, PGE2 to EP2 receptor, VEGF to VEGFR-1 and VEGFR-2, Ang1 (angiopoietin 1) to Tie2 receptor. Mast cells are attracted to chronic inflammation site by CXCL8/IL-8 interactions with CXCR1 and CXCR2 and resides there with the help of CCR2, CXCR2, and CXCR3 interaction with their correspond-

ing ligands^[110]. The plasminogen activator inhibitor-1 (PAI-1) mediated mast cell recruitment to gliomas has been studied where the level of PAI-1 is proportional to infiltration rates^[111]. Secretion of CXCL12 from glioma cell also induce mast cell chemotaxis through CXCR4 interaction^[112]. Anti-SCF and anti-c-Kit antibodies were able to limit mast cell invasion in H22 tumors (a mouse hepatocarcinoma cell line) in mice^[113].

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are produced from the common myeloid progenitors, and fall into two major categories namely, granulocytic or polymorphonuclear (PMN) MDSCs and monocytic MDSCs (M-MDSCs). Typically, neutrophils and monocytes are stimulated through the entry of bacterial as well as viral pathogens, expression of TLR ligands whereas extended cytokines production due to chronic infection, inflammation, autoimmune diseases, and cancer cause MDSCs stimulation^[114].

Tumor-derived factors recruit MDSCs to suppress host immunity within the TME through expression of cytokines (TGF- β) as well as surface molecules (PDL1 and PDL2). They promote vascularization through secreting VEGF and β FGF isoform, and tissue remodelling through tumor supportive stromal elements such as MDSC arginase-1, iNOS, and MMP-7/MMP-9/MMP-12 (metalloproteinase) activity^[60]. MDSCs phenotype may switch easily under influence of TAM in response to tumor-associated hypoxia in TME. The F4/80 marker can be used to differentiate murine intra-tumoral MDSCs, such as F4/80⁺ for PMNMDSCs, F4/80^{low}/dim for M-MDSCs and F4/80⁺ for M-MDSCs from TAM in mouse whereas CD11b⁺CD33⁺CD14⁺HLA-DR^{lo} and CD11b⁺CD33⁺CD15⁺CD66⁺HLA-DR^{lo} are a marker for M-MDSC and PMN-MDSC, respectively^[115].

The role of MDSCs in the formation of the pre-metastatic niche has been well documented. The PMN-MDSCs isolated from late-stage cancer patients were reported with high migratory capacity and increased metabolic rate than classic neutrophils^[116]. In pre-metastatic niches, PMN-MDSCs are intended towards escaping TME that allow tumor homing at the new site. PMN-MDSCs are also known for NK cell-mediated CTCs elimination and metastasis into the lungs^[117]. M-MDSCs and PMN-MDSCs upregulate the expression of matrix MMPs (metalloproteinases) and extravasation and engraftment of CTCs along with MMP8 and MMP9 respectively^[114].

Conclusions

The TME encompasses different specialized microenvironments, described in the previous sections, including the hypoxic niche, acidic niche, immune, metabolic, and mechanical microenvironments that crosstalk with each other. The classification of the tumor microenvironment mainly defines the interactions among immune cells, stromal cells, extracellular matrix, cytokines, chemokines, and tumor cells. The complexity of TME is immense as it consists of various cellular as well as acellular components. Here, we have discussed role of various immune cell types in TME. TME is an intricate ecosystem that affects each side of cancer biology and its treatment where many mechanisms are not yet completely understood. The metabolic and immune

profile of tumors has been observed to affect the phenotypic profile due to the excess of lactic acid production, more glucose consumption, and the potential infiltration of lymphocytes in the TME. Tumor infiltrating lymphocytes (TILs) in TME includes high CD3⁺, CD8⁺, CD4⁺, and Foxp3⁺ T cells and represents a prognostic indicator for immunosuppression but more studies are required to understand the mechanism and molecular players engaged in immune infiltration. A more comprehensive approach may provide a better opportunity to translate basic understanding of various components of TME into therapeutic advancements. Therapeutic intervention is possible based on the recognition of microenvironment drivers and tumor subsets along with their molecular profiles.

Abbreviations

ADCC, Antibody Dependent Cell Cytotoxicity; Ang I, Angiopoietin I; APC, Antigen Presenting Cell; APM, Antigen Processing Machinery; CAF, Cancer Associated Fibroblast; CAM, Cancer Associated Macrophage; CCA, Cholangiocarcinoma; cDC, conventional Dendritic Cell; ceRNA, competitive endogenous Ribonucleic Acid; CTCs, Circulating Cancer Cells; DC, Dendritic Cell; DEGs, Differentially Expressed Genes; dIL - 1R, decoy Interleukin-1 Receptor; ECM, Extracellular Matrix; EMT, Epithelial to Mesenchymal Transition; ER, Endoplasmic Reticulum; FGF-Fibroblast Growth Factor; GIST, Gastro Intestinal Stromal Tumor; HGF, Hepatocyte Growth Factor; HIF, Hypoxia Inducible Factor; ICAM, Intercellular Cell Adhesion Molecule; ICB, Immune Checkpoint Blockade; IDO, Indole 2, 3-dioxygenase; IGF, Insulin-like Growth Factor; IL, Interleukin; Th1, helper T lymphocyte 1; INF-, Interferon-gamma; lncRNA, long non coding Ribonucleic Acid; LXR, Liver X Receptor Alpha; M-CSF, Macrophage Colony Stimulating Factor; MCT, Mono Carboxylate Transporter; MDSC, Myeloid Derived Suppressor Cell; MMP, Matrix Metalloproteinase; moDC, monocyte derived Dendritic Cell; MPO, Myeloperoxidase; NETs, Neutrophil Extracellular Traps; NGF, Nerve Growth Factor; NK, Natural Killer; NSCLC, Non Small Cell Lung Cancer; PAI-1, Plasminogen Activator Inhibitor-1; pDC, plasmacytoid Dendritic Cell; PD-L1, Programmed Death Ligand 1; PGE2, Prostaglandin E2; PMN, Polymorphonuclear; SCLC, Small Cell Lung Cancer; SIR, Signal Regulatory Protein Alpha; TANs, Tumor Associated Neutrophils; TCA, Tricarboxylic Acid; TGF- β , Transforming Growth Factor beta; Th2, helper T lymphocyte 2; TILs, Tumor Infiltrating Lymphocytes; TLS, Tertiary Lymphoid Structure; TME, Tumor Microenvironment; TNBC, Triple Negative Breast Cancer; TNF- α , Tumor Necrosis Factor alpha; Treg, Regulatory T-cell; TRM, Tissue Resident Macrophage; VCAM, Vascular Cell Adhesion Molecule; VEGF, Vascular Endothelial Growth Factor.

Conflict of interest

There is no conflict of interest in the manuscript. I hereby permit the use of figures and tables attached to the manuscript.

Authors' contributions

AKS and SKY conceptualized, supervised, and co-wrote the

manuscript. SM and GS critically revised and co-wrote the manuscript. AB and NB facilitated with compilation of relevant literature, manuscript writing, figure preparation and participated in discussions.

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