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An (Im)Penetrable Shield- How the Tumor Microenvironment Protects Cancer Stem Cells

THERESA RELATION[A, B], MASSIMO DOMINICI[C], EDWIN M. HORWITZ [A, D, E]

a) The Research Institute at Nationwide Children's Hospital, Columbus, OH, USA, b) Ohio State University College of Medicine, Medical Scientist Training Program, Columbus, OH, USA, c) University of Modena & Reggio Emilia, Department of Medical and Surgical Sciences of Children and Adults, Modena, Italy, d) The Ohio State University College of Medicine, The Division of Hematology/Oncology/BMT, Columbus, OH, USA, e) Nationwide Children's Hospital, Departments of Pediatrics and Medicine, Columbus, OH, USA. Corresponding Author: Center for Childhood Cancer and Blood Diseases, The Research Institute at Nationwide Children's Hospital, Columbus, OH 43205, USA., E-mail address: Edwin.horwitz@nationwidechildrens.org Fax: (614) 355-2927; Grants: This work was partially funded by the CancerFree Kids Foundation and the Research Institute at Nationwide Children's Hospital

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ABSTRACT

Cancer stem cells (CSCs) are defined by their unlimited self-renewal ability and their capacity to initiate and maintain malignancy, traits that are not found in most cells that comprise the tumor. Although current cancer treatments successfully reduce tumor burden, the tumor will likely recur unless CSCs are effectively eradicated. This challenge is made greater by the protective impact of the tumor microenvironment (TME), consisting of infiltrating immune cells, endothelial cells, extracellular matrix, and signaling molecules. The TME acts as a therapeutic barrier through immunosuppressive, and thereby tumor-promoting, actions. These factors, outside of the cancer cell lineage, work in concert to shelter CSCs from both the body's intrinsic anticancer immunity and pharmaceutical interventions in order to maintain cancer growth. Emerging therapies aimed at the TME offer a promising new tool in breaking through this shield to target the CSCs, yet definitive treatments remain unrealized. In this review, we summarize the mechanisms by which CSCs are protected by the TME and current efforts to overcome these barriers. STEM CELLS *2017; 00:000–000*

SIGNIFICANCE STATEMENT

Cancer stem cells differ from bulk tumor cells due to their ability to create a heterogeneous tumor population, unlimited self-renewal, and proliferation. The tumor microenvironment (TME) provides a shelter for these cells through the presence and actions of stroma, vasculature, extracellular matrix, signaling molecules, and immune cells. The TME has increasingly been recognized as an important component of therapy resistance and tumor progression. Unlike tumor cells, the critical components that comprise the TME and their roles in tumor progression are common between different cancers. A thorough understanding of cancer stem cell-TME interactions is vital to the development of innovative therapeutics.

INTRODUCTION

Despite an enormous effort to cure cancer, the last 50 years' increase in long-term survival is primarily the result of behavioral changes (e.g. less cigarette smoking) rather than the rational development of pharmaceuticals or biologics to target the cancer [1]. The underlying explanation largely lies in our incomplete understanding of cancer biology. Two major breakthroughs in the last 20 years have fundamentally al-

tered our view of cancer. First, we now recognize that cancer stem cells (CSCs) are the origin of many cancers. Second, the tumor microenvironment (TME) is a critically important protector of malignant cells, especially CSCs. Thus, greater knowledge of both CSC biology in the TME and the impact of each TME component on cancer persistence may shed light on novel therapeutic approaches. Here, we review the current understanding of CSCs in the TME and briefly highlight potential interventional targets.

Cancer Stem Cells

Lapidot et al first identified cancer stem cells in acute myeloid leukemia using severe combined immune-deficient mice [2]. Since then researchers have replicated this finding in multiple blood and solid cancers, including chronic myelogenous leukemia [3], brain [4], breast [5], ovarian [6], head and neck [7], colon [8], liver [9], pancreas [10], and prostate cancers [11], suggesting that CSCS are a general characteristic of many cancers. Alternatively referred to as tumor-initiating or cancer stem-like cells, CSCs exhibit three defining characteristics: self-renewal, unlimited proliferation, and the ability to create a heterogeneous population [12]. This third trait is achieved (while maintaining stemness) through asymmetric division [13], similar to healthy tissue-specific adult stem cells. For a more expansive discussion of the history and complexity of CSC research, please see Kreso and Dick [14], Nassar and Blanpain [15], and Reya et al [16].

Two theories have emerged to explain the origin of cancer stem cells. The stochastic model contends that cancer cells are intrinsically and biologically comparable. CSCs emerge as a result of environmental influences that alter the cell's phenotype. In this model, all cancer cells have the potential to emerge as CSCs based on environmental cues [16]. Conversely, the hierarchy model states that tumors consist of specific subsets of cells. Only a small number of cells in any tumor have gained the mutations necessary for stemness, and differentiation of these cells' progeny results in all other tumor subsets [17]. Chaffer and Weinberg merged these models through the concept of cellular plasticity for describing solid tumors. In this model, transit-amplifying cells can de-differentiate into CSCs. This bidirectional differentiation allows for a specific subset of tumor-initiating cells while also acknowledging the potential for differentiated cells to enter this pool if they undergo mutations in stem-like genes [18]. Such distinction is important, as a limited number of CSCs may be present within the tumor at any one time but this model predicts that any cancer cell could revert into a CSC under the right conditions.

The CSC phenotype differs from the tumor bulk in critical ways. While the majority of cancer cells exhibit limited proliferative capacity, CSCs maintain the ability to divide indefinitely but can reside in a comparatively more quiescent state [12]. Traditional cancer treatments target actively dividing cells, including radiation and most chemotherapeutic agents. These therapies therefore reduce tumor bulk as a whole but do not effectively kill CSCs, often leading to tumor recurrence. For example, breast CSCs demonstrate paclitaxel resistance [19] and lower ROS expression, which are critical for ionizing radiation to induce DNA damage [20]. Additionally, glioma CSCs upregulate checkpoint regulators ATM, Rad17, and Chk1/2 after radiation, activating repair pathways that increase survival [21]. Together, aberrant ROS scavenging, DNA damage and DNA repair pathways have been reported in multiple CSC lineages [22]. Although progress has been made in using metronomic chemotherapy regimens [23], the DNA damage responses in CSCs remain an obstacle to achieving chemotherapy-induced remission [24]. Additionally, even if CSCs are eradicated, other cancer cells have the potential to revert to CSCs and repopulate the tumor (according to the cellular plasticity model of CSCs) [18]. This process is critically important in combating cancer recurrence. Identifying and removing the factors that facilitate this de-differentiation remains a central challenge in cancer treatment.

Current research has focused on developing drugs that target signaling pathways involved in stemness, including γ-secretase inhibitors, cancer stem cell vaccines, Wnt ligand modification/antagonist upregulation, and antibodies against CSC cell surface markers [25]. However, these therapies require understanding CSC biology, which may differ among cancers. For example, therapies aimed at CD44+ CSCs, a common marker in cancers such as head and neck, breast, and colorectal cancers, may not effectively target CD133+ CSCs common to glioma and lung cancer [26]. Additionally, the tumor microenvironment impedes drug penetration into the tumor and suppresses the immune system's activation against cancer cells [27]. Therefore, an approach that can circumvent these obstacles by modulating the tumor microenvironment and assaulting common tumor-supportive components would be a critical tool in cancer therapy. Our ability to attain this goal is reliant on expanding knowledge of the microenvironment's role in cancer cell survival.

Tumor Microenvironment

The tumor microenvironment (TME) is composed of stroma, vasculature, extracellular matrix (ECM), signaling molecules, and immune cells. Increasingly, the TME has been recognized as an important component of therapy resistance and tumor progression. The tumor microenvironment is heterogeneous and dependent on location within the tumor, such that the TME at the tumor periphery may differ significantly from the TME within the tumor core [28]. These differences are in part due to immune cell infiltration, tumor cell necrosis, interstitial pressure, and randomly occurring mutations within the tumor cells [29]. Although each tumor builds its own unique TME, the critical components that comprise the TME and their roles in tumor progression are common between different cancers.

The TME offers a promising avenue for new cancer treatments because, in contrast to CSCs, stromal components show relatively low mutation rate [12], making evasion of an effective therapeutic agent less likely. Unlike CSC therapy which may require specification for each cancer type [26], many of the TME protective mechanisms are shared across tumor types. Therefore, the TME represents a broad set of factors common to many tumors that influence tumor development, posing an attractive target for therapy development. Below, each component of the TME and its contribution to cancer persistence is examined.

Extracellular Matrix: Tumor cells are embedded within a dense ECM composed of collagen, glycoprotein, proteoglycans, polysaccharides, and proteins [30], which differs from normal ECM by its density and molecular composition. Increased matrix deposition and fibrosis (desmoplasia) physically prevents treatments such as chemotherapy, biologics, and other pharmaceuticals from direct contact with tumor cells [31]. Hyaluronan and collagen increase tension in the ECM. This leads to growth-induced solid stress, in which cancer cell proliferation is confined by ECM rigidity and puts pressure on blood vessels, leading to hypoxia and decreased perfusion of systemic drug therapy [32].

ECM also promotes tumor growth by growth factor and cytokine sequestration. When the ECM is cleaved by pericellular proteases such as matrix metalloproteases (MMPs) [33], molecules such as E-cadherin and pro-hepatocyte growth factor are released into the TME to stimulate cell survival and migration [34]. The ECM also has direct effect on CSCs through molecules such as MMP-3, which binds the Wnt ligand Wnt5b and causes expansion of mammary epithelial stem cells and hyperplasia [35]. Similarly, MMP-14 degrades collagen, remodels the ECM, and releases heparin binding-epidermal growth factor to activate EGFR pathway, leading to lung cancer growth [36]. Thus, the ECM physically protects CSCs from treatment and provides a repository of pro-tumorigenic cytokines.

Angiogenesis: Tumoral angiogenesis, in which endothelial cells and associated pericytes sprout new blood vessels from existing ones and extend into the tumor [37], occurs rapidly but is disorganized, with decreased pericyte coverage, weak pericyte adhesion to the endothelium, and convoluted blood flow [38]. Rapid cellular proliferation combined with high oxygen consumption rate in cancer causes inadequate nutrient delivery to the tumor, resulting in compressed blood vessels and hypoxia. Hypoxia activates HIF1α in tumor cells, a transcription factor that positively regulates alternative angiogenic signaling such as PIGF, bFGF, and PDGF pathways [39] and stimulates aerobic glycolysis, both of which correlate with poor patient prognosis and metastasis [40]. Vascular leakiness also allows hormones and other locally secreted factors to diffuse through the tumor with little resistance [41]. Pro-tumorigenic immune cells within the tumor mass (described herein) can thus locally secrete immune-suppressive cytokines such as IL-10, IL-17A, and TGF-β that bathe the tumor despite inadequate vascular networks which systemically delivered drugs are unable to penetrate [42].

Angiogenesis is directly impacted by CSCs through signaling between CSCs and angiogenic endothelial cells in several cancers, including brain [43], breast [44], and liver [45] malignancies. CSCs that release angiogenic factors into the TME to promote vascularization include HCC progenitor cells [45], VEGF, SDF-1, and HIF expression in glioma stem cells [43], and VEGF, PDGF, and Ang-1 in breast CSCs [44]. By promoting angiogenesis, CSCs promote tumor progression through increased nutrient delivery.

Metabolism: Insufficient blood flow to the tumor due to disorganized angiogenesis creates a unique and demanding metabolic environment. Concentrations of energy sources are commonly lower in the tumor compared to normal tissues, forcing the cancer cells to switch to glycolysis in order to survive [46]. Activated immune cells also rely on glycolysis for energy, to proliferate, and to carry out effector functions (ex: IFN-γ release and mTOR activity [47]), creating competition between the two populations.

In the model proposed by Kareva and Berezovskaya, cancer cells in the solid tumor core rely primarily on glycolysis, while tumor cells at the periphery are able to perform aerobic metabolism. T cells (described herein) and glycolytic cancer cells upregulate expression of glucose transporters such as GLUT-1 and SGLT-1 to increase uptake [48]. Activated T cells are able to uptake glucose at the tumor periphery without significant competition from aerobic tumor cells. However, once the T cells penetrate the tumor core or eradicate peripheral tumor cells to expose glycolytic cells within the tumor, T cells must compete with cancer cells for the available glucose. Tumors that are able to out-compete immune cells thus may grow in the absence of functioning T cells [48].

CSCs in solid tumors surround themselves with non-stem cancer cells (their progeny) which engage in this resource competition, thus protecting the CSCs from T cell targeting. This may contribute to the observation that CSCs are most frequently found within hypoxic regions of the tumor where glycolysis is prevalent [49]. Metronomic chemotherapy is designed to target this CSC defense by using smaller doses of chemotherapy administered frequently but not continuously, allowing layers of the tumor to be removed sequentially [49]. This treatment schedule was found to be more effective in treating mouse pancreatic adenocarcinoma [50], melanoma [51], and triple-negative breast cancer [52] as compared to standard chemotherapy and anti-angiogenic therapy, suggesting that the immune cells are able to attack CSCs as they are revealed without reaching T cell exhaustion [49].

Interestingly, Chang et al observed that differences in glucose uptake between tumor cells do not necessarily correlate with proliferation, suggesting that tumors may select for rapid glucose uptake based on the im-

munosuppressive effects [47]. In addition to outcompeting immune cells for glucose, cancer cells acidify their environment through glycolysis. Low pH impedes HIF1α degradation, which increases angiogenesis and VEGF signaling. This in turn increases the local myeloid-derived suppressor cell population which secretes immunosuppressive signaling molecules, such as nitric oxide and reactive oxygen species, or differentiates into tumor-associated macrophages (described herein) which support tumor growth [53]. While much remains unknown about the mechanisms through which tumors evade the immune system, there is much to suggest that manipulating cancer cell metabolism may provide multiple benefits that permit greater immune activation against the tumor.

Mesenchymal stem/stromal cells: Defined by their ability to differentiate into multiple stromal cell lineages, MSCs are found throughout the body and traffic to specific tissues, including tumors, in the context of remodeling [54]. Tumors are considered "wounds that do not heal" due to chronic inflammation, neovascularization, and immune cell infiltration [55]. Growth factors secreted into this environment such as IL-10, VEGF, and GM-CSF facilitate MSC collection. This phenomenon, which in turn reduces antitumor T cell activity by MSC release of TGF-β into the TME, has been observed in metastatic melanoma, head and neck, prostate, and breast cancers [56,57].

MSCs act directly and indirectly on cancer stem cells to increase chemoresistance. Roodhart et al showed that MSCs contribute to tumor progression by secreting fatty acids that increase chemoresistance in colon carcinoma [58]. In acute lymphoblastic leukemia, MSCs secrete chemoattractants and pro-survival growth factors in response to tyrosine kinase inhibitor treatment, which facilitate cancer cell chemoresistance [59]. Similarly, exosomes (small extracellular vesicles released from cells upon fusion of an intermediate endocytic compartment with the plasma membrane [60]) derived from MSCs increased 5-fluorouracil resistance *ex vivo* and *in vivo* in gastric cancer animal models [61]. Cuiffo et al demonstrated that cell-cell contact between breast cancer cells and MSCs increased CSC properties through miRNA upregulation [56]. Together, these findings demonstrate that MSCs support tumor progression through many varied mechanisms, some likely not yet described.

Fibroblasts: When activated by direct contact with leukocytes or secreted factors such as TGF-β, PDGF, and GM-CSF [62], cancer-associated fibroblasts (CAFs) promote tumor growth, increase angiogenesis, degrade ECM to release embedded signaling molecules, and promote epithelial to mesenchymal transition (EMT) and metastasis [63]. CAFs are derived from MSCs, normal fibroblasts, or epithelial cells. They highly express surface markers such as α-smooth muscle actin (αSMA), fibroblast activation protein (FAP), Thy-1, and S100A4 protein [64]. CAFs are defined functionally by their high division rate and high ECM deposition, and have been described as "activated myofibroblasts that cannot regress to the inactivated state" [65].

CAFs are enriched in human tumors following chemotherapy [66], which leads to increased CSC regulation through paracrine signaling. Erez et al [67] showed that CAFs from early neoplastic murine skin lesions exhibit a distinctive inflammatory gene signature, including IL-6, IL-1β, and CXCL2, a finding that was replicated in human squamous cell carcinoma. Tumor cells co-injected with CAFs grew faster and were more vascularized than tumor cells injected alone [67]. Similarly, CAF inflammatory mediators increased metastasis in hepatocellular carcinoma [68]. CAFs in non-small-cell lung cancer were found to trigger IGF1R pathway in cancer cells, leading to increased stemness marker Nanog expression and greater level of sphere formation *in vitro* and metastasis *in vivo [69]*. In hepatocellular carcinoma, CAFs secrete HGF to increase tumor cell self-renewal capacity [70]. CAFs have also been shown to contribute to stemness in glioblastoma, leukemia, and gastric cancer through secretion of TGF-β, STAT1 and NF-κB [66,71]. Because of their role in tumor progression, CAFs may prove a valuable target in transforming the TME towards tumor suppression.

Immune Modulation

The immune system is tightly regulated to protect the body, but dysregulation paradoxically contributes to tumor progression and invasion. Rather than inhibiting tumor growth and progression, the immune system is co-opted to protect the tumor against the body. The tumor actively regulates the TME to protect itself from clearance by infiltrating immune cells, including T_{reg}, macrophages, dendritic, natural killer, T, and B cells [27,72]. Tumor evasion also occurs through prolonged cancer cell-mediated NF-κB activation and signaling, which has been demonstrated to drive immune inhibition and tumor progression in lung, breast, and prostate cancers [57,73]. By re-directing the immune system to recognize and attack tumor cells, immunotherapies have the potential to create lasting remission. These approaches can be divided into innate and adaptive immune modulators; here we focus on a selection of key components.

Innate Immunity: Innate immune activation is rapid and can target a broad range of stimuli, but chronic inflammation has been linked to tumor progression and angiogenesis through a persistent wound-like state [62]. As important members of the innate immune system, natural killer (NK) cells and macrophages are appealing targets for investigators working to tip the balance of the TME towards tumor eradication.

When activated, NK cells either release granzymes and perforin to permeabilize target cells or activate the death receptor (FAS, TRAIL) pathways, resulting in apoptosis [74]. In addition to their own cytotoxic activity, NK cells magnify the immune response by releasing interferon-γ, which in turn promotes the Th1 (pro-inflammatory helper T cell) immune response, increases

antigen-presenting cell activity, activates macrophages, and reduces neoplastic cell proliferation [75]. NK cells simultaneously receive activating and inhibitory signals, the balance of which determines the cells' function [75].

Recent work has highlighted the importance of NK cells in targeting CSCs. Using freshly isolated patient samples from breast cancers, sarcomas, and pancreatic cancers, Grossenbacher et al demonstrated that CSCs upregulate stress ligands in response to radiation therapy, which are then preferentially targeted by NK cell immunotherapy [76]. A similar preference for CSCs by NK cells was revealed in colon cancer, melanoma, and glioblastoma [77]. In bladder cancer, chemoresistant CSCs treated with activated-NK cell supernatants demonstrated decreased stemness (as measured by ALDH activity and mRNA panel for stem markers) and increased cisplatin sensitivity [78]. Together, these findings reveal that NK cells can target and kill CSCs if they are able to penetrate the tumor and overcome the tumor's defense mechanisms.

Tumor cells deploy multiple mechanisms to evade NK-mediated immune killing. Tumors that either release the cytotoxic lymphocyte-activating molecule NKG2DL into circulation or downregulate its expression on tumor cells, as occurs in melanoma, hepatic carcinoma, breast, and hematologic cancers, block effective NK cell-mediated tumor targeting [74,79]. Certain cancers, such as leukemia, upregulate MHC class 1 or HLA-G expression to inhibit NK cell activation [80]. Limited infiltration into solid tumors also limits NK cytotoxic ability [81]. NK cell-based immunotherapy aims to overcome these obstacles by (1) facilitating NK infiltration into the tumor, (2) increasing NK activation inside the tumor, and (3) enhancing NK-mediated cytotoxicity [82].

Like NK cells, macrophages are one of the body's first-line defenses against foreign invaders. Traditionally, macrophages were thought to terminally differentiate from monocytes into either the M1 macrophage phenotype, characterized by cytotoxic anti-tumor activity and type 1 interferon responses, or the M2 phenotype, which supports the tumor via immunosuppression and growth factor-mediated angiogenesis [83]. However, continued research has shown that macrophages can switch between activation states throughout their lifespan [28,84,85], with conversion from M1 to M2 mediated by growth factors such as TGF-β [42]. These two activation states represent two extremes of the spectrum. Each individual cell exists along a continuum between the two poles, and its placement along that continuum can change in response to stimuli.

Once a macrophage enters the tumor it becomes a tumor-associated macrophage (TAM), which typically resembles the M2 phenotype and differs from circulating macrophages in critical ways [62]. TAMs are recruited by the tumor-derived chemokine CCL2 and molecules such as VEGF, PDGF, and TGF-β. They then produce these growth factors themselves in an amplification loop which contributes to angiogenesis and survival [42]. TAM-mediated tumor support occurs through: pro-tumorigenic growth factor release, angiogenic stimulation, tissue remodeling, ECM enzymatic cleavage, and reduction of adaptive immune responses such as inflammation and T cell maturation [86]. In relation to CSCs, TAMs have been shown to contribute to chemoresistance by releasing MFG-E8 protein to reduce cisplatin toxicity (lung and colon cancer) and by reducing CSC tumor initiation and STAT3 activation (pancreatic cancer) [87]. Together, these functions demonstrate how cancer co-opts macrophages to support tumor progression and therapy resistance. Recent studies in HCC, fibrosarcoma, melanoma, glioma, pancreatic, and breast cancers have shown that it is possible to reprogram TAMs to their tumor-destructive counterparts, and such reprogramming results in reduced angiogenesis and vascular normalization [38,88]. These findings support further research into the mechanisms of TAM-mediated tumor progression and methods to reverse their effects.

Adaptive Immunity: Long-term cancer antigen immunity represents the "Holy Grail" in cancer immunotherapy, as it would mean the body has developed lifelong, effective immune surveillance precluding tumor recurrence. However, this goal remains elusive due to complex immune evasion such as immunoediting, where immunogenic cancer cells are killed while non-immunogenic cells proliferate [88]. As cancer mutations arise over time, the immune system keeps tumor development in check until the non-immunogenic cells can escape and form a tumor mass. By selecting for cells that evade it, the immune system thus creates an enemy equipped with the very tools needed to defeat it [89]. In this section, we will focus on T cells to illustrate these challenges.

Like macrophages, the role of T cells as either pro- or anti-tumorigenic is largely dependent on environmental stimulation [42]. For a full review of T cell subtypes, see Hanahan and Coussens [38], Palucka and Coussens [90], Chen and Flies [91]. Inflammatory T cell cytokine secretion, most importantly IFN-γ, suppresses tumor development such that increased T cell infiltration in solid tumors correlates with better prognosis [92]. This effect is abrogated by the presence of immunosuppressive T cells, such as CD4+ Th2, Th17 [62], and regulatory T cells (T_{Reg}), which suppress enrichment of other types of T cells in the tumor through both direct contact and by secreting TGF-β and IL-10 [57]. T_{Reg} are physiologically important because they block the immune system from recognizing self-antigens; cancer co-opts this mechanism, allowing the tumor to develop without T cell attack of cancer-associated neoantigens [38].

Also like macrophages, T cells can interconvert between phenotypes based on exposure to stimuli [93]. CD4+ Th17 and Th2 cells can switch from producing IL-17 to IFN-γ through a combination of metabolic, epigenetic, and cytokine signaling pathways [94]. Memory T_{Reg} (CD4+ Foxp3+ CD25high CD27+ CD45RA−) can shift

to a Th17-like phenotype in humans, expressing IL-17 and CCR6 [95]. By inducing T_{Reg} to the Th17 phenotype through increased IL-6 production in dendritic cells, mice with B16 melanoma tumors demonstrated increased antitumor activation [96]. A subset of T_{Reg} in highly inflammatory cancers such as colorectal carcinoma and breast cancer produce adenosine or prostaglandin E_2 to reduce inflammation and tumor development, in contrast to the typical tumor-supportive role of T_{Reg} [97]. T cell plasticity has also been found in graft versus host disease [98], atherosclerosis [99], and inflammatory skin diseases [100], indicating the potential for a common pathway across diseases.

Tumors have developed multiple mechanisms to circumvent cytotoxic T cell activity and amplify T_{Reg} signaling. While T cells primed against tumor antigens may exist in patients, they are blocked from attacking the tumor either physically by the ECM [101] or through immune suppression. The ECM physically impedes T cell accumulation within the tumor, inhibiting antigen recognition [88]. Tumor evasion occurs via manipulation of the Fas ligand (FasL), programmed cell death protein 1 (PD1), and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) pathways. FasL, expressed in solid tumor vascular networks, induces apoptosis in bound cells, including T cells. Tumors with high FasL expression show low cytotoxic T cell infiltration but high T_{Reg} activity due to high T_{Reg} expression of c-FLIP, an apoptosis inhibitor [88]. PD1/PDL1 interaction between tumor ligand and T cell receptor causes T cell inhibition/apoptosis and tumor proliferation [102], but paradoxically PD1 activation on T_{Reg} produces increased immunosuppressive activity [103]. Long-term T cell exposure to tumor antigens leads to high PD1 receptor expression and eventually T cell exhaustion. Evasive measures also include increased PD1 expression on tumor cells after exposure to IFN-γ released by invading leukocytes [89]. Like PD1, CTLA-4 negatively regulates T cell checkpoint progression [88].

The importance of the TME in T cell activation is evident in results from chimeric antigen receptor- T cell (CAR-T) therapy, in which T cells are genetically modified *ex vivo* to recognize tumor-associated antigens. Subsequently, infused T cells will be activated upon tumor antigen binding and kill target cells *in vivo [102]*. CAR-T therapy has shown considerable success in treating leukemias but has shown limited efficacy in solid tumors [102], with the exception of promising data coming from studies of CAR-T therapy in neuroblastoma [104] and sarcoma [105]. The readily evident difference between solid and liquid tumors is the separation between CSCs protected in the TME away from the bulk of malignant cells in blood cancers versus the unity of these elements in solid tumors. Therefore, the observed difference in efficacy supports the assertion that the TME blocks immune-mediated tumor clearance [81]. If investigators are able to improve immune cell penetration and remove the TME barriers to immune clearance, then therapies such as CAR-T may be more effective in solid tumors, reaching the levels seen in blood cancers [106].

Conclusions

The TME provides shelter for tumor cells, and especially CSCs, through regulation of the stroma, vasculature, extracellular matrix, signaling molecules, and immune cells. Cancer has evolved to out-maneuver the body's natural defenses against aberrant growth, and without targeting these defenses cancer is likely to recur. Additionally, even if the entire current complement of immune-evasive measures are successfully targeted by new treatments, new, as-of-yet unknown mechanisms are likely to arise to counteract therapeutic efforts; hence, a continuously evolving, comprehensive understanding of TME biology is important to prepare ourselves for the future. Breakthroughs in basic biology will undoubtedly lead to both strategies for outpacing tumor evolution, which aims to evade pharmacologic and biologic therapies, and to more effective approaches for eradicating CSCs.

Author Contributions

T.R.: Conception and Design, Manuscript writing, M.D.: Final approval of manuscript, E.M.H.: Conception and Design, Financial Support, Manuscript writing, Final approval of manuscript

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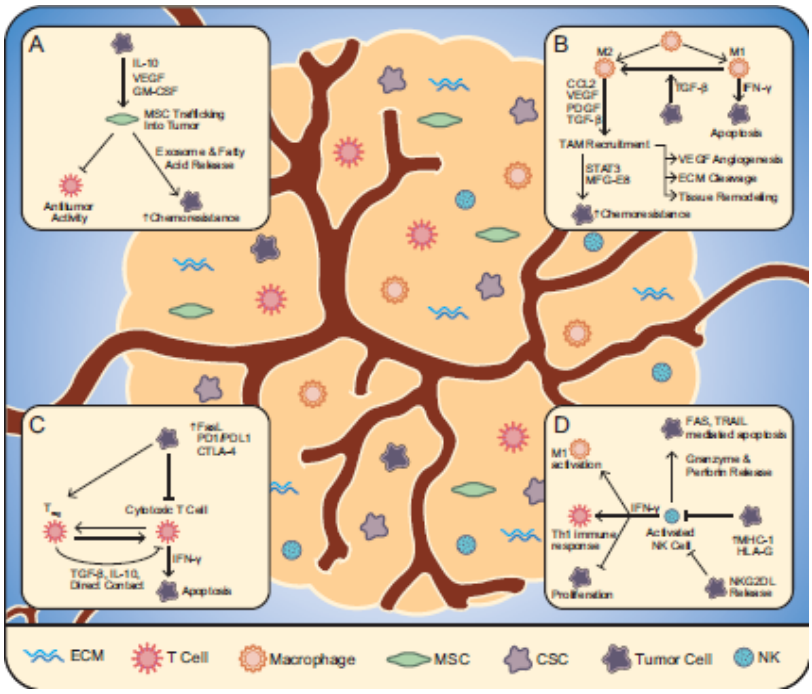
Figure 1. The Tumor Microenvironment. Tumor cells are embedded in a complex microenvironment served by tortuous, disorganized blood vessels and composed of immune cells, ECM, fibroblasts, and MSCs. (A) MSCs traffic into the tumor in response to cancer cell secretion of cytokines such as IL-10, VEGF, and GM-CSF. Once inside the tumor, MSCs release exosomes and fatty acids into the TME. This results in increased chemoresistance. MSCs also decrease anti-tumor T cell activity. (B) Cytotoxic T cells release IFN-γ, which suppresses tumor development. T_{reg} suppress inflammatory T cells through TGF-β, IL-10, or through direct contact. T cells can interconvert between cytotoxic and regulatory T cell phenotypes. FasL, PDL1, and CTLA-4 upregulation on tumor cells blocks cytotoxic T cell activity but increases T_{reg} immunosuppressive activity. (C) Macrophages exhibit phenotypes along a spectrum between tumor-suppressive M1 and tumor-supportive M2. M1 mediate tumor cell apoptosis through IFN-γ and can convert to M2 through factors such as tumor-secreted TGF-β. M2 macrophages play many roles, including: ECM cleavage, tissue remodeling, angiogenesis, chemoresistance, and TAM recruitment. (D) Tumor cell release of NKG2DL and upregulation of MHC I and HLA-G suppresses NK cell activation. Activated NK cells release granzymes and perforin to induce Fas and TRAIL-mediated apoptosis in cancer cells. IFN-γ released by NK cells triggers a Th1 immune response, decreased tumor cell proliferation, and increased M1 activation.



Graphical Abstract

Cancer stem cells (CSCs) are embedded within a complex tumor microenvironment with many components exerting influence over the CSCs. The extracellular matrix (ECM), mesenchymal stem/stromal cells (MSCs), and certain subtypes of T cells and macrophages promote CSC growth. Natural killer (NK) cells and other subtypes of T cells and macrophages inhibit CSC proliferation.