Tumor Microenvironment: Coconspirator in Tumorigenesis

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Abstract

The tumor microenvironment is pivotal for tumor establishment, evasion of host immune surveillance, growth, invasion, and metastasis by providing a nutrient meshwork rich in lympho-vasculature, cellular and metabolic coconspirators of the tumor, and a signaling network for cross talks. Of note are a number of players that support or impede tumor growth directly by themselves or indirectly through their products. Given the immensity of the topic and limitation of the scope of this chapter, we choose to discuss the following well-studied players and products that are the focus of research and resource commitment: (1) fibroblasts and cancer-associated fibroblasts, (2) endothelial cells with angiogenesis, (3) immune cells, and a couple of cellular products, i.e., (4) secretomes, and (5) exosomes. We recognize that under each of the five subtitles lies a world of evidence that embodies numerous never-ending topics. Each of them deserves separate in-depth exploration. We hence set out to briefly introduce their basic biological functions, discuss their roles in tumor growth and mechanisms of interaction with tumor cells and among themselves, summarize representative research and milestones of achievements, and finally address their relevant clinical implications and advances.

Learning Objectives
- Understand the notion of the tumor microenvironment (TME) and the basic roles in tumorigenesis
- Learn the names and tumorigenic roles of cancer-associated fibroblasts (CAF), secretome, and exosome
- Comprehend the importance of endothelial cells and angiogenesis in tumorigenesis
- Know different types of immune cells, e.g., tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), and regulatory T cells (Treg), and their respective roles and functions in tumorigenesis and clinical immunotherapy

3.1 Introduction

Tumor invasion features the departure of tumor cells from the primary normal microanatomic compartment after in situ proliferation to infringe into neighboring territory. During this invasion process, the tumor cells are supported by the surrounding specialized environment to support their growth. Such an environment is frequently referred to as tumor microenvironment (TME). TME is the compartment where tumor cells survive and thrive. By coordinating the interactions and communications among different components via complicated mechanisms, the TME is essential in creating primary niches for the tumor cells and is pivotal for sustained tumor growth, evasion of host immune surveillance, generation of neo-vasculatures, promotion of invasion, and metastasis.

TME consists of physical and chemical components in addition to the tumor cells. TME denotes the stroma with lymph vasculature and immune cells. Stroma is the connective tissue that comprises both noncellular extracellular matrix (ECM) and cellular components. Chemically, the noncellular components of ECM consist of ground substances that form the framework for tumoral and non-tumoral tissue to organize and network with each other. The noncellular components of ECM also consist of functional molecules, including enzymes, metabolic products, and signaling molecules such as chemokines, auto/juxta/paracrine hormones, and growth factors. In aggregate, these proteins are

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called secretomes. In contrast, the recently emerging concept of exosomes represents portions of cellular compartments that are shed into the ECM. The cellular components of TME consist of multiple cell types, i.e., fibroblasts, endothelial cells, and immune cells [1].

In this chapter, we limit our review to the fundamental roles of the most relevant components of the TME as coconspirators in tumorigenesis. These coconspirators include fibroblasts and cancer-associated fibroblasts (CAFs), secretomes and exosomes released by CAFs and tumor cells, endothelial cells with angiogenesis, and the immune cells. We also describe the interactions among these different coconspirators and address issues relating to the molecular mechanisms and events. Lastly, we discuss their potential clinical implications.

3.1.1 Fibroblast and CAFs

Fibroblasts are the most cellular component of the stroma. In contrast to fibrocyte, the suffix "-blast" implies immaturity and the potential to function as precursors to differentiate into other cell types. Embryonically, the fibroblast is derived from mesoderm with a subset derived from the neural crest. Functionally, fibroblasts synthesize a variety of proteins, including collagens, fibronectin, and laminin, to constitute the ECM and basement membrane. In addition, they produce various types of ground substances (glycosaminoglycans, proteoglycans, and glycoproteins) and release cytokines, juxta- or paracrine hormones or growth factors, proteases, etc., to maintain and modulate the matrix framework. Fibroblasts can also migrate and proliferate in response to different signals, e.g., leukotrienes and growth factors. Although they lack specific markers, the fibroblasts are morphologically recognized by their spindle shape, oval to elongated nuclei, and their microanatomical location in an organ. When the fibroblasts are activated and/or proliferate, the cytoplasm is plump, bright eosinophilic on H & E stained sections as collagen laying fibroblasts in diseased conditions. The fibroblasts are known for their plasticity. Transformation into myofibroblasts has been well described in tissue repair. Lastly, as it has been well known, in vitro experimental models also showed that human fibroblasts could be reversely transformed into pluripotent stem cells [2].

Since the first descriptions of CAF were brought forth [3], there has been an explosion in knowledge regarding CAFs' roles in ECM remodeling, angiogenesis, signal networking and transduction, harnessing and modulating host immune system, and clinical potential in therapy. While progress continues to be made in this field, challenges remain. Recently, a group of experts in the field of CAF biology summarized and issued a Consensus Statement to elucidate relevant issues of CAFs, including definition, tissue generation, functions, heterogeneity and plasticity, and potential clinical benefits [4].

Although most CAFs are believed to be derived from local fibroblast activation or genetic alterations, other cells of origins have also been described. Several cells are able to "de-differentiate" into CAFs, which include adipocytes [5], endothelial cells [6], and bone marrow-derived mesenchymal stem cells [7]. Epithelial cell as the origin of CAFs has been one of the most extensively studied mechanisms called epithelial-mesenchymal transition (EMT). EMT is associated with tumor progression and metastasis [8]. Both benign and malignant epithelial cells can generate CAFs through EMT [9]. Although the transformation from cancer cells to CAFs is well described, it is interesting to note the nonoverlapping genetic alterations between cancer cells and CAFs [10]. Such discrepancies, however, appear to be due to genetic alterations occurring at early precursor stages with loss or gain of these alterations in subsequent subclonal evolution of tumor cells.

Analogous as they may appear, CAFs are different from normal fibroblasts. Two experimental models are most exemplary that describe the differences: (1) In their mouse model of prostate cancer, Olumi et al. [11] reported that CAFs directed tumor progression by inducing prostatic intraepithelial neoplasia in normal prostate epithelial cells. Under the same experimental conditions, such tumor progression was not observed by normal fibroblasts. These findings were displayed by both in vivo tissue recombination system and in vitro co-culture system. (2) In their breast cancer experimental model systems, Dumont et al. [12] showed that CAFs were protumorigenic and supported tumor dissemination by inducing a mesenchymal-like phenotype of both premalignant and malignant mammary epithelial cells. In comparison, fibroblasts isolated from benign reduction mammaplasty helped maintain the tumor cells epithelial morphology and hindered tumor growth and dissemination.

Once in place, CAFs exert their roles in regulating tumor growth, invasion, and metastasis in various ways through interactions with other ECM components and tumor cells. One school of thought that contributed to the pool of evidence was by implicating the role of physical forces in the tumorigenic process. Using the breast as the model organ, Paszek and Weaver [13] illustrated that the "tumors are rigid because they have a stiff stroma". The stiffness of the stroma was created by increased interstitial tension via infiltrating immune cells and activated resident fibroblasts that will increase the production of macromolecules, e.g., collagens, fibronectin, laminin, and ground substances. The stiffness was further enhanced by cross-linking enzymes [14]. In response, such increased tension would feedback to the tumor cells and increase their invasiveness [15]. One CAF product, i.e., the lysyl oxidase (LOX) family, deserves attention for their roles in contributing to the stiffness of tumor stroma, tumor growth, and progression [16].

CAFs' functions are diverse, which require heterogeneity and plasticity among CAFs to carry out different functions under different conditions. Interestingly, there have been both experimental and clinical model systems that have provided supporting evidence of such specialization. Investigators showed that some CAFs could mutually convert their functional phenotypes under different instruction signals released by different tumor subclones [17]. The molecular events that are implicated behind the functional switch of CAFs phenotypes are still unclear.

Various strategies have been developed to target CAFs at the cellular level for clinical therapeutic purposes based on the knowledge accumulated over the past decades. By targeting fibroblast activation protein (FAP), which CAFs selectively produce, Ostermann et al. [18] demonstrated the antitumor effect of monoclonal antibody (mAb) FAP5-DM1 on xenograft immunodeficient mouse models for lung, pancreas, and head and neck cancers. Sibrotuzumab, a mAb, was tried on patients with advanced colorectal cancer for clinical phase I and early phase II trials, although eventually was not successful [19, 20]. However, the setbacks did not stop investigators from further pursuing alternative means. By conjugating promelittin-containing FAP-cleavable sequences to pegylated phospholipids and anchoring them to reduced graphene oxide (rGO) nanosheets, Kim et al. [21] reported that the resulting nanosheets, PL-rGO, showed tumor inhibitory effect both on in vitro culturing and in vivo mouse models. On the other hand, by taking advantage of CAFs heterogeneity and plasticity, other innovative methods are being devised to reprogram and salvage CAFs back to their "normal" phenotypes to help achieve tumor suppression effects [4].

Progress made in molecular genetics has helped advance our understanding of CAFs and their cross-interactions with cancer cells. The role of Braf, its associated ERK–MAPK signaling pathway, and clinically approved the application of Braf inhibitor for melanoma have been well known [22]. The findings that Hirata et al. [23] published on *BRAF* mutant melanoma cells' response to PLX4720 was most revealing. After the initial response of melanoma cells to PLX4720, a rapid tolerance was developed. Such tolerance was because of PLX4720's effect on TME, leading to "paradoxical" activation of CAFs with more matrix production and remodeling. As a result, an increased integrin β1/FAK/Src signaling was seen in melanoma cells. After adding a FAK inhibitor to the anti-Braf regimen, the investigators showed effective suppression of ERK signaling, and hence more effective tumor treatment.

3.1.2 Secretome

Secretome denotes the collection of proteins released by a cell into the extracellular space as defined by TME. These proteins are of different functional types involved in physio-logical and pathological processes, including enzymes, metabolic products, chemokines, auto/juxta/paracrine hormones, growth factors, etc. In the context of TME, the Matrix MetalloProteinases (MMPs) family and TGF-beta (TGFβ) are the two major secretory proteins of the secretome that have remained at the center stage of attention over the past decades.

Since its first description in early 1960 by Gross and Lapiere [24], extensive knowledge has accumulated on MMPs over the past decades. These achievements ranged from bioengineering of the first cDNA clone, to protein crystal structure characterization, mouse model establishment, and approval of the first MMP inhibitor for clinical application. MMPs are a family of secreted zinc-dependent endopeptidases, 24 identified so far in humans, comprising collagenases, stromelysins, matrilysins, gelatinases, and others. MMPs are expressed by both tumor cells and CAFs and are known for their roles in tumor invasion as groundbreakers to degrade ECM components at the leading edge of the tumor. Additionally, MMPs also have regulatory functions on cell adhesion molecules processing which eventually impact cell migration and invasion. The roles of MMPs at different stages of cancer survival, growth, invasion, and metastasis were reviewed by Gonzalez-Avila et al. [25] in depth.

Given the roles of MMPs in cancer invasion and metastasis, MMPs have been applied as targets for MMPs inhibitors in clinical trials and as biomarkers for prognostic purposes. Dozens of clinical trials have been conducted, some showing encouraging preclinical data. However, progress on MMP inhibitors has been slow, with none demonstrating antitumor effects in clinical trials. The value of MMPs for their clinical application has been best reflected as biomarkers for prognostic purposes. Two assays exemplify MMPs biomarkers' roles. One of the assays is the 70-gene MammaPrint assay [26]. MammaPrint was approved by the US Food and Drug Administration and regulators in the European Union as an adjunct prognostic assay for patients with stage I/II breast cancer who are below 61 years of age with negative or 1–3 lymph nodes positive disease. MMP9 is listed as 1 of the 5 genes under "altered extracellular matrix adhesion and remodeling" of the "Tissue Invasion and Metastasis" category. In addition, MMP9 is also included as 1 of 6 genes under "altered expression of known angiogenesis effectors" of the "Sustained Angiogenesis" category. The other assay is the Oncotype DX assay [27]. The Oncotype DX assay has been most widely used and well received by clinicians mainly because of the convenience of using formalin-fixed paraffin-embedded tissues and its easy-to-interpret recurrence score-based reporting. MMP11 is included as one of the 16 cancer-related genes.

Of the many growth factors, TGF-beta (TGFβ) has been known as a central player for its complex roles in homeostasis and tumorigenesis by its signaling via canonical SMAD

pathway and several non-canonical pathways. On the one hand, TGFβ promotes the transdifferentiation of fibroblasts to CAF and recruits fibroblasts to the tumor. On the other hand, CAFs secrete large amount of TGFβ to affect tumor growth by enhancing cross talk between cancer cells and CAF, and by mediating metabolic activities of tumor. Similarly, another paracrine growth factor, hepatocyte growth factor (HGF), also deserves attention for its contributory roles to promote cancer progression. By overexpression of HGF and TGFβ, Kuperwasser et al. [28] showed that normal and malignant human breast tissues could be reconstructed in mice. Therapeutically, it is not difficult to find clinical trials targeting the CAF-induced signaling molecules, especially TGFβ and HGF inhibitors and mAb. The results are promising but mixed [29].

In contrast, there has been evidence showing biphasic roles of TGFβ in both promoting and hindering tumor growth and progress. In their study on skin tumors using transgenic mouse models, Cui et al. [30] found that TGFβ inhibited benign tumor formation and enhanced invasive carcinomas' progression. Other CAF secretory proteins also demonstrated such inhibitory roles on cancer growth. In the study on colon cancer secretomes using in vitro cell culture, xenograft mouse models, and clinically resected colon cancers, Chen et al. [31] revealed novel molecular signature secretory proteins that exhibited negative modulatory effects on colon cancer cell growth.

In addition to proteinase enzymes and signaling molecules, other aspects of secretomes impacting cancer biology include senescence-associated secretome, inflammatory secretome, angiogenesis secretome. The functions of these diverse secretomes are intertwined. For instance, senescence is a mechanism that prevents and blocks cancer cell proliferation and, therefore, is regarded as a tumor suppressor mechanism. As reviewed in depth by da Cunha [32], driven by NF-κB, the secretome of cells in a senescent state can contain as many as 80 bioactive molecules. These molecules include pro-senescent, pro-apoptotic, and antiangiogenic protein molecules. In association with p16 and SASP (senescence-associated secretory phenotype) that incite immune cells, these secretomes facilitate tissue regeneration and senescent cell removal. On the other hand, however, these molecules also include aforementioned MMPs and growth factors, plus pro-inflammatory leukotrienes and angiogenic factors. As stated by Rodier and Campisi [33], cellular senescence might take part in complex biological processes that include tumor promotion.

3.1.3 Exosome

Other mechanisms involving CAF interactions and cross talk among themselves and cancer cells include effects of metabolites and secretion of exosomes. Exosomes and transfer of information between cancer cells and other cellular components of TME have emerged as a novel and important mechanism that has fueled many research opportunities in recent years. It is worthwhile to mention that given the key roles exosomes play, the term "Exosomics" was used to help carry the notion and its associated importance.

Exosomes are known as a type of small extracellular vesicles (30–150 nm) that form via the cellular process of endocytosis. The endosomes thus formed are shed by the parent cells into ECM with a double-layered lipid membrane. As part of the parent cell, exosome represents a portion of the cellular cytoplasm and membrane containing parent proteins, lipids, and nucleic acids. The proteins in exosomes consist of endocytic proteins and protein molecules that are both membranous (receptors, adhesion molecules, channels) and cytoplasmic (cytoskeletons, enzymes, players of different pathways). Interestingly, on examination of the lipid contents, Llorente et al. [34] found significant differences between exosomes and parent cells. The exosomes lipids were highly enriched in a subset of lipids, e.g., glycosphingolipids, sphingomyelin, cholesterol, and phosphatidylserine. Other non-enriched lipid species were selectively included. Such a pattern of lipid composition helped raise the possibility of a yet-to-be elucidated sorting mechanism and potential tumor biomarkers.

Although largely cytoplasmic, it was demonstrated that exosomes contain different types of DNA species, i.e., single-stranded DNA, mitochondrial DNA, and double-stranded nuclear DNA. Thakur et al. [35] convincingly demonstrated the presence of double-stranded genomic DNA in exosomes and provided further experimental evidence showing that exosomal DNA represents the entire genome and carries detectable mutations that reflect parent tumor cells. Such findings were received with enthusiasm and opened doors for many research opportunities, notably in non-small cell lung carcinomas. Taking advantage of commercial DNA isolation products that have been made available, researchers were able to detect major EGFR mutations with improved sensitivity on liquid biopsy and pleural effusions using exosomal-derived DNA.

In the recent years, exosomal RNA has been extensively investigated due to technical advances and the availability of next-generation sequencing-based RNA sequencing. Various RNA species have been characterized, including coding mRNAs and noncoding RNAs. Through deep RNA sequencing, Nolte-'t Hoen et al. [36] uncovered a variety of noncoding RNAs, including microRNAs (miRNA), small nuclear RNAs (snRNAs), circular RNAs (circRNAs), etc. Interestingly, like lipids, a perplexed sorting mechanism also exists by selectively including certain RNA species but excluding others in the exosomes [37]. There have been different schools of thought trying to address this issue, includ-

ing the presence of recognizable RNA signal sequences and regulatory pathways.

Research activities exploring the roles of exosomal RNA have been advancing on different fronts from neurodegenerative diseases to different types of cancer. In one translational study aiming for therapeutic purposes against pancreatic ductal adenocarcinoma (PDAC), Kamerkar et al. [38] demonstrated state-of-the-art design of their experiment by taking advantage of the unique features of exosome as a delivery system and molecular features of PDAC. As mentioned above, exosomes are a portion of cellular cytoplasm and membrane that contains transmembrane proteins. CD47 is an integrin-associated transmembrane protein that functions to protect the cells from being phagocytosed by monocytes and macrophages. It, therefore, enhances endocytosis and delivery of the cytoplasmic content to the recipient cells [39]. As PDAC frequently harbor mutations in KRAS gene, investigators developed exosomes derived from normal fibroblast-like mesenchymal cells to carry short interfering RNA to functionally knock down this oncogenic KRAS G12D mutation. This exosome-based treatment by combined use of these features successfully achieved tumor suppression in mouse models of PDAC with a significant increase in overall survival.

The creation of a dedicated program met the enthusiasm in extracellular RNA research under the US National Institutes of Health, the Extracellular RNA Communication Consortium (ERCC). Another program is the International Society for Extracellular Vesicles (ISEV), which surveyed extracellular vesicles and RNA. The ISEV survey showed that although relatively new, the field has generated great interest and commitment both in resource and funding. However challenges remain in elucidating the mechanisms behind RNA production, transfer, and function in recipient cells.

3.1.4 Endothelial Cells and Angiogenesis

Angiogenesis and lymphangiogenesis is an integral part of TME. The clinical implications of TME can be dated back about half a century ago when Dr. Judah Folkman published his article " Tumor angiogenesis: therapeutic implications" [40]. Briefly, tumor growth is accompanied by neovascularization, which is defined by angiogenesis and lymphangiogenesis essential to supply the necessary nutrients and oxygen to support tumor growth, proliferation, and metastasis. Angiogenesis is a dynamic process in response to angiogenic stimulation involving the participation of multiple cell types and factors in the TME. Central to the angiogenic process is the endothelial cells that can come from existing vessels from which new vessels sprout or originate from bone marrow-derived mesenchymal stem cells or even tumor stem

cells. In turn, endothelial cells can exhibit different phenotypes, including "dedifferentiating" into CAFs as aforementioned under the CAFs section [6]. Behind the cellular components are multiple factors that impact the angiogenic process, including hypoxia, tumor cell burden, inflammation, genetic alterations of tumor cells, and signaling molecules. Among the many known factors is vascular endothelial growth factor (VEGF) and integrin.

Of the prolific angiogenic growth factors (VEGFs, FGF, PDGFs), VEGF has been the focus of attention because of its pivotal roles in angiogenesis. Bevacizumab (Avastin), the mAb against VEGF, is the most widely used therapeutics in oncology and other FDA-approved anti-VEGF drugs. The family of VEGF have several members, i.e., VEGF A-E and PIGF (Placenta Growth Factor), and a number of isoforms due to alternative splicing. VEGF-A is the major player in regulation of angiogenesis and thus is frequently referred to as VEGF. VEGF-C and VEGF-D are primarily implicated in regulation of lymphangiogenesis, which is recognized as another mode of vascularization in tumors and as alternate route for cancer cell dissemination [41]. Overexpression of the VEGF genes was detected in both tumor and CAFs and the proteins are secreted as soluble growth factors in the ECM that function via binding with VEGF receptors on the surface of endothelial cells or other target cells. The binding triggers a cascade of signaling events in the VEGF regulated pathways that eventually lead to proliferation of endothelial cells and formation of new blood vessels. It is worth mentioning, however, that the tumor neovasculature thus formed are irregular and defective. As a result, the defective vasculature will expose multiple cell types to the TME with various stimulating signals and eventually trigger a cascade of events leading to more aggressive tumor behavior [42].

Knowledge of VEGF led to an investigation using mAB to block its function, which was successfully tested on xenograft mouse models by Kim et al. [43]. The preclinical success led to clinical trials using a humanized murine antibody, i.e., A.4.6.1 and eventual FDA approval of Bevacizumab in 2004 for previously untreated metastatic colorectal cancer and subsequently for patients with non-squamous non-small cell lung carcinoma among other malignancies. The success also provided opportunities for FDA approval of other VEGF inhibitors, including multiple tyrosine kinase inhibitors (TKI); Ramucirumab, which targets the VEGF receptor (VEGFR2); Ziv-aflibercept, which targets a chimeric VEGF (VEGF-A, VEGF-B, PIGF)

Integrin is another TME molecule that deserves attention because of its roles as hub for crosstalk between cells and ECM as well as roles in modulating tumor angiogenesis. Integrin comprises two subunits, i.e., α and β subunits, that form a heterodimer to combine with target receptors found in substances of ECM, which include fibronectin, vitronectin, tenascin, fibrillin, etc. Detection of integrin αvβ3 in endothe-

lial cells helped link its role to angiogenesis and offered research opportunities for targeted therapy in several different tumors, including glioblastoma (GBM) [44]. GBM has rich vasculature and high-level integrin αvβ3 expression and matrix protein vitronectin. Taking advantage of these features, MacDonald et al. [45] demonstrated antiangiogenic efficacy of αv integrin antagonist in mouse models of GBM. The successful story helped devise Cilengitide, the first integrin antagonist, and clinical trials. Although the Phase III clinical trial failed to show improved therapeutic outcome in newly diagnosed GBM with methylated MGMT promoter, findings accumulated highlighted the potential of targeting integrins for the treatment of GBM [46]

3.1.5 Immune Cells

Dense inflammatory infiltration, tumor infiltrating lymphocytes (TILs) in many solid tumors have been observed and studied for a long time. The TILs are thought to be an immune response against tumor cells as part of immune surveillance and elimination of tumor cells that are foreign to the immune system. This hypothesis is supported by observations such as patients with immunosuppression (AIDS; organ transplant) developing certain types of cancers. On the other side, inflammation has been known to be tumorigenic, as exemplified by association of inflammatory bowel disease and colorectal adenocarcinoma, viral hepatitis and hepatocellular carcinoma, H. pylori gastritis and lymphoma, etc. Such polarity highlights the intricacy between the host's immune system and cancer.

Along with tumor cells, CAFs, endothelial cells, and other mesenchymal cells, immune cells represent one of the significant cellular types in the TME. They include neutrophils, tumor-associated macrophages (TAM), myeloid-derived suppressor cells (MDSC), dendritic cells (DC), natural killer (NK) cells, or lymphocytes as adaptive immune response. The immune cells can be resident to the TME or recruited into TME by migration via different signaling pathways and mechanisms. Functionally, immune cells can be cytotoxic to tumor cells, e.g., CD8+ cytotoxic T lymphocytes (CTL) and NK cells, or they can be immunosuppressive and protumorigenic. Since tumor-suppressive or cytotoxic functions of immune cells including neutrophils, DCs, CTL, and NK cells have been well documented in the literature, in the following paragraphs, we focus our discussion on three types of immune cells that possess immunomodulatory functions: (1) TAMs, (2) MDSCs, and (3) Regulatory T (Treg) cells.

TAMs are believed to differentiate from monocytes and are recruited from blood to TME by tumor emitted or CAF-derived signals. TAMs are known for their polarizing phenotypes in the tumorigenic process; i.e., M1 macrophages are pro-inflammatory and cytotoxic to tumor cells, M2 macrophages are anti-inflammatory and are friendly to tumor cells. It is worth mentioning that M1 and M2 macrophages should not be simply viewed as physically distinct and separate subclonal cell populations, but instead, they represent functional plasticity that can switch from one phenotype to another upon different signaling they receive from TME at different tumorigenic stages. The concept that TAMs phenotype can mutually switch was supported by DeNardo et al. [47] using their mouse models in which suppression of M2 function of TAM helped improve the tumor toxicity role of M1 type.

The unique polarizing phenotypes of TAMs also caught investigators' attention to extrapolate their inherent clinical significance and implications. Progress is being made on several fronts including prognosticating different types of solid tumors by quantifying TAMs' density and M1/M2 ratio using established immunohistochemical markers or by determining TAMs' locations to correlate with the tumor's clinical behavior [48]. Advances in therapeutics are also noticeable, including suppressing tumor growth by inhibiting M2 TAMs or promoting switching of M2 to M1 TAMs; immunotherapies by PD1/PD-L1 signaling blockade; targeted therapies by mAb and inhibitors; genetic modifications, etc.

MDSCs have been increasingly recognized as a major regulator of host immune response to evade host immunosurveillance. They are mobilized and infiltrate tumors to disrupt or hinder the processes of TAMs polarization, CTL and NK cells cytotoxicity, and dendritic cells antigen presentation. MDSCs were discovered from an early study of inflammation on tumor-bearing mouse models in which a subset of myeloid cells expressing CD11b and Gr-1 were revealed [49]. Since there is no mouse Gr-1 ortholog in humans, MDSC is functionally and phenotypically classified into two major subsets, polymorphonuclear (PMN) and monocytic (M)-MDSC. Criteria to define different types of MDSCs were suggested based on a combination of a set of differentially expressed surface markers, including CD11b, CD14, CD15, etc. Functionally, MDSCs' capabilities to suppress NK cell, lymphocytes, especially CTLs, were demonstrated by a number of experimental models. MDSCs also produce several molecules to help suppress immune responses, e.g., arginases, NO, and ROS. Although knowledge on MDSCs has been improving in recent years, clinical advances in utilizing MDSCs as a prognostic marker and as a therapeutic target by immunomodulatory therapies have not been significant. In addition, the involvement of MDSCs is also being studied in infectious disease, autoimmunity, aging, obesity, pregnancy, and transplantation.

Treg cells are another immune cell type in the TME that possess immune-modulatory functions and functionally demonstrate some overlap with MDSCs in regard to the suppression of tumor-associated antigen presentation and cytotoxic T cell function. The discovery of Treg cells represented efforts of a generation of immunologists that started with the hypothesis in the 1970s that a type of suppressive T cells needs to be present to regulate and maintain homeostasis of the host immune system. The breakthrough came with the finding of CD25 as the surface marker for Treg in 1995. With the subsequent discovery of Treg cells specific forkhead helix transcription factor (Foxp3) as necessary and sufficient for the immune suppressive function, the CD4+CD25+Foxp3+ lymphocytes were therein established as Treg cells [50].

Treg cells are a heterogeneous population and can be either naturally occurring/thymus-derived or induced/peripherally derived. Several mechanisms have been identified on how Treg cells exert their suppressive functions on effector T cells by intercellular interactions, secretome, metabolic interruptions, or by regulating FOXP3 expression. The improved understanding of Treg cells led to many clinical trials. In organ transplantation medicine, researchers took advantage of Treg cells' immune-modulatory functions to infuse patients with ex vivo expanded Treg cells to prevent graft-versus-host disease [51]. On the other hand, investigators also utilized the essence of Treg cells' protumorigenic effects to devise immunotherapeutic strategies by depletion of Treg cells in the hope of augmenting antitumor immune responses by effector T cells. Such depletion strategies can be achieved by direct infusion of Treg-depleted donor lymphocytes to patients with hematopoietic malignancies or by functionally decreasing Treg cells via blocking their migration to TME, targeting immune checkpoint (CTLA-4, PD-1, etc.) and other molecules of Treg cells. With FDA approval of ipilimumab, an anti-CTLA-2 mAb, as the first-line treatment of several solid tumors, including non-small cell lung cancer, therapies targeting immune-modulatory cells hold great promise. However, given the heterogeneity, plasticity, and diverse effects of Treg cells on tumor, more studies are needed on their tumorigenic mechanisms in order to provide new strategies for tumor immunotherapy and develop more effective and less toxic antitumor compounds.

3.2 Conclusion

There have been significant advances in the field of TME with many new discoveries. This chapter set out to cover the most important achievements in both historical perspective and recent progress in TME literature. Discussions were focused on five key coconspirators in TME, namely CAFs, associated secretomes, exosomes, endothelial cells, and immune cells, with emphasis on their fundamental biological roles and functions in tumorigenesis. While each of these coconspirators appears to function as an independent entity, their functions are intertwined and overlap in promoting tumorigenesis. The field is still evolving, but the definitions, basic functions, and roles of the conspirators in TME in tumorigenesis were highlighted. We have tried to review the most current research models, milestones, cutting-edge technologies in targeted therapies, and their roles in providing personalized therapies and potentials for clinical applications.

Study Questions
- With the accumulation of knowledge on CAFs, what directions do investigators need to pursue in the future?
- What can clinical scientists make the best use of CAFs' secretome?
- How does the notion of Exosomics enlighten us?
- What aspects of tumor angiogenesis remain to be further investigated?
- With the advent of immunotherapy, immune cells in TME are getting the attention and resource commitment they deserve. What do we have to do to maintain the momentum?

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