

Review on Tumour Microenvironment Cell Types Associated with Metastatic Cancer

Srirupa G. Choudhary^{1, #}, Pravin D. Potdar²

¹ Department of Biotechnology, Pondicherry Central University, Puducherry 605014, India

² Department of Molecular Medicine and Biology, Jaslok Hospital & Research Centre, Mumbai, Maharashtra 400026, India

Abstract: The Tumour microenvironment (TME) is a highly dynamic and perpetually altering entity of cancer development, progression, invasion, and dissemination. TME is interspersed with cellular components such as immune cells, blood vessels and stromal cells, and non-cellular components comprising extracellular matrix (ECM) and exosomes. Different cancer types possess distinct phenotypes of TME. The intricate processes of TME facilitate uncontrolled bulk formation, and chaotic vascularization, supporting oxygen and blood availability, along with the removal of metabolic wastes and aberrant metastatic colonization and seeding. Tumour masses are embedded with diverse innate and adaptive immune system cell milieu, capable of performing both pro and anti-tumorigenic effects. The nexus underpinning cancer cells and associated TME, such as cell-cell or cell-ECM interactions, play a key role in determining safe and effective therapeutic interventions. This review mainly emphasizes different roles of various cell types involved in the tumour microenvironment in the progression and promotion of metastatic potencies of tumour cells. We have further suggested that the targeting of all these cell types in the tumour microenvironment may give better cancer therapies in the near future.

Keywords: Tumor microenvironment; Metastasis; Cancer; Cancer therapeutics; Extracellular matrix.

1. Introduction to TME and Metastasis Process

The most predominant cause of cancer-pertinent mortality is metastasis. Metastasis is defined as a cascade of events ranging from tumour enlargement and progression, intravasation of neoplastic cells into circulating bloodstream as Circulating Tumor Cells (CTCs) via the trans-endothelial cells, distant extravasation, metastatic colonization and spread^[1,2]. The non-random metastatic cascade is identical in diverse types of cancers regardless of the final colonization site. This is exemplified by the Epithelial-Mesenchymal Transition (EMT) program. The EMT plays a crux in the departure of cancer cells from primary tumours. Epithelial-mesenchymal transition cites the depletion of epithelial traits and attainment of mesenchymal characteristics. This transition is essential for re-directing the carcinoma cells to surrounding parenchyma tissues and intravasate via the endothelial cells into the bloodstream. Certain EMT-inducible transcription factors such as Snail, Slug, Twist and Zeb1 modulate this transformative stage. Related studies unfold more complicated dynamics between EMT and metastasis. CTCs traverse into the bloodstream as single cells or in clumped groups. CTCs also permit neutrophils and platelet infiltration to escape immunosurveillance. Cancer cells remain passive at secondary sites to facilitate adjustment to the new

niche environment^[3].

An array of factors ranging from genetics, immune cell involvement, transcriptional and others modulate cancer progression. Nonetheless, it is exceptionally challenging to unwind the intricate process of metastatic metabolism, proliferating tumour reprogramming and plasticity^[4].

Some of the critical drivers of metastatic phases are coordinately synchronized spatiotemporally. In case the primary tumour is tissue-specific polarized, metastatic dissemination is inevitable. The leading hallmarks of metastatic dissemination are uncontrolled cell migratory behaviour, restructuring of extracellular matrix base, leaky and permeable blood vascularization at the tumour site, and neoplastic cell spread in circulation, lymph, perineural and perivascular routes. Some prime angiogenesis in distinct types of cancers includes innervations via lymphatic, extra-vascular and hematogenous routes.

Dormant metastasis is an underrated cancer characteristic owing to the lack of suitable experimental models. Recently, *in vivo* models have been selected wherein early-stage tumour-derived Metastatic Initiating Cells (MICs) are potent enough for seeding dormant metastasis and spontaneously generate spread, thus mimicking disease course in patients. Numerous animal models unearth the findings of the biology of MICs and dormant metastatic exits.

Colonization and expansion at distant secondary sites are the most devastating metastasis stage, both clinically and biologically. Evading organ-specific barriers and productive host-tissue ecosystem interactions greatly facilitate this extensive wildfire spread. Some organ-specific metastatic colonization sites include the lungs, liver, bone, and central nervous system^[5].

Correspondence: Srirupa G. Choudhury, E-mail: srirupafeb20@gmail.com Department of Biotechnology, School of Life Sciences, Pondicherry Central University, Kalapet, Puducherry 605014, India.

Received 25 February 2023; Revised 17 March 2023; Accepted 18 May 2023; Available Online 13 June 2023

DOI: 10.54457/DR.202302001

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2. Tumor Microenvironment (TME)-A milieu reprogramming metastatic invasion and dissemination

TME is a one-of-a-kind heterogenous double-edged sword interspersed with cancerous cells, stromal cells, and immune cells capable of secreting macromolecular metabolites. Such growth factors and molecules are directed to navigate uninhibited cellular growth, intravasate into endothelial-lined blood vasculature, and cell migration in clusters. As circulating tumour cells (CTCs) extravasate into secondary sites, metastatic colonization and establishment occur in distant organ systems^[6]. Abnormal genetic predisposition drives tumour initiation and seeding; however, almost two decades of extensive research indicate that the TME niche controls neoplasm's structural and functional phenotypic plasticity^[7].

The hallmarks of the TME include the following: (1) Hypoxic niche: Intra-tumoral hypoxia is chiefly characterized by redox adaptation and cancerous outgrowth (2) Immunological microenvironment: TME is composed of immune-infiltrating cells such as T lymphocytes, B lymphocytes, natural killer (NK) cells, myeloid-derived suppressor cells (MDSCs), mast cells, dendritic cells (DCs), tumour-associated neutrophils, cancer-associated fibroblasts (CAFs), tumour-associated macrophages (TAMs), vascular endothelial cells, cancer-associated pericytes and bulk malignant cells (3) Lactate, Reactive Oxygen Species (ROS) and Lipid Metabolism: Lactate metabolism enhances angiogenetic factors and creates an intra-tumoral acidic environment, enhancing macrophage polarization towards a pro-inflammatory and pro-tumour characteristic. Lipid metabolism is associated with cancer recurrence and promotion of pre-metastatic niche in ovarian cancer. ROS affects the regulation of immune components of TME, such as myeloid-derived suppressor cells (MDSCs), TAMs, CAFs and stromal cells. However, a phenomenon called ROS addiction is characterized by the co-evolution of cancer cells and ROS^[8].

3. Immunology-associated influence on TME

The complex rogue nature of TME is characterized by blood vessels, lymphatic vessels, and nerve innervations interspersed with immune cells and stromal cells. The immune entity helps in the thriving of the self-sustained tumour microenvironment. Immune surveillance, ECM remodelling and vascularization are key components of a well-designed TME that facilitates the interplay of tumour and stromal cells. Immune cells and related soluble metabolites/growth factors that are innervated surrounding the tumour always impact tumour progression and disease. There are four significant approaches pertaining to this phenomenon. (1) Tumour-draining lymph nodes (TDLNs) are the primary sites where a nexus is formed between antitumor immunity and immunosuppression. These are the primary locations where cancer-associated antigens are presented to naïve T cells, and an antitumor immune response is exerted. TDLNs are potential therapeutic targets of a few of the specific cancer tumour microenvironments. Studies in colorectal cancer patients shed light on colonizing regulatory T cells in the tumour-draining lymph nodes (TDLNs). However, a dearth of the same in the tumour site indicates disease amplification and spread. (2) Spleen, a secondary lymphoid

organ, on the other hand, is situated beyond the realms of the TME. The latest scientific findings unveil that splenectomy enhances antitumor immune functions by elevating the functionalities of FoxP3⁺ regulatory T lymphocytes at the metastatic sites. This improves a number of immunotherapeutic interventions. (3) Bone marrow and TME are imperceptibly cross-linked, accelerating cancer development, neovascularization and a multitude of underlying mechanisms. Bone marrow is a reservoir that aids priming of tumour antigen-specific CD4⁺ T and CD8⁺ T cells, inducing antitumor immune response and cancer progression. (4) Systemic inflammation is considered an immune exercise and is prevalent in lower incidences of lung cancer development. Growth factors namely tumor-induced cytokines prime TME and accelerate inflammatory pathways and cancer development^[9].

Immunotherapy is taking over the cancer treatment market with a considerable success rate. However, limitations exist as some cancer patients do not respond positively to it. Many cancer subtypes of triple-negative breast cancer patients respond aberrantly to different metabolic inhibitors, where one acts to inhibit glycolysis and another response to inhibit lipid biosynthesis. Recent findings reinforce that metabolic reprogramming and metabolism of TME are adjunct to immunotherapy^[10].

4. Neurogenic control on the TME

The interplay between the nervous system and cancer cells via the parasympathetic or sympathetic nerves sets tumour progression and dissemination in motion. Haematological malignancies, solid tumours such as pancreatic cancers and gastric tumorigenesis are reported to be in neural regulation, mediating cancer invasion and metastasis^[9].

The heterotypic tumour microenvironment (TME) is dispersed with infiltrating T lymphocytes exhibited with loss of PPAR-gamma coactivator 1 α (PGC1 α). PGC1 α is functionally responsible for mitochondria biogenesis, metabolically rewired by the chronic AKT signalling transduction pathway. However, newly developed and metabolically reprogrammed and activated PPAR-gamma coactivator 1 α (PGC1 α) tumour-specific T cells exude a higher degree of intertumoral homeostasis and effector function^[10]. NOS2 and COS2 are inflammasomes responsible for tissue restructuring and adaptation to cancer stem cell characteristics (CSCs) and endow metastatic phenotypic plasticity to breast cancer patients, respectively. Dysregulation of this NOS2/COS2 physiologic axis leads to critical changes in intra-tumoral and intercellular communication with the immune system and their adaptation to the hypoxic tumour microenvironment^[11].

5. Influence of the cellular and non-cellular TME entities modulating cancer progression

The TME is composed of non-cellular components such as extracellular matrix (ECM), basement membrane (BM) and cellular components, namely adipocytes, cancer-associated fibroblasts, tumour endothelial cells, tumour-associated macrophages, tumour-infiltrating immune cells, pericytes and a plethora of growth factor signalling molecules like IL-6, IL-1 β , FGF-2, PDGF that are capable of modulating tumour motility and chaotic metastatic

seeding at distal organs (Fig.1). In this review, the main aim underscores the elucidation of diverse types of cellular and non-cellular components interspersed in the tumour microenvironment and regulating the entire landscape of the deadly disease.

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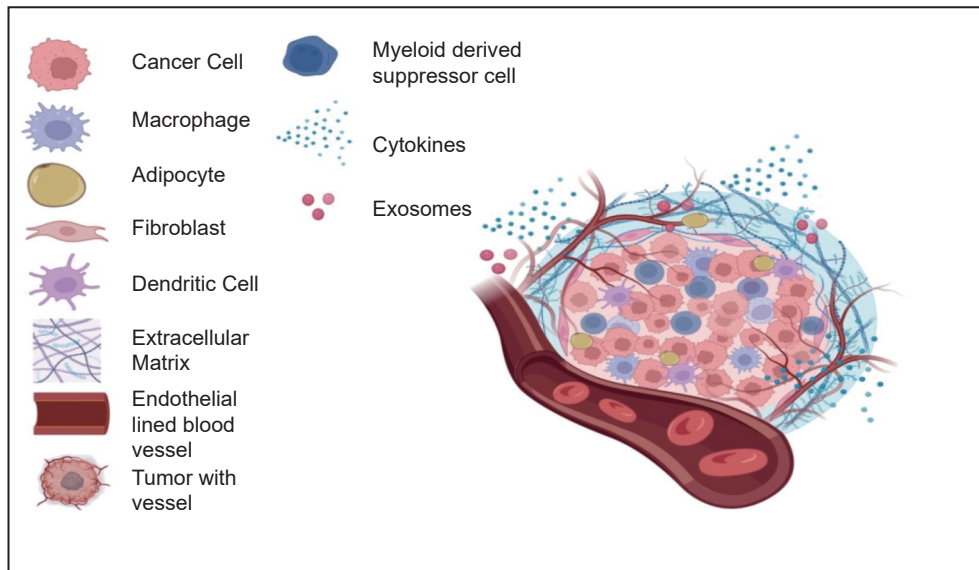


Fig. 1. A brief Tumor Microenvironment (TME) outlook depicting different cell types responsible for metastatic cascade.

5.1. Dendritic Cells (DCs)

DCs are primarily derived from bone marrow hematopoietic progenitor cells destined to enter either myeloid or lymphoid lineages^[12]. Such cells are more popularly deemed as the critical sentinels of the immune system, with the prime role of cementing the gap between the innate and adaptive immune systems. In 1973, the Nobel laureate Ralph Steinmann pioneered this concept and eventually received the Nobel Prize in 2011^[13]. Dendritic cells are the most professionally potent antigen-presenting cells (APCs) with adaptive immune functionalities, ranging from uptake, processing, and presentation of antigenic peptides, including tumour-associated antigens (TAAs). This, in turn, stimulates naïve antigen-specific CD4⁺ and CD8⁺ T cells. This activity is validated by the study of DC-deficient animals where downregulation/knocking out of Batf3, an indispensable transcription factor (TF), confirms the significance of DCs in tumour immunology^[14]. Apart from presentation to T lymphocytes via Major Histocompatibility Complex (MHC I) and (MHC II) molecules, DCs can produce cytokine and growth factors. Such secretions mediate interaction with other heterogeneous immune cell populations^[15]. In the homeostatic signalling pathways, DCs play a leading role in the induction and maintenance of immunological memory. A higher degree of plasticity is evident in such populations due to differential morphogenetic and functional phenotypes^[16]. Dendritic cells are majorly classified into three categories, namely conventional/classical DCs (cDC), plasmacytoid DCs (pDC) and monocyte-derived DCs (moDC). cDCs are further subdivided into two subtypes: cDC1 and cDC2. This is in accordance with the presence of TFs and surface molecules. pDCs secrete type I interferon, and moDCs are derived from monocytes and reside in localized tissues^[17].

Conventional or classical DCs are subdivided into two subtypes, namely cDC1 and cDC2. cDC1 is highly specialized in exogenous antigen presentation to CD8⁺ T cells via MHC I. Their

developmental events are tightly regulated by interferon regulatory factor 8 (IRF8) and leucine zipper transcriptional factor ATF-like 3. On the other hand, endogenous antigens are presented by cDC2 to activate CD4⁺ T cells via MHCII. Interferon regulatory factor 4 (IRF4) modulates the development of this subset^[13]. pDCs are multifaceted with potential implications of detecting viral nucleic acids via (TLR)-7 and -9, autoimmune diseases, namely systemic lupus erythematosus (SLE) and psoriasis, and cancer, albeit controversially. This DC subset is initially localized in lymphoid organs and then circulates in the bloodstream to reach the lymph nodes^[15]. pDCs specialize in the secretion of tons of type I interferons, preventing tumorigenic proliferation, metabolic rewiring of neoplastic tissues, induction of angiogenesis and metastatic spread^[15]. Studies, however, contrastingly correlate that melanoma, breast and ovarian cancers have poorer prognostic outcomes accompanied by heightened cell proliferation. This is due to the generation of suppressive TME phenotypes and decreased cytotoxic activity of CD8⁺ T cells^[18]. Infection and inflammatory events guide the production of moDCs and aid in differentiating CD4⁺ T-cells towards an evolving T helper cell phenotype^[15]. Context-dependent tasks, namely extravasation into endothelial lining tissues and differentiation into monocyte-derived macrophages, are performed by human-derived CD14⁺/CD16⁺ monocytes. These monocytes secrete IL-10, maintain immunological tolerance, and migrate to damaged and inflamed areas where pro-inflammatory mediators like TNF- α and IL-23 are secreted^[16].

DCs possess an orchestrated and spatial-temporal effective antitumor response. Some of the streamlined coordinated activities follow as such: antigen uptake and processing, activation in response to extrinsic signals; maturation and trafficking to lymphoid tissues; antigen presentation and priming of naïve T lymphocytes via MHC I/II-TLR-ligand interaction; infiltration of T cells into the TME, mediated by DC-derived chemotactic signals and

finally in-situ crosstalk between effector T lymphocytes and cytokines in the TME niche^[19].

DC Activation in TME: Immediately after TLR-ligand interaction, many metabolic alterations arise within the DCs. Those are elevated glucose uptake and lactate production guided by the phosphoinositide 3-kinase (PI3K) /serine/threonine kinase (AKT) pathway and the TANK binding kinase 1 (TBK1)-I κ B kinase ϵ (IKK ϵ) pathway. NADPH, citrate, and reactive oxygen species (ROS) are generated from the pentose phosphate pathway, tricarboxylic acid cycle and electron transport chain, respectively. Citrate coupled with NADPH are the precursors of fatty acid synthesis. The number of Golgi and endoplasmic reticulum (ER) in DCs rises due to the conversion of citrate into acetyl CoA and subsequent incorporation into FAS. ROS, on the other hand, safeguards antigens from degradation in DCs. Thus, the idiosyncrasy underpinning citrate usage in DCs supports activation, maturation, differentiation, expansion, and specialization in biological actions^[17].

5.2. Cancer-Associated Fibroblasts (CAFs)

Former studies reveal CAFs are activated, reprogrammed, and metabolically rewired in the TME. Thus, tracing the cellular origin of the CAF subsets partially confirms the functions of such a heterogeneous population. Mapping of such studies shed light upon oncological therapeutic interventions^[20]. CAFs are a group of the most abundantly available heterogeneous stromal cells near the TME^[21]. The heterogeneity factor is attributable to the various cellular precursors^[22]. CAFs are both phenotypically and epigenetically distinct from normal tissue-resident fibroblasts. These cells remain endlessly programmed and exhibit biological roles by secretion of soluble factors and formation of extracellular matrix (ECM)^[23]. Tissue-resident fibroblasts, commonly christened as quiescent fibroblasts, are the direct cellular precursors of CAFs. Such fibroblasts bear different regulators for different cancers and are activated via a myriad of modulators, namely fibroblast growth factor 2 (FGF-2), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), transforming growth factor (TGF)- β , reactive oxygen species (ROS) and stromal-derived factor-1 (SDF-1)^[24]. Resident fibroblasts differentiate into myofibroblasts and aid in tissue repair^[21]. Mesenchymal stem cells are alternative precursors of CAFs. Mesenchymal markers such as α -SMA and FAP are abundantly expressed due to the transdifferentiation of epithelial cells and endothelial cells into CAFs via epithelial-to-mesenchymal transition (EMT) or endothelial-to-mesenchymal transition (EndoMT)^[22]. During malignancy, microRNAs (miRNAs) aid in the activation of CAFs. This is demonstrated in lung adenoma-carcinoma. Small endogenous noncoding RNAs specifically knock out target gene expression and act as potential biomarkers in cancer diagnosis^[25].

Within the proximity of the TME, CAFs promote tumorigenic growth and invasion, reconstruction of ECM base, and induce vasculature, namely; angiogenesis and lymph angiogenesis, infiltration of immune cells and support neoplastic inflammation^[21]. During tumorigenesis, CAFs robustly act in the turnover and cross-linking of collagen, forming tumour desmoplasia and elevated non-plasticity. Interaction between CAFs and ECM deposition leads to metastatic invasion mediated by matrix metalloproteinase-dependent and independent mechanisms^[21]. An intricate interplay between CAFs, cancer cells and macrophages stimulate angiogenesis. Comito et al. indicated that polarized M1 macro-

phages were differentiated into M2 polarization de novo with the assistance of CAFs and prostate carcinoma (PCa) cells. This, in turn, drives the vascularization interspersed within the PCa tumour^[26]. Previous investigations shed light upon the enhancement of tumour angiogenesis and lymphomagenesis, degradation of ECM deposition and metastatic invasion, performed by co-culturing fibroblasts and nonsmall cell lung cancer tumour cells^[25]. CAFs are involved in chemoresistance and maintain stemness in breast cancer cells. Additionally, Wnt-pathway induces functional diversification of colorectal CAFs and reinforces cancer progression^[27]. In the TME niche, tumour cells are metabolically rewired and reprogrammed to meet their continual demand of uninhibited cell expansion. This is possible due to elevated amounts of ATP, deficit oxygen levels and numerous nutrients. The Warburg effect is exhibited in CAFs and is one of the most intelligible cancer metabolic modalities, delineating CAFs-tumour cell synchronization. Such an effect encourages uninterrupted cellular proliferation, spermatogenesis and immune escape^[20]. Certain signalling pathways, namely EGFR signalling, TGF- β signalling, Hippo signalling and Wnt signalling, modulate the plasticity of CAFs and direct future targeted therapies^[22].

5.3. Cancer-Associated Adipocytes (CAAs)

Adipocytes are stromal cells localized in various tissues and have functional phenotypic factors in the TME. In-vitro, in-vivo and clinical data reveal that adipocytic factors augment tumour progression. Peritumoral adipocytes exhibit higher levels of fat-derived factors, lowered differentiation markers and induction of metabolic rewiring of cancer cells, subsequently termed Cancer Associated Adipocytes (CAA)^[28]. Research examinations unfold the synchronized connection between breast cancer and CAAs and how the neoplastic-driven state formats the TME. The TME has the functional phenotypes of contactless proliferation, vascularization, invasion, and metastatic dissemination. CAAs reside in the invasive entrance of mammary gland tumours, accompanied by lower levels of lipid deposition and over-secretion of inflammatory cytokines and enzymes. Such cells are small and irregularly shaped with abnormal expression of adipokines, namely leptin, adiponectin, CCL2, and CCL5^[29]. Adipocytes adjoining the ovarian-omental metastatic cancer cells are known as CAAs, capable of secreting macromolecules/metabolites to provide energy to cancerous masses. Such excellent adipogenic phenotypic flexibility directly links inflammation circuitry and CAAs^[30]. Secreted protein acidic and rich in cysteine (SPARC) is an ECM protein that reprograms differentiation of adipocytes and contextually represses adipogenesis, resulting in the fatty bone marrow and osteoporosis^[31].

5.4. Tumor-Associated Extracellular Vesicles (TAEV)

Current, in-depth investigations reveal that TME stromal cells and cancer cells interact via non-classical, secretory vesicles, commonly called extracellular vesicles (EV). EVs comprise macromolecules such as lipids, nucleic acids and proteins that act over many ranges to regulate intercellular crosstalking and maintain the orchestration of homeostasis and diseased states^[32]. The primary mediators cementing communication interplay between the CAFs, and neoplastic cells are Tumor-Derived Extracellular

Vesicles. (TDEV) TDEVs result in ECM restructuring and matrix metalloproteinases (MMPs) release. In the case of rectal cancers, EVs secreted by carcinoma cells stimulate fibroblasts to differentiate into CAFs which in turn are reprogrammed for dysregulated growth and tumour formation. Additionally, EVs produced from ovarian cancers upregulate KLF6/NF- κ B signal transduction axis and reinforce tumorigenesis^[33]. TDEVs are also capable of inducing systemic energy metabolism. This is well exemplified by elevated levels of miR-122, a breast cancer EV that reduces glucose intake by downregulating the glycolytic enzyme pyruvate kinase. This mechanistic strategy dovetails uninhibited metastatic spread. However, inhibition of miR-122 lowers neoplasm *in vivo*^[34].

5.5. Tumour-Associated Endothelial cells (TAEs)

The Tumor Endothelial Cells are characteristically different from normal endothelial cells concerning molecular, morphological, and physiological signatures. Molecularly, tumour endothelial cells are perpetually transcriptionally activated (e.g., Myc-targeting) with aberrant levels of RNA content due to increased nucleotide biosynthesis and glycolysis. In terms of morphological aspects, the cells are heavily fenestrated with intercellular connections to adjacent cells. Heavily packed endothelial cells act as an impediment to seamless functioning and facilitate leakiness^[35]. Tumour vascularization is guarded by endothelial progenitor cells. Such cells within the TME are connected to anomalous pericytes, which may lead to leakiness between the cell-to-cell interconnections and allow dispersion of CTCs, indicating metastasis^[36]. VEGF and FGF-2 are some of the cytokines derived from the TME, inhibiting endothelial cells' ability to upregulate chemoattractant expression (i.e., CXCL7, CXCL10.) and adhesion molecules (ICAM1 and VCAM1). This, in turn, promotes immune surveillance escape and uncontrolled cellular growth^[37].

5.6. Tumour-Associated Macrophages (TAMs)

The TME niche is embedded with the vital element known as the Tumor-Associated Macrophages (TAMs), a set of heterogeneous populations whose origin and half-lives differ in greater magnitudes. Some long-lived, tissue-localized TAMs belong from the embryo; however, bone marrow is the source of short-lived, disseminated monocyte-derived macrophages that are attracted towards TME via chemotactic factors known as CCL2, CCL5 and Macrophage-Colony Stimulating Factor (M-CSF)^[2].

Due to the heterogeneous population, TAMs in the TME niche have diverse roles in cancer development. TAMs have a direct role in tumour proliferation. This is mediated by the secretion of growth factors, namely Epidermal Growth Factor Receptor (EGFR), aiding in uncontrolled cellular growth. In the TME-recruited macrophages, elevated signaling pathways such as Wnt/ β -catenin regulate tumour invasion in liver cancer progenitor cells. Also, TAMs upregulate metastatic invasion by secretion of ECM degrading enzymes such as MMPs and cathepsin, allowing evasion of immunosurveillance. IL-1ra acts as an immunosuppressive cytokine secreted by TAMs and supports tumour stemness and invasive spread. The prime element of TAM biology is immune escape and repression. In-depth investigations illustrate that macrophages derived from bone marrow monocytes lower the

activity of the STAT3 signal transduction pathway through stimulation of hypoxia-induced activation of CD45 phosphatase, facilitating transdifferentiation into TAMs^[38]. Targeting of TAMs is the primary focus of cancer therapeutic modalities with two mainstream approaches: (1) TAM depletion using anti-neoplastic drugs like trabectedin and monoclonal antibodies such as CCL2/CCR2, CSF-1/CSF-1R, CXCL12/CXCR4 and (2) TAM rewiring using agonists like TLRs, CD40, inhibitors of NF- κ B, HDAC, and mi/siRNA^[39].

5.7. Cancer Stem Cells (CSCs)

Initiation of tumours is heralded by two mechanistic principles: driven either by transformed differentiated cells or altered tissue-localized stem cells. This transformative process is facilitated in response to radiation, toxins, inflammation and during tissue regeneration. Also, over-expressed oncogenes and repressed tumour suppressor genes lead to the recurrence of hypo-differentiation and neoplastic transformation^[40]. The CSC theory hypothesizes that tumorous masses are analogous to organ systems, coupled with atypical bulk tumour growth, perpetual self-renewability, and homeostasis^[41]. The discovery of CSCs in acute myeloid leukaemia in 1997 drove further identification in solid cancers, namely hepatic, pancreatic, ovarian, head and neck, and glioblastoma^[42]. The five most common mechanisms govern CSC biology: (1) Developmental pathways, (2) Epigenetics, (3) Epithelial-Mesenchymal Transition (EMT), (4) Cell cycle regulation and apoptosis, (5) Stem cell factors^[43].

CSCs are a heterogeneous stromal population and are naturally unfavourable to target. These are aberrantly resistant to cytotoxic therapy, leading to tumour deregulation and relapse. To maintain the stemness of CSCs, the CSC niche of the TME plays a prime role. Erwei Song et al. tried to eradicate CSC from the TME effectively. His lab intensively studies breast cancer samples with the surface molecules CD10⁺ and GPR77⁺ CAFs predominating the CSC niche. Such CAFs enrich the CSC niche and induce chemoresistance by interaction with the IL-6 and IL-8, which in turn relies on NF- κ B signalling^[44]. The TME comprises immune cells, ECM, CAFs, MSCs, ECs, stem and differentiated cancer cells and a complex network of growth factors and cytokines, collectively termed as secretome. All these components synchronize neoplastic angiogenesis, immune surveillance, and metastasis. The interplay between CSCs and TME is orchestrated by the release of the encapsulated exosomes and microvesicles, growth factors (IL-6, IL-8, and VEGF) hormones, metabolites (matrix metalloproteinases) and chemokines. All these entities significantly stimulate the TME and its metabolism, radio, and chemotherapy resistance^[45].

5.8. Extracellular Matrix (ECM)

The ECM is embedded with hundreds of distinctly unique macromolecular complexes, namely glycolipids such as proteoglycans, heparan sulphate, hyaluronan and versican, collagen and glycoproteins such as fibronectin, elastin, and laminins. ECM is divided into subcategories: interstitial matrix and basement membrane. Interstitial matrix is a 3D model-like structure, encircling tumour clonal tumour cells, intermingled with stromal cells and strengthening the structural integrity of tissues and organ sys-

tems. On the other hand, the basement membrane is a dense sheet-like structure that acts as a barrier for an in-situ carcinoma initiation and development^[46]. Maintenance of TME is looked upon by ECM, which also modulates metastatic niche. ECM plays a role in cellular motility and adhesion out of the TME and acts in the secretion of chemokines and angiogenic factors, inducing an inflammatory state. Continuous inflammatory outcomes shape stromal fibroblast cells into myofibroblasts, promoting growth factor deposition, enhancing contraction and favouring stiffness^[36].

Pancreatic Ductal Adenocarcinoma (PDAC) is characterized by desmoplastic stromal fibrotic outgrowth that hinders chemotherapeutic agents from acting into TME. The thickened state of tumorous mass mediates a hypoxic environment and rewires metabolic plasticity, supporting profuse tumour proliferation and widespread dissemination^[47].

ECM is a suitable therapeutic target for the treatment of cancers. Tyrosine Kinase Inhibitors (TKIs) with EGFR mutation are applied to ECM for treating NSCLC. (Nonsmall cell lung carcinoma) Lin et al. shed light on the tumour vaccine concept with the EDA domain of fibronectin recruiting macrophages, inhibiting angiogenesis, and averting lung metastases in a murine breast cancer model. L19 is a monoclonal antibody labelled 123I that prime antigen, EDB fibronectin and aids in imaging brain, colorectal and lung cancers. Also, clinical phase trials of nanoparticle-loaded albumin-bound (NAB) paclitaxel are currently in vogue for treating pancreatic, biliary duct and breast cancer. Nanoparticles loaded with diverse chemical drug constituents are reprogrammed for controlled release, with minimum toxic effects and higher efficiency. Thus, ECM dysregulation influences cancer progression, and dissemination and targeting of the ECM and TME interconnection are one of the salient features of cancer therapy.

5.9. Exosomes

Exosomes are miniature extracellular vesicles measuring (40–160 nm) shuttling between the tumour mass and microenvironment. It is filled up with lipids, nucleic acids, and proteins and harbours miRNAs, which in turn triggers uncontrolled metastatic invasion^[48]. A mechanistic model of breast cancer exosomes loaded with miR-105 depicts the induction of the oncoprotein MYC. This activates MYC signalling within the vast heterogeneous population of cancer-associated fibroblasts (CAFs) for metabolic rewiring. In the case of a nutrient-rich environment, miR-105-influenced CAFs, glucose, and glutamine metabolism fuels cancer cells. Contrastingly, nutrient-deficient CAFs transform lactic acid and ammonia into energy-rich metabolites^[49].

A hypoxic environment in glioma and colorectal cancer facilitates exosome secretion harbouring substantial amounts of miRNA-210-3p. This, in turn, causes apoptotic escape and switches the cell cycle phases from G1 to the S phase by down-regulation of CELF2 expression^[50]. Exosomes are ideal diagnostic and prognostic tools in cancer biology, and their constituent entities are commonly used as biomarkers for accelerating metastatic invasion. Biomarkers include hypoxia-modulated mRNAs and proteins, namely MMPs, IL-8, and PDGFs are rich in oxygen-deficient glioma cells. Cancer patients with elevated levels of such biomarkers are found to have poorer survival rates^[51]. Non-coding RNAs such as long noncoding RNAs (lncRNA), micro RNAs (miRNAs), and circular RNAs (circRNAs) are deposited

inside the exosome's vesicles, mediating neovascularization and tumour reprogramming^[52]. Overall, exosomes are essential components of TME with potential clinical applications for curing the devastating disease.

6. Targeting Tumor Microenvironment for Cancer Therapy

Nowadays, the oncology market is inundated with numerous therapeutic regimens ranging from chemotherapy, surgery, radiation therapy and immunotherapy. Although the ever-booming pharmaceutical industries have furnished a series of new drugs against the invasive disease, not much help is garnered. This is due to the mutational devastation faced by distinct types of carcinomas. Drug targets such as EGFR, p53 and c-Myc are mutated, and thus multi-drug resistance culminates, bringing about disease recurrence, chaotic relapse, and poor diagnostic significance^[53].

The tumour microenvironment, tumour vicinity cells, and bulk tumour exterior and interior cells play a strong connexion, modulating tumour biology. Therefore, a handful of seamless strategies are found to target the efficient TME as follows: (1) Targeting Cancer-Associated Fibroblasts (CAFs). (2) Targeting Extracellular Matrix (ECM). (3) Modulation of Hypoxia and Acidosis. (4) Subversion of Neovascularization. (5) Impact on Tumor-Derived Extracellular Vesicles and Chronic Inflammation. (6) Prevention of macrophage infiltration and differentiation. and (7) Activation of anti-tumour immunity^[53].

DC vaccination is found to be helpful in prostate cancer, and hence, Provenge therapy is established. The Provenge protocol involves culturing monocytes from prostate cancer patients, differentiation into DC, activation with PAP antigens and re-introduction back into the patients. A first-generation, antibody-based therapy, namely an Immune Checkpoint Blockade (ICB), targets explicitly infiltrated immune cells localized within the vicinity of the TME. During T-cell activation and function, this therapy's functionality encompasses blocking receptor/ligand signalling (CTLA4 and PD1). Scientific findings suggest that this ICB therapy works wonders in only selected patients prior to cancer biomarker identification in those subjects^[1].

Also, Antiangiogenic therapy (AA) modulates VEGF/VEGFR signalling axis pathway, inhibiting angiogenesis, and allowing oxygen depreciation and metabolic waste accumulation to occur, subsequently preventing pre-metastatic niche seeding. Bevacizumab is a neutralizing antibody against VEGF-A, possessing a decoy receptor called Aflibercept. A tyrosine kinase inhibitor named Sorafenib is used, and an antibody named Ramucirumab blocks VEGF binding to its receptor. AA therapy coupled with monoclonal antibody PDL-1 has shown considerable success in renal and hepatocellular carcinoma^[1].

Further detailed experimental investigations shed light on other therapeutic strategies, such as CAR-NK cells, liver stellate cells and fibroblasts, which have already reached the stage of pre-clinical trials. Continual efforts are devising strategies to target TME therapeutically, encircling the cancerous immune-infiltrated masses. However, to date, FDA-approved repurposed medical interventions exhibited considerable efficacy^[1], as shown in Table 1.

Table 1. A handful of FDA-approved therapeutic agents targeting different cancer types^[54-58].

Therapeutic Agents	Molecular Targets	Mechanism of Action	Cancers Indicated	Clinical Status	References
Bortezomib (Velcade)	26S Proteasome	26S Proteasome inhibitor causing ER stress	Multiple myeloma	FDA approved	[54]
Sorafenib (Nexavat)	VEGFR	TKI of VEGFR2, PDGFR β , Raf	Renal and hepatocellular carcinoma	FDA approved	[55]
Sunitinib (Sutent)	VEGFR	TKI of VEGFR1–3, PDGFR, c-Kit	Pancreatic neuroendocrine tumours, renal cell carcinoma	FDA approved	[56]
Bevacizumab (Avastin)	VEGF	Humanized mAB against VEGF	Metastatic colorectal and renal cell carcinoma	FDA approved	[57]
Trastuzumab (Herceptin)	HER2	HER2 Targeted Therapy	Breast Cancer	FDA approved	[58]

7. Conclusion and Future Perspectives

Most cancer treatments are deemed ineffective due to generous amounts of benign and neoplastic tumours entering the relapse and recurrence mode, metastatic dissemination, colonization, and establishment. Also, therapeutic targeting to the metastatic hot-spot region is not always found to exhibit immune surveillance and killing of cancer cells.

From many research findings, it is clear that tumour cells recruit non-malignant cells in and around their vicinities and induct upon them hostile parameters, namely nutrient and oxygen deficiencies, waste product accumulation and acidity. This subsequently leads to cancer amplification, dissemination and metastasis.

The roles of various cellular and non-cellular components in the TME are explained in depth in this review. This sheds light on the microstructural anatomy and physiology of the tumour microenvironment. Targeted therapeutic intervention into the TME is the latest resort to treating different kinds of cancer.

From comprehending the ever-evolving interactome scenes within the tumour ecosystem to designing novel and innovative solutions for cancer therapy, extensive study is impending to be unlocked and implemented to understand the whole universe of tumour biology. Lab-on-chip devices and newer and novel 3D spheroids are tailored to reprogram and restructure cancer cell physiologic settings in such embodiments and replicate the respective biological phenomena enhancing the plasticity of the TME. Hence, the tumour ecosystem or the tumoral niche is therapeutically exploited as a novel strategic platform for cancer therapeutic purposes.

Abbreviations

AA, Antiangiogenic therapy; APCs, Antigen Presenting Cells; BM, Basement Member; CAA, Cancer-Associated Adipocytes; CAFs, Cancer-Associated Fibroblasts; CAR-NK, Chimeric Antigen Receptor-Natural Killer Cells; circRNAs, Circular RNAs; CSCs, Cancer Stem Cells; CTCs, Circulating Tumor Cells; DCs, Dendritic Cells; ECM, Extracellular Matrix; EGFR, Epidermal Growth Factor Receptor; EMT, Epithelial-Mesenchymal Transition; EV, Extracellular Vesicles; FDA, Food Drug and Administration; FGF 2, Fibroblast Growth Factor-2; HDAC, Histone Deacetylases; HGF, Hepatocyte Growth Factor (HGF); ICB, Immune Checkpoint Blockade; IFN, Interferon Regulatory Factor 8; lncRNAs, Long non-coding

RNAs; M-CSF, Macrophage-Colony Stimulating Factor; MDSCs, Myeloid-Derived Suppressor Cells; MHC, Major Histocompatibility Complex; MICs, Metastatic Initiating Cells; miRNAs, MicroRNAs; MMPs, Matrix Metalloproteinases; NAB, Nanoparticle Loaded Albumin Bound; NK, Natural Killer Cells; NSCLC, Non-small cell lung carcinoma; PCa, Prostate Carcinoma (PCa); PDAC, Pancreatic Ductal Adenocarcinoma; PDGF, Platelet-Derived Growth Factor; PGC1, PPAR-gamma coactivator 1 (PGC1); PI3K, Phosphoinositide 3-kinase (PI3K); ROS, Reactive Oxygen Species; SDF-1, Stromal-Derived Factor-1; SLE, Systemic Lupus Erythematosus; SPARC, Secreted Protein Acidic and Rich in Cysteine; TAA, Tumour-associated Antigens; TAEs, Tumor-Associated Endothelial Cells; TAMs, Tumor-Associated Macrophages; TBK1, TANK binding kinase 1; TDEV, Tumor Derived Extracellular Vesicles; TFs, Transcription Factors; TKIs, Tyrosine Kinase Inhibitors; TLR, Toll-Like Receptor; TME, Tumor Microenvironment; TNF, Tumor Necrosis Factor; VEGF, Vascular Endothelial Growth Factor.

Conflict of interest

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

PDP contributed to the consensus concept and design. SGC and PDP were responsible for data acquisition. SGC is responsible for manuscript drafting. All authors approved the final version of the manuscript.

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