



The Dysregulation of the Secretome in the Tumor Microenvironment: A Potential Source for Biomarker Discovery and Personalized Cancer Treatment

Authors: Jacqueline Bersano
Submitted: 3. June 2024
Published: 17. June 2024
Volume: 11
Issue: 3
Affiliation: Charles River Laboratories GmbH, Freiburg im Breisgau, Germany; University of Freiburg, Freiburg im Breisgau, Germany
Languages: English
Keywords: Cancer Secretome, Tumor Microenvironment, Tumor-stroma Crosstalk, Biomarkers, Therapeutic Targets
Categories: News and Views, Life Sciences, Medicine
DOI: 10.17160/josha.11.3.991

Abstract:

The cancer secretome includes all proteins released by cancer cells and the components of the tumor microenvironment. Dysregulation of secretome emerged as a major contributor to cancer initiation and progression. Cancer cells and stromal cells communicate through different secretory pathways and work together to promote tumor growth. Understanding how this tumor-stroma crosstalk drives cancer development and studying changes in the secretome can help identify new biomarkers and therapeutic targets. Considering the challenges in diagnosing several cancer types in the early stages and providing personalized treatment, it is crucial to thoroughly study cancer processes and their key players to develop effective biomarkers and targeted treatments.

JOSHA

josha.org

**Journal of Science,
Humanities and Arts**

JOSHA is a service that helps scholars, researchers, and students discover, use, and build upon a wide range of content



The Dysregulation of the Secretome in the Tumor Microenvironment: a Potential Source for Biomarker Discovery and Personalized Cancer Treatment

Jacqueline Bersano

jacqueline.bersano@gmail.com

Charles River Laboratories GmbH, Freiburg im Breisgau, Germany; University of Freiburg, Freiburg im Breisgau, Germany

Abstract

The cancer secretome includes all proteins released by cancer cells and the components of the tumor microenvironment. Dysregulation of secretome emerged as a major contributor to cancer initiation and progression. Cancer cells and stromal cells communicate through different secretory pathways and work together to promote tumor growth. Understanding how this tumor-stroma crosstalk drives cancer development and studying changes in the secretome can help identify new biomarkers and therapeutic targets. Considering the challenges in diagnosing several cancer types in the early stages and providing personalized treatment, it is crucial to thoroughly study cancer processes and their key players to develop effective biomarkers and targeted treatments.



1. INTRODUCTION

Cancer refers to a large group of diseases where the body's cells undergo a multi-stage process and acquire malignant features [1]. The high complexity of genetic aberrations acquired by cells and the derived alterations on their proliferation and homeostasis processes can render extremely difficult an early diagnosis of the disease and the development of successful treatments [2].

Estimates of the World Health Organization (WHO)'s cancer agency show that cancer is still a leading cause of death, with its worldwide burden predicted to rise by approximately 50% in the next two decades [3,4].

In recent years, a significant increase in cancer research funding driven by the public and private sectors has led to the progression of personalized medicine. This approach aims at developing more targeted therapies, thus providing each patient with the most appropriate treatment and dose, while taking into account the heterogeneity of tumors [5–7].

These increasing efforts have resulted in significant improvements in the prognosis of several tumor subtypes. However, a large number of new oncology drugs do not prove meaningful clinical benefits or patients eventually develop resistance to these therapies [5,8]. Identifying novel biomarkers which better predict patient response to therapies is one of the essential actions to put into force [9]. Therefore, patient stratification to predict response to treatment is a key element of personalized medicine [7].

Early diagnosis of cancer through the detection of cancer-specific markers is also of vital importance in reducing negative outcomes. However, most cancers are detected in advanced stages when distant metastases compromise the efficacy of treatments [10].

Biomarkers are indicators used to detect cancerous cells or predict response to treatment [11]. The discovery and use of biomarkers are crucial for personalized medicine because measuring indicators specific to each patient's tumor can improve early detection of the disease and the selection of clinically effective treatments [8,11].

Secreted proteins are very promising biomarkers since they reflect the functionality of the cells. The secretome entails all proteins released by a cell, tissue or organism, which regulate normal physiological processes. In pathologic conditions, including



cancer, the dysregulation of secretome plays an important role in most of the hallmarks of cancer by contributing to cancer cell proliferation, survival, and differentiation [12]. The secretome is an indicator of the state of the cells at different stages of the disease and also reflects the various phases of pathological conditions. The presence or absence of secreted proteins can be analyzed in clinically relevant biological fluids of patients and can be advantageous for the diagnosis, prognosis, and therapeutic monitoring of cancer [13].

Moreover, the term secretome refers to not only the proteins secreted by cancer cells but also the complete set of proteins found in the tumor microenvironment (TME) [14]. Today the concept of tumor as a stand-alone process is outdated by evidence that proves how the interaction between tumor and stromal cells drives cancer initiation and progression [15]. Tumor-stroma communication is largely mediated by the secretion of growth factors, enzymes, cytokines, and chemokines that, as cited above, compose the secretome [12].

Therefore, identifying cancer-specific factors secreted by the tumor and its surrounding components can shed light on crucial tumorigenic processes and be used as biomarkers to improve the success of oncology treatments [9]. This review will discuss how the cancer secretome could serve as a pool of biomarkers and therapeutic targets, and how important it is to consider the tumor microenvironment in order to understand how cancer and stromal cells communicate and drive cancer progression.

2. SECRETOME IN CANCER

2.1 Secretome

The term “secretome” was introduced in 2000 by Tjalsma et al. and defines all proteins, including cytokines, growth factors, adhesion molecules, proteases, and receptors, released by a cell, tissue, or organism through various secretory pathways [14,16]. Secreted proteins act locally or systemically and are released in the extracellular matrix (ECM) via classical and non-classical pathways.

When they are synthesized, proteins present a signal peptide at their N-terminus which directs them to the Golgi apparatus in coat protein complex II (COPII)-coated vesicles. The proteins are then transported to the cell surface where they are released in the microenvironment through the fusion of the Golgi-derived vesicles



with the plasma membrane. This is the classical pathway by which proteins are secreted [12].

Proteins can also be released in the extracellular space through a non-classical pathway which consists of at least four different mechanisms [14]. These non-classical exports are all ER/Golgi-independent and use vesicular or non-vesicular transport mechanisms. In the vesicular mechanisms, proteins are integrated into intracellular vesicles, which are endosomal compartments, that fuse with the plasma membrane and release their content in the extracellular space. The non-vesicular mechanisms use different types of transport, such as self-sustained protein translocation, through plasma membranes and ABC transporter-mediated secretion [12,14].

2.2 Secretome in cancer-related processes

The secretome is involved in several biological processes like cell signaling and communication, and immune response. The secretome is largely involved in cancer-related processes by promoting malignant cell survival and migration, immune suppression, epithelial-mesenchymal transition (EMT), and angiogenesis [12,14]. Through these processes, cancer secretome sustains the proliferation of tumor cells from early phases of tumorigenesis, promotes tumor cell escape from the immune system, and contributes to cell acquisition of a motile and mesenchymal phenotype, which increases their ability to migrate and invade distant organs [12,17].

Inflammation is strictly connected to carcinogenesis. Among the hallmarks of cancer proposed by Hanahan and Weinberg in 2001, the evasion of immune response and inflammatory environment are recognized as signatures connected with inflammation [2,12]. The connection between inflammation and cancer involves two pathways that activate various types of oncogene by mutation, promote chromosomal rearrangement or amplification, and inactivate tumor-suppressor genes. This leads to the activation of transcription factors, in particular nuclear factor- κ B (NF- κ B), signal transducer and an activator of transcription 3 (STAT3), and hypoxia-inducible factor 1 α (HIF1 α), which produce cytokines, chemokines, cyclooxygenase-2 (COX-2) and prostaglandins. These secreted factors ultimately promote cancer cell survival and proliferation by activating the same transcription factors which increase the levels of inflammation and the development of a cancer-related inflammatory microenvironment [18].



Once activated, NF- κ B regulates the activation of several inflammatory cytokines which sustain cell growth and proliferation [19]. The inflamed TME is largely populated by T cells, mast cells, and tumor-associated macrophages (TAMs) [12]. TAMs are differentiated macrophages that have a significant impact on tumor progression. Together with other immune cells, TAMs secrete chemotactic, tumor-promoting growth factors, and angiogenic factors that sustain the recruitment of additional immune cells, cell migration, and remodelling of the tumor microenvironment [20].

The remodeling of the ECM is also one of the crucial steps of cancer progression which allows cancer cell movement. Matrix metalloproteinases (MMPs) are proteases upregulated in tumor cells during matrix degradation and contribute to creating passages through the ECM. Other factors such as ECM components, epidermal growth factor (EGF), insulin-like growth-factor-1 (IGF1), and transforming growth factor-beta (TGF- β) further induce cell proliferation and migration and are secreted by cancer cells [21,22].

Secretory factors participate also in the EMT, which is the starting process of metastasis through which cells switch from non-motile epithelial to motile mesenchymal cells, assuming more malignant properties [23]. During EMT cancer cells lose their cell-to-cell adhesions, pass through the tumor membrane, and enter the bloodstream, thus invading distant organs. TGF- β is the main promoter of EMT since it represses the production of epithelial markers, E-cadherin, and stimulates N-cadherin, which is one of the principal markers of mesenchymal cells [19]. The crosstalk between cancer cells and the TME plays an important role during EMT because neoplastic cells secrete cytokines that induce fibroblast activation into cancer associated fibroblasts (CAFs). Fibroblasts are the most abundant cells in the tumor stroma and have key roles during cancer initiation and progression [24]. Upon induction by the cancer cells, CAFs release MMPs, growth factors, and cytokines including TGF- β , promoting proliferation, migration, invasion, and metastasis [12].

The tumor-stroma interaction drives also the remodelling of new vasculature, termed angiogenesis, which ensures to cancer cells an adequate provision of oxygen, nutrients and metabolic exchange essential for their survival and proliferation [2,21]. Hypoxia is a well-known regulator of angiogenesis since it induces cancer cells to secrete vascular endothelial growth factor (VEGF) and other pro-angiogenic factors, which lead to the generation of disorganized and chaotic vasculature [21].



2.3 Detecting biomarkers in the secretome in biological fluids

Given its involvement in the principal processes of cancer initiation and progression, the secretome has the valuable potential to be used to detect the presence of cancer cells, disease monitoring, and patient selection. Measuring the expression of specific proteins holds also the advantage of considering the TME since the cancer secretome includes all factors secreted not only by cancer cells but also by components of its microenvironment, such as ECM proteins, growth factors, inflammatory cytokines, enzymes, microvesicles, and exosomes. Cancer arises due to an accumulation of mutations, insertions, deletions, and chromosomal translocations in the genome of the cells. Since the secretome also comprises degraded protein products released by cancer-related mutated species, its analysis can be particularly useful in diagnosis [10,12,25].

In clinical applications, cancer-specific proteins can be measured in small amounts in biological fluids such as blood (plasma and serum), saliva, interstitial fluid, and urine [12]. This makes their use as biomarker sources very advantageous because their collection is non-invasive, low-risk, and less expensive than tissue biopsies. Moreover, biofluids represent the tumor from a genomic, transcriptomic, and proteomic point of view, and due to the simplicity of their collection, they can be collected multiple times and reflect different stages of the disease [10,14].

Plasma and serum are the most commonly used biological fluids for clinical diagnosis mainly because their closeness to all tissues makes their proteomic signature highly representative of the molecules released by the tissues [12,26]. However, a disadvantage to consider in their use is the high range of protein concentration that complicates the analysis of low abundant proteins, like cytokines, that have more the potential of being cancer-specific markers [10,14].

In recent years, tumor interstitial fluid (TIF) has gained attention as a precious source of biomarkers, considering its reduced complex matrix simultaneously free of high-abundance interfering species and rich of cancer-related factors [10]. Meanwhile, the latest progress in proteomic analyses and bioinformatic tools are promising steps for a more comprehensive and unbiased characterization of secretome. Proteomics is widely used for the identification, measurement, characterization, and analysis of proteins, while quantitative mass spectrometry (MS)-based techniques are valid tools for discovering biomarkers [27].



Their combination in Liquid Chromatography Mass Spectrometry (LC-MS)-based proteomics is a valuable approach to analyze intracellular signaling pathways, protein-protein interactions or to identify drug targets, and post translational modifications (PTMs). With the use of LC-MS-based proteomics, we can quantify thousands of low-abundant proteins involved in cancer-related processes, without the need to select specific proteins in advance [27,28]. Considering its rapid advancements together with bioinformatic tools, identifying potential biomarkers in cancer secretome, for instance in TIF, will become more accessible and easier. Currently, specific secreted factors including carcinoembryonic antigen (CEA), prostate specific antigen (PSA), alpha-fetoprotein (AFP), CA 125, CA 15-3, and CA 19-9 are already used in clinical diagnosis [29]. Searching for biomarkers from cancer secretome in liquid biopsy analysis shows potential in understanding the role of secreted proteins in cancer, providing new diagnostic and prognostic biomarkers, and potentially therapeutic targets [10,28].

3. TUMOR-STROMA INTERACTION

3.1 The tumor microenvironment (TME)

Studying the impact of the cancer secretome on cancer progression and identifying potential biomarkers for cancer diagnosis and prognosis necessitates considering the role of the tumor microenvironment (TME) and the communication between tumor and stromal cells.

Today it is well recognized that cancer is not a stand-alone process that arises uniquely due to the accumulation of mutations in cancer cells. In 1889, Paget et. Al described the “seed and soil” hypothesis which proposed that tumor metastasis was the result of interactions between tumor cells and their organ environment [30,31]. Following studies elucidated that in tumorigenesis, cancer cells circumvent anti-tumorigenic activities exerted by immune cells and other components of the stroma and acquire the ability to corrupt their microenvironment into one with tumor-promoting functions. In subsequent stages of tumor development, cancer cells can metastasize and survive in distant organs only when they succeed in creating an appropriate microenvironment that further sustains their proliferation and invasion [30–32].

The TME comprises all cellular components of the tumor stroma that surround the cancer cells, such as inflammatory cells, fibroblasts, endothelial cells, pericytes,



lymphocytes, macrophages, but also ECM components and soluble factors [12]. The interaction between the TME and the cancer cells is mediated by the secretion of factors, including growth factors, MMPs cytokines, and chemokines [30,31].

In this cross-talk, cancer cells recruit TME components, that after activation assume malignant features and functions and in turn secrete factors that rule the crucial phases of cancer initiation and development, including tumor cell proliferation, migration, and invasion, ECM remodeling and degradation angiogenesis, immune suppression, EMT and metastasis [20,24,33].

3.2 Cancer-associated fibroblasts

Fibroblasts are major components of the connective tissue and regulate its synthesis. They are non-epithelial, non-vascular, and non-inflammatory cells with an elongated structure and a spindle-shaped morphology [24,34]. Fibroblasts produce ECM components (type I, type III, and type V collagen, and fibronectin) and regulate basement membranes by synthesizing type IV collagen and laminin. They also express MMPs, thus regulating ECM degradation [35].

Fibroblasts are activated during tissue injury by growth factors such as TGF- β , EGF, fibroblast growth factor 2 (FGF2), and platelet-derived growth factor (PDGF), which are secreted from injured epithelial cells, monocytes and macrophages. The activation of fibroblasts can also be mediated by direct cell-cell communication and reactive oxygen species (ROS) [24].

When activated, fibroblasts play important functions during wound repair and start to synthesize ECM components which contribute to scar formation and tissue fibrosis. Activated fibroblasts are termed “myofibroblasts”, they express α -smooth-muscle actin and acquire a contractile morphology and stress fibers, together with proliferation and migration capabilities. Moreover, they release cytokines, chemokines, growth factors, etc. through which they recruit immune cells [24,36]. The activation of fibroblasts is also found in cancers. While during non-cancerous conditions, fibroblast activation ceases once tissue integrity is restored, cancer-associated fibroblasts (CAFs) maintain their activated status and secrete an excess of cytokines and ECM components. This dysregulation of physiological wound healing and chronicity of inflammatory responses are typical of tissue fibrosis and scarring and led to the definition that tumors are “wounds that never heal” [24,34].



CAFs actively participate in tumor growth, angiogenesis, tissue invasion, metastasis, immune response suppression, and anti-cancer therapy resistance [34]. By overexpressing ECM components, CAFs alter the composition and porosity of the ECM. The increased ECM stiffness acts as a physical obstacle for immune cells, but can also induce enhanced recruitment of monocytes and their differentiation to macrophages having a tumor-supporting role [20]. CAFs are also essential for cancer invasion since they express MMP2 and MMP9, able to degrade ECM components (type IV collagen, and laminin), and MMP1 and MMP3, which promote EMT by cleaving E-cadherin-mediated adhesions [24,35]. By secreting proteases and exerting contractile forces, CAFs create gaps in the basement membrane, which can be crossed by migrating cancer cells [37]. During EMT, CAFs also activate HGF and Wnt2/ β -catenin pathways, which promote the transition of cells from endothelial to mesenchymal and motile.

HGF and TGF- β are factors predominantly secreted by CAFs. HGF promotes collagen degradation in the matrix, thus supporting cancer cell migration and invasion, while TGF- β is one of the main regulators of fibroblast activation and ECM remodeling. In the initial phases of carcinogenesis, TGF- β plays an antitumorigenic role, whereas, with the advancement of cancer, it activates several signaling pathways that promote EMT and sustain cancer cell proliferation and invasion [24,38]. These are the Smad signaling, the phosphatidylinositol-3-kinase–Akt pathway, and the RHOA and p38 MAPK signaling [24]. CAFs can also induce angiogenesis by secreting several growth factors, including VEGF, PDGF, and FGF2 [24].

CAFs have an immunosuppressive role during cancer since they recruit immune cells and contribute to their differentiation into tumor-promoting immune cells. Through the expression of inflammatory mediators (IL-6, IL-11, CXCL1, CXCL2), CAFs limit T cell proliferation and activate myeloid cell recruitment and differentiation to tumor associated macrophages (TAMs).

The recruitment of myeloid cells by CAFs is linked to therapy resistance to anti-programmed cell death protein 1 (PD)-1 immune checkpoint therapy [34]. Furthermore, the upregulation of immunosuppressive cytokine, growth factor, and chemokines production creates an environment which shields the cells from chemotherapeutic agents or suppresses their activity [39].



3.3 The extracellular matrix (ECM)

The extracellular matrix (ECM) is a non-cellular structural component of the TME made up of proteins synthesized and deposited outside the cell surface. This network of proteins such as collagens, glycoproteins, and proteoglycans, provide structural and functional integrity to connective tissues and organs [37,40]. The ECM mediates cell-cell communication in the TME through the activation or suppression of intracellular signaling pathways [41]. ECM components are ligands for cell surface receptors, such as integrins, which are gatekeeper proteins responsible for activating a signaling network that ultimately regulates cell proliferation, differentiation, and migration [37,41,42]. The aberrant expression of ECM components driven by CAFs leads to a dysregulation of these signaling pathways and alterations in the ECM [41].

In cancers, the activation of intracellular signaling induced by integrins promotes cancer cell invasion and metastasis through the RhoA/Rac signaling, stops growth suppressors p15 and p21 and inhibits apoptosis via FAK signaling [43,44].

Integrins promote cancer cell migration and invasion also by activating TGF- β 1,2 and 3, and PDGF which in turn stimulate the synthesis of collagen and fibronectin and angiogenesis. ECM stiffness also induces angiogenesis by VEGF signaling [40,45].

ECM components are also part of the secretome and their excessive secretion is mainly regulated by CAFs. In tumorigenic alteration, fibrillar collagen is the principal ECM component overexpressed, followed by fibronectin, tenascin C, and hyaluronan. These proteins undergo post-translational modifications (PTMs) which determine how the matrix interacts with other molecules and cellular receptors. However, in the tumorous tissue, PTMs alter ECM architecture leading for instance to the linearization of collagen fibers that appear linearised, perpendicularly oriented, and support the migration of cancer cells [42].

3.4 Immune cells

Avoiding immune suppression is one of the hallmarks of cancer through which cancer cells escape from immune surveillance and destruction. While the tumor progresses, cancer cells acquire the ability to reprogram also the immune cells, in the same way as they remodel other cells of the TME, such as CAFs [2,46]. The immune cells of the TME with a tumor-promoting or a tumor-antagonizing role are T



cells, B cells, macrophages, neutrophils, dendritic cells (DCs), eosinophils, myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells.

T cells belong to the adaptive immune system, but in cancer, they can have a dual function. CD8⁺ T cells exert a cytotoxic activity towards cancer cells through granzyme and perforin-mediated apoptosis or via FASL-FAS-mediated cell death. However, in cancer, CD8⁺ T cells are exhausted or dysfunctional and unresponsive to tumor-specific antigens [37,46,47]. Their cytotoxic activity is also suppressed by IL-10, TGF- β , and IL-35 secreted by regulatory T cells (Tregs) [48].

CD4⁺ T cells have diverse subsets that exert different types of functions. The Th1 subtype of CD4⁺ T cells plays an anti-tumorigenic role since it promotes the activation of CD8⁺ T and B cells and directly kills cancer cells through the secretion of interferon γ (IFN γ) and tumor necrosis factor α (TNF- α).

The role of the Th2 subtype is less defined, but evidence shows it can help cancer progression by secreting anti-inflammatory mediators. Tregs are a subset of CD4⁺ T cells with an immunosuppressive function. In normal tissue, they regulate immune tolerance and inflammation, but in the TME they inhibit effector lymphocytes, including CD4⁺ T helper and CD8⁺ cytotoxic T cells [45,46,48].

Tregs exert their functions via the master transcription factor forkhead box protein P3 (FOXP3), which is crucial for maintaining immune homeostasis, but in the cancer context mediates anti-cancer immunity response [46,48].

The role of B cells in the TME is still under investigation. B cells can produce anti-inflammatory and pro-angiogenic mediators, thus promoting tumor growth, MDSCs recruitment, and angiogenesis. On the other side, they have an anti-tumor activity via antibody-dependent cell cytotoxicity and complement activation [37,46].

Macrophages are differentiated from monocytes, which have key functions in immune response regulation and can exert pro- or anti-inflammatory effects, thus suppressing or supporting tumor progression. In the early phases of cancer initiation, macrophages secrete proinflammatory factors that recruit other cells of the immune system and exert antitumor functions. After this initial stage, cancer cells become able to evade immune surveillance and induce immune cells like macrophages to assume malignant features and functions [12,20,46]. This activation of macrophages into tumor-associated macrophages (TAMs) is mediated by factors secreted by cancer cells and the TME, namely the macrophage colony-stimulating factor 1 (CSF-1) and other interleukins [12]. TAMs sustain angiogenesis, metastasis,



and escape from the immune response by secreting anti-inflammatory cytokines and MMPs. Factors secreted by macrophages include the EGF, which mediates neovascularization and modulates immune response and pro-angiogenic factors, such as MMPs and VEGF, PDGF, FGF, and TGF- β [49].

These molecules contribute to the proliferation and migration of endothelial cells, matrix remodeling, and the formation of new vessels, thus promoting cancer cell migration and invasion [46,49].

Neutrophils represent the first immune defense and 50-70% of all circulating leukocytes. In several solid tumors, neutrophils differentiate toward a tumor-associated phenotype and have pro-tumorigenic functions by suppressing the immune response and remodeling the ECM [37,50]. CAFs are the main modulator of tumor-associated neutrophils (TANs) differentiation through the secretion of CXCR2 and stromal cell-derived factor 1 alpha (SDF-1 α). CAFs can also suppress T cell activation mediated by TANs via the STAT3 signaling pathway [20].

DCs are antigen-presenting cells (APCs) involved in the initiation and regulation of the adaptive immune system since they recognize antigens and present them to T cells. By doing this, they could exert anti-tumor immunity functions [37,46]. However, in the tumor context, factors such as CCL2, CXCL1, CXCL5, and VEGF are secreted by cancer and stromal cells and suppress their maturation [51]. Furthermore, cancer cells can also block DC activity by expressing the immune check-point inhibitor PD-1 [52].

Eosinophils are involved in parasite infections and allergic diseases and in cancer can have a dual functionality by interacting with NK and T cells. Also here, secreted proteins play a crucial role. Eosinophils sustain NK cell migration and activation through the secretion of CCL5, CXCL10, and IL-12. Eosinophils act as APCs, thus promoting CD4⁺ T cell proliferation, and release cytokines, such as CCL5, CXCL9 and, CXCL10 which induce the recruitment and cytotoxic activities of CD8⁺ T cells. Nevertheless, eosinophils also exert pro-tumorigenic activities by activating Treg cells and suppressing T cell responses through Programmed cell death ligand-1 (PD-L1) [46,53].

MDSCs are immunosuppressive cells which inhibit T cells, NK cells, B cells, and DCs immune activities. They secrete ROS, nitric oxide (NO), MMPs, like MMP9, and anti-inflammatory cytokines and in the TME they lose their phagocytic activity.



Therefore, they support cancer angiogenesis, invasion and immune tolerance [46,54].

NK cells are cytotoxic cells part of the innate immune system. They identify and eliminate stressed cells, including cancer cells, that do not express MHC class I molecules by releasing perforin and granzymes which cause apoptosis [46]. Although NK cells antagonize cancer cells, as the tumor progresses the secretion of TGF- β and other factors by CAFs impair NK functions [46,54]. CAFs also support cancer cell escape from NK-mediate killing by secreting MMPs. MMPs reduce the expression on tumor cells of MHC class I chain-related proteins A and B (MICA/B), which are essential for NK cells in identifying malignant cells [55].

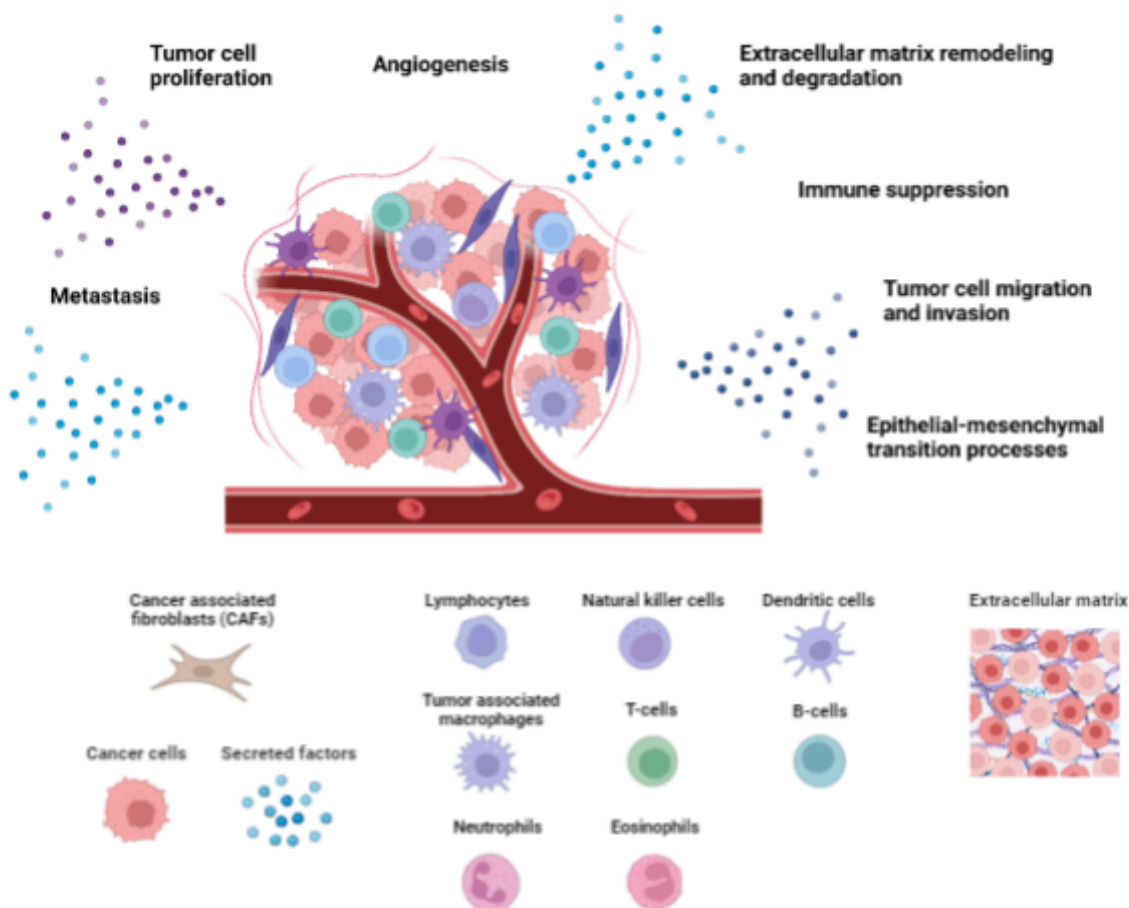


Figure SEQ Figure 1* ARABIC 1. The tumor microenvironment (TME): The TME comprises tumor cells, stromal cells such as fibroblasts and immune cells which adopt tumor promoting phenotypes and functions and support tumor growth. The interaction between tumor cells and the stroma primarily occurs through the secretion of various cytokines, growth factors, and chemokines. These signaling molecules play a pivotal role in crucial stages of cancer initiation and progression, including tumor cell proliferation, migration, invasion, angiogenesis, extracellular matrix modification, immune system suppression, epithelial-mesenchymal transition, and metastasis.



3.5 Clinical implications and future perspectives

Today, it is well recognized the importance of targeting cells of the tumor stroma to restore the anticancer immune response, eliminate the tumor, and prevent metastasis.

In contrast to conventional therapies, targeted cancer drugs are meant to attack only cancer cells, by targeting specific cell-secreted factors, receptors, surface antigens, or signal transduction pathways that control cancer cell cycle progression, cell death, angiogenesis, and metastasis [56].

Antibodies that target specific angiogenic factors, such as Bevacizumab (monoclonal antibody against VEGF), Cetuximab and Panitumumab (monoclonal antibodies against EGFR), Trastuzumab and Pertuzumab (monoclonal antibodies against HER2), have been approved against different type of cancers (breast, colon and lung) during the years [25,57].

These treatments improve prognosis at most in combination with inhibitors of tyrosine kinase receptors (TKRs), including erlotinib, sunitinib, and regorafenib, that inhibit multiple receptors (PDGFR, VEGFR, or FGFR). One of the drawbacks of these therapies is the activation of alternative pathways that lead to tumor relapse and drug resistance, which further demonstrates the need to detect cancer-specific biomarkers for patient selection and prognosis assessment [25,58].

One of the strategies to overcome tumor relapse is trying to target specific factors involved in cancer-related processes. The results from preclinical and clinical trials of these agents showed mixed results. Approaches that target IL-6, CCL2 or the CCL2 receptor (CCR2) revealed antitumor efficacy in preclinical tests, but not in clinical trials [25,55,59]. Better results were obtained with inhibitors of SDF-1/CXCL12 and IL-8 antagonists, which showed good prognosis in clinical trials. New therapies are also focusing on combining current therapies with agents targeting specific factors involved in EMT in order to enhance tumor sensitivity or targeting both the ECM and MMPs, with the aim of improving the correct diffusion of drugs and blocking metastasis, respectively. However, there is still an urgent need to deeply investigate the underlying mechanisms of the signaling pathways involved in achieving success in the clinical translation. Meanwhile, the heterogeneity of tumors is a crucial factor that determines the reason why not all



patients benefit from the same treatments. This reinforces the need to find robust biomarkers to select patients that will benefit from these treatments [21,25].

In recent years, therapies that target the immune system have become very promising and prove to prolong the survival of patients [25,60]. These therapies include immune checkpoint inhibitors, cytotoxic T lymphocyte-associated protein-4 (CTLA-4) antibodies, and chimeric antigen receptor T (CAR-T), cancer vaccines [61]. Evidence showed that immune checkpoint inhibitors are highly effective against cancer. Their mechanism of action consists of blocking the two main immune checkpoints, cytotoxic T lymphocyte antigen-4 (CTLA-4) and PD-1, which act as negative regulators of T-cell activation. In recent years, immune checkpoint inhibitors became the first-line treatment for an increasing number of cancers since they proved to significantly improve the overall survival in metastatic diseases [45,62].

However, the use of checkpoint inhibitors leads to a higher risk of severe autoimmune complications and the occurrence of resistance [61].

CAR-T cells are another innovative approach where patient T cells are genetically engineered to recognize and suppress targeted cancer cells. Combining immune checkpoint inhibitors with CAR T cells holds promise to revolutionize cancer immunotherapy because it guarantees the presence of immune cells and their functional activity [63].

CAR-T cells able to secrete specific cytokines have also been developed. CAR-T cells secreting IL-15 revealed decreased apoptosis and PD-1 expression, while those secreting IL-19 decreased TAMs and T-reg cells [63]. The major limitations of CAR-T cells are the immunosuppressive TME which impairs the persistence of CAR-T cells and adverse events, such as cytokine release syndrome (CRS) and neurotoxicity [61,63].

The use of secretome-targeting drugs in combination with immunotherapies has shown to be beneficial for targeting various types of cancer since it can ensure a synergistic effect. Targeted agents inhibit specific molecules that promote tumor growth and progression and decrease tumor-associated immunosuppression, which can be beneficial for immunotherapy to enhance cytotoxicity [64,65].

The combination of immune checkpoint inhibitors and specific secreted factors is also under investigation in preclinical and clinical trials. These consist of the administration of IL-12 and IL-15 (immuno-stimulatory cytokines able to activate NK



cells), engineered MSCs to locally deliver IL-2 (a key cytokine for T cells) into the TME, and the use of IL-2 variants engineered to enhance T cell profusion and cytotoxicity [37].

In recent years, the number of new treatment options against different types of cancer rapidly increased, thus providing a diverse range of targeted drugs and immunotherapies that offer more personalized and successful therapeutic approaches. As a consequence, the variety of combination therapies has significantly expanded, demonstrating improved clinical outcomes and the ability to overcome treatment resistance. Nevertheless, identifying biomarkers and selecting the optimal combination strategies still needs further exploration [65].

4. CONCLUSIONS

This review illustrated how the tumor-stroma crosstalk regulates the principal tumor hallmarks through the secretion of several growth factors, cytokines, ECM proteins, enzymes, and extracellular vesicles, and that deeper investigations could help elucidate how signaling pathways drive cancer initiation and progression.

Future studies must consider not only proteins secreted by tumor cells but also those released by components of the TME and thoroughly explore the exchange of signals between tumor cells and stromal cells. The secretome is a precious source of cancer biomarkers that could revolutionize the development of diagnostic and prognostic approaches, therapies, and the prediction of therapeutic responses.



References

1. What Is Cancer? - NCI. Published September 17, 2007. Accessed April 21, 2024. <https://www.cancer.gov/about-cancer/understanding/what-is-cancer>
2. Hanahan D, Weinberg RA. The Hallmarks of Cancer. *Cell*. 2000;100(1):57-70. doi:10.1016/S0092-8674(00)81683-9
3. Global cancer burden growing, amidst mounting need for services. Accessed April 22, 2024. <https://www.who.int/news/item/01-02-2024-global-cancer-burden-growing--amidst-mounting-need-for-services>
4. Mao JJ, Pillai GG, Andrade CJ, et al. Integrative oncology: Addressing the global challenges of cancer prevention and treatment. *CA: A Cancer Journal for Clinicians*. 2022;72(2):144-164. doi:10.3322/caac.21706
5. Hoeben A, Joosten EAJ, van den Beuken-van Everdingen MHJ. Personalized Medicine: Recent Progress in Cancer Therapy. *Cancers (Basel)*. 2021;13(2):242. doi:10.3390/cancers13020242
6. Sullivan R. The gross imbalances of cancer research must be addressed. *Nature*. 2023;621(7977):S15. doi:10.1038/d41586-023-02609-2
7. Carrigan P, Krahn T. Impact of Biomarkers on Personalized Medicine. In: Nielsch U, Fuhrmann U, Jaroch S, eds. *New Approaches to Drug Discovery*. Vol 232. Handbook of Experimental Pharmacology. Springer International Publishing; 2015:285-311. doi:10.1007/164_2015_24
8. Kunnumakkara AB, Bordoloi D, Sailo BL, et al. Cancer drug development: The missing links. *Exp Biol Med (Maywood)*. 2019;244(8):663-689. doi:10.1177/1535370219839163
9. Ileana Dumbrava E, Meric-Bernstam F, Yap TA. Challenges with biomarkers in cancer drug discovery and development. *Expert Opinion on Drug Discovery*. 2018;13(8):685-690. doi:10.1080/17460441.2018.1479740
10. Cevenini A, Orrù S, Imperlini E. Secretome Proteomic Approaches for Biomarker Discovery: An Update on Colorectal Cancer. *Medicina (Kaunas)*. 2020;56(9):443. doi:10.3390/medicina56090443



11. Louie AD, Huntington K, Carlsen L, Zhou L, El-Deiry WS. Integrating Molecular Biomarker Inputs Into Development and Use of Clinical Cancer Therapeutics. *Front Pharmacol.* 2021;12. doi:10.3389/fphar.2021.747194
12. da Cunha BR, Domingos C, Stefanini ACB, et al. Cellular Interactions in the Tumor Microenvironment: The Role of Secretome. *J Cancer.* 2019;10(19):4574-4587. doi:10.7150/jca.21780
13. Stastna M, Van Eyk JE. Secreted proteins as a fundamental source for biomarker discovery. *Proteomics.* 2012;12(4-5):722-735. doi:10.1002/pmic.201100346
14. Karagiannis GS, Pavlou MP, Diamandis EP. Cancer secretomics reveal pathophysiological pathways in cancer molecular oncology. *Mol Oncol.* 2010;4(6):496-510. doi:10.1016/j.molonc.2010.09.001
15. Role of the tumor microenvironment in tumor progression and the clinical applications (Review). Accessed April 23, 2024. <https://www.spandidos-publications.com/10.3892/or.2016.4660>
16. Tjalsma H, Bolhuis A, Jongbloed JD, Bron S, van Dijk JM. Signal peptide-dependent protein transport in *Bacillus subtilis*: a genome-based survey of the secretome. *Microbiol Mol Biol Rev.* 2000;64(3):515-547. doi:10.1128/MMBR.64.3.515-547.2000
17. Hanahan D, Coussens LM. Accessories to the Crime: Functions of Cells Recruited to the Tumor Microenvironment. *Cancer Cell.* 2012;21(3):309-322. doi:10.1016/j.ccr.2012.02.022
18. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature.* 2008;454(7203):436-444. doi:10.1038/nature07205
19. Frontiers | Tumor-Induced Inflammatory Cytokines and the Emerging Diagnostic Devices for Cancer Detection and Prognosis. Accessed May 20, 2024. <https://www.frontiersin.org/journals/oncology/articles/10.3389/fonc.2021.692142/full>
20. Wang J, Li D, Cang H, Guo B. Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. *Cancer Med.* 2019;8(10):4709-4721. doi:10.1002/cam4.2327



21. Ritchie S, Reed DA, Pereira BA, Timpson P. The cancer cell secretome drives cooperative manipulation of the tumour microenvironment to accelerate tumourigenesis. *Fac Rev.* 2021;10:4. doi:10.12703/r/10-4
22. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol Life Sci.* 2020;77(9):1745-1770. doi:10.1007/s00018-019-03351-7
23. Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: a systematic review. *Mol Cancer.* 2019;18(1):63. doi:10.1186/s12943-019-0983-5
24. Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer.* 2006;6(5):392-401. doi:10.1038/nrc1877
25. Cancer stem cell secretome in the tumor microenvironment: a key point for an effective personalized cancer treatment | Journal of Hematology & Oncology | Full Text. Accessed May 22, 2024. <https://jhoonline.biomedcentral.com/articles/10.1186/s13045-020-00966-3>
26. Dunphy K, O'Mahoney K, Dowling P, O'Gorman P, Bazou D. Clinical Proteomics of Biofluids in Haematological Malignancies. *International Journal of Molecular Sciences.* 2021;22(15):8021. doi:10.3390/ijms22158021
27. Methods and biomarkers for early detection, prediction, and diagnosis of colorectal cancer - ScienceDirect. Accessed May 23, 2024. <https://www.sciencedirect.com/science/article/pii/S0753332223005759>
28. An Introduction to Analytical Challenges, Approaches, and Applications in Mass Spectrometry-Based Secretomics - PubMed. Accessed May 23, 2024. <https://pubmed.ncbi.nlm.nih.gov/37597723/>
29. The cancer secretome: a reservoir of biomarkers | Journal of Translational Medicine | Full Text. Accessed May 23, 2024. <https://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-6-52>
30. Augsten M. Cancer-associated fibroblasts as another polarized cell type of the tumor microenvironment. *Front Oncol.* 2014;4:62. doi:10.3389/fonc.2014.00062



31. Langley RR, Fidler IJ. The seed and soil hypothesis revisited - the role of tumor-stroma interactions in metastasis to different organs. *Int J Cancer*. 2011;128(11):2527-2535. doi:10.1002/ijc.26031
32. Stromal cells in the tumor microenvironment: accomplices of tumor progression? | Cell Death & Disease. Accessed May 24, 2024. <https://www.nature.com/articles/s41419-023-06110-6>
33. IJMS | Free Full-Text | Fibroblasts in the Tumor Microenvironment: Shield or Spear? Accessed May 24, 2024. https://www.mdpi.com/1422-0067/19/5/1532?type=check_update&version=1
34. Monteran L, Erez N. The Dark Side of Fibroblasts: Cancer-Associated Fibroblasts as Mediators of Immunosuppression in the Tumor Microenvironment. *Front Immunol*. 2019;10:1835. doi:10.3389/fimmu.2019.01835
35. Javarsiani MH, Javanmard SH, Colonna F. Metastatic components in colorectal cancer. *J Res Med Sci*. 2019;24:75. doi:10.4103/jrms.JRMS_957_18
36. Ziani L, Chouaib S, Thiery J. Alteration of the Antitumor Immune Response by Cancer-Associated Fibroblasts. *Front Immunol*. 2018;9. doi:10.3389/fimmu.2018.00414
37. Visser KE de, Joyce JA. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. *Cancer Cell*. 2023;41(3):374-403. doi:10.1016/j.ccell.2023.02.016
38. Asif PJ, Longobardi C, Hahne M, Medema JP. The Role of Cancer-Associated Fibroblasts in Cancer Invasion and Metastasis. *Cancers*. 2021;13(18):4720. doi:10.3390/cancers13184720
39. Feng B, Wu J, Shen B, Jiang F, Feng J. Cancer-associated fibroblasts and resistance to anticancer therapies: status, mechanisms, and countermeasures. *Cancer Cell Int*. 2022;22:166. doi:10.1186/s12935-022-02599-7
40. Interactions between extracellular matrix and growth factors in wound healing - PubMed. Accessed May 25, 2024. <https://pubmed.ncbi.nlm.nih.gov/19320882/>



41. Hastings JF, Skhinas JN, Fey D, Croucher DR, Cox TR. The extracellular matrix as a key regulator of intracellular signalling networks. *Br J Pharmacol.* 2019;176(1):82-92. doi:10.1111/bph.14195
42. Winkler J, Abisoye-Ogunniyan A, Metcalf KJ, Werb Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. *Nat Commun.* 2020;11(1):5120. doi:10.1038/s41467-020-18794-x
43. Li S, Sampson C, Liu C, Piao H long, Liu HX. Integrin signaling in cancer: bidirectional mechanisms and therapeutic opportunities. *Cell Commun Signal.* 2023;21(1):266. doi:10.1186/s12964-023-01264-4
44. Pickup MW, Mouw JK, Weaver VM. The extracellular matrix modulates the hallmarks of cancer. *EMBO Rep.* 2014;15(12):1243-1253. doi:10.15252/embr.201439246
45. JCI Insight - The evolving relationship of wound healing and tumor stroma. Accessed May 25, 2024. <https://insight.jci.org/articles/view/99911/figure/2>
46. Lei X, Lei Y, Li JK, et al. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. *Cancer Letters.* 2020;470:126-133. doi:10.1016/j.canlet.2019.11.009
47. Schietinger A, Philip M, Krisnawan VE, et al. Tumor-Specific T Cell Dysfunction Is a Dynamic Antigen-Driven Differentiation Program Initiated Early during Tumorigenesis. *Immunity.* 2016;45(2):389-401. doi:10.1016/j.immuni.2016.07.011
48. Li J, Tan J, Martino MM, Lui KO. Regulatory T-Cells: Potential Regulator of Tissue Repair and Regeneration. *Front Immunol.* 2018;9. doi:10.3389/fimmu.2018.00585
49. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer.* 2004;4(1):71-78. doi:10.1038/nrc1256
50. Galli F, Aguilera JV, Palermo B, Markovic SN, Nisticò P, Signore A. Relevance of immune cell and tumor microenvironment imaging in the new era of immunotherapy. *J Exp Clin Cancer Res.* 2020;39(1):89. doi:10.1186/s13046-020-01586-y



51. Michielsen AJ, Hogan AE, Marry J, et al. Tumour tissue microenvironment can inhibit dendritic cell maturation in colorectal cancer. *PLoS One*. 2011;6(11):e27944. doi:10.1371/journal.pone.0027944
52. Krempski J, Karyampudi L, Behrens MD, et al. Tumor-infiltrating programmed death receptor-1+ dendritic cells mediate immune suppression in ovarian cancer. *J Immunol*. 2011;186(12):6905-6913. doi:10.4049/jimmunol.1100274
53. Eosinophil-lymphocyte interactions in the tumor microenvironment and cancer immunotherapy - PMC. Accessed May 27, 2024. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9554620/>
54. Mao X, Xu J, Wang W, et al. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. *Mol Cancer*. 2021;20(1):131. doi:10.1186/s12943-021-01428-1
55. Chomarat P, Banchereau J, Davoust J, Palucka AK. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. *Nat Immunol*. 2000;1(6):510-514. doi:10.1038/82763
56. Shuel SL. Targeted cancer therapies: Clinical pearls for primary care. *Can Fam Physician*. 2022;68(7):515-518. doi:10.46747/cfp.6807515
57. Min HY, Lee HY. Molecular targeted therapy for anticancer treatment. *Exp Mol Med*. 2022;54(10):1670-1694. doi:10.1038/s12276-022-00864-3
58. IJMS | Free Full-Text | Receptor Tyrosine Kinase-Targeted Cancer Therapy. Accessed May 28, 2024. <https://www.mdpi.com/1422-0067/19/11/3491>
59. Xu M, Wang Y, Xia R, Wei Y, Wei X. Role of the CCL2-CCR2 signalling axis in cancer: Mechanisms and therapeutic targeting. *Cell Prolif*. 2021;54(10):e13115. doi:10.1111/cpr.13115
60. Emens LA, Ascierto PA, Darcy PK, et al. Cancer immunotherapy: Opportunities and challenges in the rapidly evolving clinical landscape. *European Journal of Cancer*. 2017;81:116-129. doi:10.1016/j.ejca.2017.01.035
61. A guide to cancer immunotherapy: from T cell basic science to clinical practice | Nature Reviews Immunology. Accessed May 28, 2024. <https://www.nature.com/articles/s41577-020-0306-5>



62. Miranda Poma J, Ostios Garcia L, Villamayor Sanchez J, D'errico G. What do we know about cancer immunotherapy? Long-term survival and immune-related adverse events. *Allergologia et Immunopathologia*. 2019;47(3):303-308. doi:10.1016/j.aller.2018.04.005
63. Tumor Secretome to Adoptive Cellular Immunotherapy: Reduce Me Before I Make You My Partner - PubMed. Accessed May 28, 2024. <https://pubmed.ncbi.nlm.nih.gov/34447383/>
64. Vanneman M, Dranoff G. Combining Immunotherapy and Targeted Therapies in Cancer Treatment. *Nat Rev Cancer*. 2012;12(4):237-251. doi:10.1038/nrc3237
65. Yang J, Kang H, Lyu L, Xiong W, Hu Y. A target map of clinical combination therapies in oncology: an analysis of clinicaltrials.gov. *Discov Oncol*. 2023;14:151. doi:10.1007/s12672-023-00758-4



About the Author



Jacqueline Bersano, Freelancer Medical and Scientific writer. Jacqueline Bersano has a PhD in Cancer Biology from the Albert-Ludwigs-Universität Freiburg and most recently worked as a QM Associate in the Regulatory team of Dr. Langer Medical.