Cancer stem cell secretome in the tumor microenvironment: a key point for an effective personalized cancer treatment

Julia López de Andrés1,2,3, Carmen Griñán-Lisón1,2,3, Gema Jiménez1,2,3,4* and Juan Antonio Marchal1,2,3,5*

Abstract

Cancer stem cells (CSCs) represent a tumor subpopulation responsible for tumor metastasis and resistance to chemotherapy and radiotherapy, ultimately leading to tumor relapse. As a consequence, the detection and eradication of this cell subpopulation represent a current challenge in oncology medicine. CSC phenotype is dependent on the tumor microenvironment (TME), which involves stem and differentiated tumor cells, as well as different cell types, such as mesenchymal stem cells, endothelial cells, fibroblasts and cells of the immune system, in addition to the extracellular matrix (ECM), different in composition to the ECM in healthy tissues. CSCs regulate multiple cancer hallmarks through the interaction with cells and ECM in their environment by secreting extracellular vesicles including exosomes, and soluble factors such as interleukins, cytokines, growth factors and other metabolites to the TME. Through these factors, CSCs generate and activate their own tumor niche by recruiting stromal cells and modulate angiogenesis, metastasis, resistance to antitumor treatments and their own maintenance by the secretion of different factors such as IL-6, VEGF and TGF-β. Due to the strong influence of the CSC secretome on disease development, the new antitumor therapies focus on targeting these communication networks to eradicate the tumor and prevent metastasis, tumor relapse and drug resistance. This review summarizes for the first time the main components of the CSC secretome and how they mediate different tumor processes. Lastly, the relevance of the CSC secretome in the development of more precise and personalized antitumor therapies is discussed.

Keywords: Cancer stem cells, Tumor microenvironment, Secretome, Growth factors, Interleukins, miRNAs, Exosomes

Introduction

The cancer stem cell (CSC) model is based on the identification of tumor cells in different stages of differentiation in a wide variety of tumors, including ovarian [1], breast [2, 3], brain [4], lung cancer [5], melanoma [6], prostate [7], colorectal [8] and liver cancer [9]. All of them are composed by a small subpopulation of cells with stem cell-like characteristics such as quiescence, slow cell cycle, expression of embryonic SC transcription factors and epigenomic regulation driven by micro-RNAs (miRNAs) [10]. Like normal SCs, CSCs can self-renew and divide asymmetrically to give rise to daughter cells, which constitute the bulk of the tumor, and this makes CSCs responsible for the maintenance and proliferation of the tumor, as observed in healthy tissues [11].

However, identification of these subpopulations has not been easy, and although several markers have been described, tumor heterogeneity and inter-patient variations make it difficult to define robust markers [12]. In general terms, the most commonly used indicators to identify CSCs are surface markers such as CD133 and CD44 [13, 14], increased activity of aldehyde
dehydrogenase (ALDH) [14, 15] and their ability to exclude Hoechst 33342 (side population) [16], and to form spheres in vitro [17].

In addition, CSCs drive tumor drug resistance due to their ability to enter a quiescent state, activate DNA repair mechanisms, reactivate drug efflux system and protect against ROS [12], ultimately being responsible for disease relapse. Therefore, the CSC model explains the poor prognosis of the disease and indicates that identifying and attacking CSCs are currently a major challenge in cancer research.

As the importance of CSCs in tumor development has been elucidated, special attention has also been paid to their environment, since the tumor niche has a strong influence on the tumor behavior. The tumor microenvironment (TME) includes stem and differentiated cancer cells, the extracellular matrix (ECM), mesenchymal stem cells (MSCs), cancer-associated fibroblasts (CAFs), endothelial cells (ECs), immune system cells, and a complex network of cytokines and growth factors [18]. All these components orchestrate tumor processes in different ways. Non-tumor and differentiated tumor cells interact closely with CSCs by modulating their activity and contributing to key tumor processes such as tumor growth, metastasis, angiogenesis and immune system evasion [18]. Indeed, TME cells also promote resistance to antitumor therapies, since the secretion of soluble factors such as interleukin-6 (IL-6), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), or transforming growth factor β (TGF-β) and ECM adhesion proteins such as integrins leads to the activation of several tumor survival pathways [19]. Additionally, the ECM has a different composition, organization and post-transcriptional modification in the TME than the surrounding normal tissue [20] and largely influences the intratumor signaling, transport mechanism, cell motility, metastasis and immune response [21, 22]. Moreover, tumor ECM shows higher density and stiffness, which can interfere on nutrient, oxygen and metabolite diffusion which in turn lead to tumor hypoxia. This stiff ECM also acts as a physical barrier to the action of chemotherapeutic and radiotherapy agents. Tumor hypoxia and the barrier capacity are related to poor treatment response [20, 23].

Importantly, CSCs do not merely adapt to the TME; they also form their own niches by recruiting and activating other cells and modify their environment in different ways [24]. Understanding the interaction of CSCs with their niche may be crucial to design effective cancer treatments and selectively target this cell subpopulation.

This review examines for the first time the main components secreted by CSCs to generate and modify their own environment and to orchestrate the tumor hallmarks. To this end, we describe the role played by CSC secretome in cell recruitment, in the interactions with tumor niche as well as in distal metastasis. Finally, the impact of CSCs on the development of resistance to current antitumor treatments and the new therapies that focus on overcoming these issues by targeting the CSC secretome are also discussed.

**Cancer stem cell secretome**

Secretome refers to all the molecules secreted by a cell or shedded from its membrane and is fundamental for cell–cell communication. Despite that the secretome has commonly been defined only by the protein fraction, the non-protein components such as lipids, miRNAs and messenger-RNAs (mRNAs) isolated in or secreted via vesicular bodies have been also incorporated into this definition [25]. There is increasing evidence that CSCs regulate different tumor hallmarks such as angiogenesis, tumor growth, metastasis, drug resistance and immune dysregulation through their secretome (Fig. 1). CSCs communicate with the TME by releasing microvesicles (MVs) and exosomes, as well as a wide range of soluble factors including chemokines, cytokines, growth factors, hormones and metabolites [26]. MVs differ from exosomes in size (MVs range from 50 to 10,000 nm while exosomes are typically 30–150 nm in diameter), their secretion mechanism and cargo [27].

Some factors involved in the communication of CSCs with their environment such as IL-6, IL-8 and IL-1β, vascular endothelial growth factor (VEGF) and various matrix metalloproteinases (MMPs) can be released free into the extracellular space or encapsulated in exosomes and MVs [28–30]. In addition, various cytokines such as CCL2 or CCL5 [31], TGF-β [32–34] and hypoxia-inducible factor 1 (HIF-1) can also be released via exosomes to the TME [35]. All these factors perform multiple functions within the tumor, since they promote the formation and activation of the TME, hypoxia, tumor vascularization and metastasis, or chemo- and radiotherapy resistance, as it will be discussed in more detail below (Fig. 1).

In addition, several studies have shown that not only soluble factors but also miRNAs strongly contribute to cancer development and progression by altering the secretome, promoting both tumorigenic and tumor suppression responses. MiRNAs are small RNAs molecules of 18–22 nucleotides with the ability to regulate cancer-related processes including cellular proliferation, cell cycle arrest, senescence, DNA damage response, apoptosis, metastasis and CSC properties. These diverse functional features are tumor and tissue specific and can have a positive or negative effect, and changes in miRNA levels may influence their function [36–38]. One recent finding is that miRNAs can be found inside exosomes or
MVs and prevent RNase degradation. Therefore, these exosome-transferred miRNAs have emerged as a new mechanism mediating the tumor–stroma crosstalk and metastasis [31, 39, 40] (Table 1). Therefore, it is clear that miRNAs secreted by CSCs could be potent regulators of the secretome involved in cancer initiation and progression. For example, miR-200 family has been shown to inhibit the epithelial–mesenchymal transition (EMT) and enhance the reverse process [41, 42].

### Table 1 Exosomes or extracellular vesicles-derived miRNAs from cancer stem cells

| miRNAs                          | Cancer type        | Functions                                                                 | References          |
|---------------------------------|--------------------|---------------------------------------------------------------------------|---------------------|
| miR-10b, miR-105, miR-9         | Breast cancer      | Invasiveness, endothelial cell migration, angiogenesis and metastasis     | [39, 43–45]         |
| miR-195, miR-203a               |                    |                                                                           |                     |
| miR-200 family                  |                    |                                                                           |                     |
| miR-21, miR-34, miR-155         | Oral cancer        | Proliferation, migration and poor prognosis                               | [44]                |
| miR-19b, miR-29c, miR-151       | Renal cancer       | EMT and metastasis                                                        | [46, 47, 48]        |
| miR-215 and miR-375, miR-17–92  | Colorectal cancer  | Relapse and poor prognosis, tumor development and metastasis             | [47]                |
| cluster, miR-200c               |                    |                                                                           |                     |
| miR-21                          | Glioblastoma       | Angiogenesis and tumor growth                                             | [36, 50]            |
| miR-139, miR-183                | Prostate cancer    | Cell proliferation and migration                                           | [48]                |
| miR-21, miR-221                 | Pancreatic cancer  | Angiogenesis, tumor growth, metastasis and migration in advanced tumor stages | [36, 49, 51]       |
| miR-146, miR-17, miR-155        |                    |                                                                           |                     |
Secretome in the interaction with stromal cells

CSCs play an essential role in tumor niche generation by recruiting and activating TME cells through different signaling pathways [41] (Fig. 2). In fact, many of these pathways result in a communication loop between CSCs and stromal cells, whereby CSCs self-regulate and regulate their environment.

Mesenchymal stem cells (MSCs)

Mesenchymal stem cells perform key functions in the development of cancer including regulation of inflammatory processes, angiogenesis, metastasis, maintenance of CSCs and tumor growth [50, 51]. For this reason, MSCs are recruited into the tumor niche by CSCs and interact with each other through a wide network of cytokines [52] (Fig. 2).

First, CSCs recruit MSCs to the sites of primary tumor growth by secreting IL-6, which also triggers other responses in the tumor niche. In turn, IL-6 induces the production of CXCL7 by MSCs [52], which has been shown to promote tumor invasiveness and metastasis in murine models [53, 54], as well as tumor growth through interaction with the tumor receptor CXCR2 [53] that, in turn, induces the synthesis of other cytokines including IL-6 and IL-8.

Moreover, CSCs in pancreatic cancer have been found to overexpress IL-1ß [55] to attract MSCs by promoting MMP-1 secretion, which in turn activates the protease-activated receptor 1 (PAR1) and G-protein-coupled signal pathways, resulting in migration and recruitment of MSCs to the tumor niche. Moreover, tumor-secreted IL-1ß induces the expression of several chemokines by MSCs [56, 57] because it promotes the expression of nuclear factor kB (NF-kB), a major regulator of chemokine expression [57]. As with IL-6, IL-1ß also interacts with other cell types and triggers other pathways in the TME directly affecting tumor growth, invasion and angiogenesis [56], by inducing the secretion of angiogenic factors by tumor and stromal cells. However, it seems that the effect of IL-1ß is related to tumor type and the TME, since a negative effect on IL-1ß-mediated tumor growth has also been reported, explained by an increased immune response [58, 59].

Finally, glioma CSCs have been shown to secrete stromal cell-derived factor 1 (SDF-1/CXCL12) in order to recruit MSCs [60]. MSCs also secrete SDF-1/CXCL12, and communication between CSCs and MSCs through SDF-1/CXCL12 leads to tumor progression through different ways, including CSs survival, tumor growth, metastasis and angiogenesis processes [61, 62].
Cancer-associated fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs) are also key actors in TME supporting tumor maintenance, angiogenesis, EMT and metastasis and producing ECM components [63]. CSCs recruit CAFs either by activating adjacent fibroblasts, CAFs or transforming MSCs through the secretion of different factors like platelet-derived growth factor (PDGF), FGF, IL-6 and TGF-β [24, 64–66] (Fig. 2). In breast cancer, it has also been described that the activation of fibroblasts to CAFs requires the activation of STAT3 by these cytokines, which results in CAFs showing higher CCL2 expression than normal fibroblasts, which induces Notch1 expression of CSCs and, therefore, promotes stemness maintenance in a bidirectional interaction [67]. On the other hand, CSCs can induce differentiation of MSCs into CAFs through the secretion of TGF-β via the activation of the TGFBR1/Smad pathway [34, 68, 69].

Furthermore, in a recent study using a murine model of breast cancer, CSCs have shown to produce the Hedgehog (Hh) ligand Sonic Hedgehog (SHH), activating the Hh signaling pathway in CAFs, which leads to increased CAF proliferation and ECM deposition and enhances the production of other factors such as ACTIVIN A, insulin-like growth factor 1 (IGF-1) and leukemia inhibitory factor (LIF), which result in CSC growth and self-renewal [70], in a feedback communication between CAFs and CSCs.

Immune cells

In the TME, immune system cells may exhibit tumorigenic or antitumor activity depending on environmental signals [71]. For this reason, the communication with tumor cells becomes an important factor. The role of CSCs is not only to recruit and activate cells in the tumor niche, but also the evasion of the immune response (Fig. 2). CSCs recruit macrophages to the tumor niche by producing pro-inflammatory cytokines and chemokines, such as CCL2 [72], IL-6, IL-4, VEGF and TGF-β [73, 74]. Once in the TME, macrophages are activated to tumor-associated macrophages (TAMs) through CSC-secreted factors such as IL4, TGF-β and macrophage inhibitory cytokine 1 (MIC-1), which also inhibits the phagocytic activity of macrophages [75, 76]. Moreover, CSC-released TGF-β [77, 78] induces the differentiation of CD4+T lymphocytes to regulatory T lymphocytes (Treg) by stimulating the synthesis of FOXP3, thereby having an immunosuppressive effect on the tumor [79, 80]. However, this is not the only pathway used by CSCs for macrophage activation [81, 82]. Treg causes a decrease in tumor immunity not only by regulating the accumulation of T lymphocytes, but also by releasing other factors with immunosuppressive roles such as TGF-β and IL-10 [83].

A major hallmark of CSCs is to evade the immune response [84] through an inadequate antigen presentation [82, 85, 86] and the ability to create a differentiated tumor cell barrier around them [87]. Additionally, CSCs secrete different molecules with protective function against both innate and adaptive immune responses such as TGF-β, IL-4, IL-6, IL-10 and prostaglandin E2 (PGE2) [73, 80, 88]. In addition, FOXP3 in Treg, whose expression is regulated by TGF-β and other factors, also inhibits the secretion of IL-2, interferon gamma and IL-4 [89]. Furthermore, CSCs secrete exosomes with an immune response modulating effect by suppressing T-cell response [90] and inhibiting dendritic cell differentiation [91]. These finding show that CSCs are capable of triggering multiple pathways for immune evasion.

In summary, the CSC secretome is responsible for the recruitment and activation of MSCs, CAFs and immune cells to the TME. In addition, they promote the function of these cell types, thus leading the regulation of inflammatory responses, tumor growth, angiogenesis, metastasis and their own maintenance.

Secretome in angiogenesis

Angiogenesis is a central process that promotes tumor development and metastasis. CSCs modulate the vascularization of the tumor niche mainly through VEGF, but other factors are also involved (Fig. 2). CSCs recruit endothelial cells (ECs) to the tumor niche and induce angiogenesis by secreting HIF-1, VEGF and SDF-1/CXCL12 [92–94]. Furthermore, MSCs migrate to the tumor niche recruited by CSCs, and once there they differentiate into ECs through the action of VEGF [95–97]. In addition, CSCs are capable of differentiating into ECs and endothelial progenitor cells (EPCs) and form vessel-like networks in a process called “vascular mimicry,” mediated also by VEGF [98–101]. Indeed, CSCs preferentially overexpress more VEGF receptors (VEGFR-1 and VEGFR-2) than their differentiated counterparts and their activation by VEGF mediates chemotaxis, tubule formation and vascular marker expression [100–103]. Likewise, CSCs show high expression of vascular–endothelial cadherin (VE-Cadherin) and Notch, both involved in the transformation to ECs and EPCs [102–106], as well as MMP-2 and MMP-9, which promote ECM remodeling thus promoting new vessel formation by CSCs [105, 106]. Moreover, CSCs overexpress CXCR4, whose SDF-1/CXCL12 ligand induces VEGF production via activation of the PI3K/AKT signaling pathway [107, 108]. This makes that stromal cells and CSCs can also modulate angiogenesis by promoting the expression of CSC-secreted VEGF [109]. CSC-secreted VEGF plays other critical roles in the tumor niche since it enhances CSCs proliferation by stimulating neuropilin-1,
a co-receptor of VEGFR2, and thus promotes tumor progression and relapse [110–112].

Furthermore, CSCs secrete TGF-β [77, 78] that can also induce the expression of VEGF and another angiogenic factor, the connective tissue growth factor (CTGF), in both epithelial cells and fibroblasts [113, 114]. Finally, a comparative study of glioma CSCs and non-SCs secretome has identified an increase in hepatoma-derived growth factor (HDGF) in glioblastoma stem-like cells (GSCs) linked to angiogenesis in vivo [115].

Taken together, these findings demonstrate that the CSC secretome modulates the generation of new vasculature in the TME by recruiting ECs and MSCs. In addition, the secretome involved in this process also enhances the maintenance of CSCs and therefore tumor proliferation and relapse.

Nonetheless, the vascular network formed to support the rapid tumor growth is aberrant, with disorganized, immature and highly permeable blood vessels, and cannot fully fulfill its functions [116]. This limits tumor perfusion, decreases oxygen supply and increases hypoxia in the tumor and prevents the arrival of immune system cells. Poor perfusion also reduces the efficacy of radiation therapy and antitumor drug perfusion to the tumor, which allows tumor survival [116]. Furthermore, the high permeability, related to reduced coverage of the pericytes and their binding to the ECs, facilitates the metastatic spread of cancer cells, mainly CSCs [117].

Secretome in hypoxia

The rapid proliferation of cancer cells and the TME aberrant vasculature cause hypoxic regions within the tumors. In response to this situation, both CSCs and non-SCs secrete HIF-1, which is stabilized only in areas with very low oxygen level [118, 119]. HIF-1 in the tumor niche is related to poor prognosis because it enhances tumor spread and CSC self-renewal [15, 120–122], promoting the stem phenotype by enhancing Notch-β-1 or ALDH1 expression. Furthermore, in several tumor types HIF-1 has been reported to promote resistance to therapy [23] and EMT phenotype [123], to remove or prevent toxic metabolic waste products [118, 124]. HIF-1 is also involved in angiogenic processes by inducing VEGF and VEGFR2 expression [125–127] and can suppress antitumor immune responses [128].

On the other hand, the hypoxia-inducible factor 2 (HIF-2) and HIF-regulated genes are preferentially expressed in CSCs compared to their differentiated counterparts, which is associated with poorer prognosis and higher CSCs proliferation and survival [129]. Furthermore, HIF-2 has been shown to be expressed at low levels of hypoxia and even at physiological levels of oxygen [119, 129]; therefore, its function is not limited to hypoxic regions. Moreover, the secretome of hypoxic tumor environments regulates miR-210 expression in a positive way, as shown by the fact that HIF-1 secreted by both CSCs and non-SCs promotes its expression. MiR-210 is an important mediator of the response to low oxygen tension and promotes mRNA degradation of normoxic genes in several cancers, particularly in pancreatic cancer. Thus, circulating miR-210 levels could serve as a useful biomarker for diagnosis in cancers with extremely hypoxic signatures [130, 131]. In addition, tumor-secrected HIF-1 induces miR-155 expression under hypoxic conditions and plays a dual role in maintaining this factor as miR-155 directly targets HIF-1 and suppresses its expression and miR-155 suppresses the translation of von Hippel–Lindau tumor suppressor (VHL) leading to increased HIF activity. This role is, however, not so contradictory, if we consider that miR-155 targets HIF-1 but not HIF-2α, which is more oncogenic. MiR-155 is also related inflammation since IL-6 induces its expression and this in turn activates the JAK2/STAT3 pathway, thus promoting tumor inflammation [132].

In conclusion, the CSC secretome regulates the response to hypoxic environments through HIF and miRNAs and its overexpression is related to poor prognosis, related to the effects on tumor maintenance and CSCs themselves.

Secretome in CSC maintenance

In addition to interacting with stromal cells, CSCs regulate their maintenance in the tumor niche, as described above. In addition, CSCs regulate their self-renewal through the autocrine secretion of TGF-β, which promotes stemness properties of CSCs in breast [133], colon [134] and ovary cancer [135] and induces self-renewal and prevents differentiation in glioma CSCs [136–138]. Indeed, CSCs secrete nodal and activin, from the TGF-β family, linked to the CSCs self-renewal in pancreatic cancer in vitro and CSCs tumorigenicity in vivo [139]. Signaling pathways involved in TGF-β-induced CSC phenotype acquisition include SMADs, AKT, SOX and MAPK, which in turn synergize with other signaling pathways to promote tumor invasion and metastasis [140]. Furthermore, CSC survival is promoted by autocrine production of different interleukins. In colon cancer, CSC-secreted IL-4 promotes their maintenance and inhibits apoptosis [76]. IL-16 also promotes tumor growth and invasion by activating CSC self-renewal and EMT [56, 141]. IL-6 can induce tumor cell dedifferentiation in breast [142, 143], colon [144, 145], and prostate [146] cancers and regulates stemness in ovarian CSCs driven by ALDH1A1 expression [147]. Similarly,
overexpression of IL-8 promotes pancreatic CSC self-renewal via IL-8/CXCR1 axis [148]. This is consistent with other studies, showing that the IL-8/CXCR1 pathway is essential for CSCs survival in breast cancer [149].

CSCs promote their maintenance and tumorigenicity through the secretion of exosomes. For example, GSCs secrete exosomes carrying chloride intracellular channel protein 1 (CLIC1), which affects tumor proliferation both in vivo and in vitro [150]. In pancreatic and colorectal cancer, exosomes released by CSCs stimulate the stemness phenotype and cell invasion and mobility [151]. In addition, CSCs deregulate miRNAs, which also affect their own maintenance. MiR-302/367 cluster was found to be strongly expressed in CSCs and strongly repressed during differentiation in most cancers, with its expression being highly correlated with the expression of CSC markers [152]. Interestingly, Rahimi et al. isolated CSCs using their stem cell-specific miR-302 expression and maintained CSCs stemness by continued selection [153]. Lastly, in colorectal cancer miR-146a increases the symmetrical division of CSCs, and miR-1246, one of the most differentially expressed miRNAs in the CSC population, is involved in self-renewal processes, tumorigenicity and drug resistance [36, 154].

Given the importance of CSC maintenance in the TME, the CSC secretome plays a key role on tumor development by promoting CSCs self-renewal, preventing their differentiation and even promoting the dedifferentiation of tumor cells to SCs.

Secretome in extracellular matrix remodeling

ECM remodeling is essential for angiogenesis, metastasis and tumor growth, and since the ECM is a reservoir of many factors, its degradation results in their release into the environment. ECM remodeling occurs principally through the activity of matrix metalloprotease-10 (MMP-10), which enhances EMT, metastasis and CSC state [155, 156]. It has been reported that CSCs overexpress MMP-9 [157, 158], which allows the activation of inactive TGF-β in ECM [159], MMP-2 and MMP-13 [105, 160], all related to increased metastatic and angiogenic capacity. In ovarian cancer cell lines, CSCs overexpress CCL5 and its CCR1, CCR3 and CCR5 receptors compared to their differentiated counterparts, resulting in increased autocrine-mediated invasion capacity by NF-kB activation and the consequently elevated MMP-9 secretion [161].

In addition, CSCs induce the production of certain ECM components such as periostin and tenasin through different factors, with TGF-β playing a central role. That ECM components promote metastasis and support SCs functions [162–166]. Periostin promotes the acquisition of a stemness phenotype in tumor cells when it binds to Wnt ligands [167, 168]. Periostin is also essential for metastatic colonization with infiltrating tumor cells being able to induce periostin expression in the metastatic niche [166]. This protein can also increase the expression of the VEGFR by ECs [169]. Lastly, tenascin is involved in vascular mimicry by enhancing the release of MMP-2 and MMP-9 by CSCs [170]. Thereby, both ECM components are important for tumor angiogenesis.

CSC secretome is involved both in ECM degradation facilitating tumor metastasis, angiogenesis and proliferation, and in the generation of specific components involved in tumor behavior.

Secretome in metastasis

Metastasis is a process that involves the dissemination of cells through lymphatic or blood vessels from the primary tumor to distant sites where colonization leads progressively to the growth of a secondary tumor. The EMT is a fundamental process for tumor invasion and involves the loss of epithelial properties and acquisition of motile and invasive phenotype [171]. The CSC secretome induces EMT and promotes metastasis in different ways. A key factor in the metastatic process is TGF-β, which correlates with the expression of SC markers in tumor cells and promotes that phenotype, as described above, through induction of EMT [77, 137, 172–174]. TGF-β has the ability to promote cell invasion and metastasis of CSCs and is not only expressed by CSCs, but also by other TME cells such as TAMs or CAFs [158, 175, 176]. However, TGF-β is a ubiquitous cytokine that is expressed in nearly every cell type and therefore has an active role in various cellular processes. Although TGF-β has been reported to promote tumor progression, invasion and metastasis in late-stage tumors, it has also been shown to act as a tumor suppressor as it can inhibit cell proliferation, induce apoptosis and mediate tumor cell differentiation in early-stage tumors. The role of TGF-β depends on tumor stage and is regulated by tumor cells, stromal cells and the immune system. The mechanisms behind the dual role of TGF-β are related to mutations in some components of the signaling pathway, but it may also be that there is no alteration of the tumor suppressor pathway, if not that it is inhibited [177, 178]. Several factors can promote tumor development including: (1) p53 mutations that activate the formation of the Smads2/3 and p63 complex that suppresses the action of p63 allowing TGF-β to promote EMT; (2) loss of Smad4 function secondary to genetic alterations; (3) overexpression of Six1 (pro-metastatic regulator); (4) oncogenic activation of the Ras-RAF-MAPK pathway; (5) hypomethylation of the PDGFβ gene; and (6) DAB2 epigenetic downregulation [178]. Moreover, miR-106b-25 inhibits p21 and Bim (pro-apoptotic factor) and is probably involved in the
positive regulation of Six1. Furthermore, miR-106b-25 can activate the tumorigenic path by inhibiting suppression growth through TGF-β and repressing the inhibitory protein Smad7 [177].

In addition, different miRNAs play a fundamental role in metastasis. Down-regulated miR-200 and let-7 miRNAs in CSCs can regulate EMT stem-like transition, self-renewal and metastasis in breast, prostate and colon cancer, and miR-34a downregulation in CSCs is related to self-renewal and asymmetric division [43, 152, 154, 179, 180]. miR-21 is a known "oncomiR" differentially up-regulated or over-expressed in CSCs and has been related to metastasis, poor prognosis, cell cycle and CSC promotion in many cancers. miR-221, miR-100, miR-10b or miR-125a upregulation in CSCs and non-CSCs can modulate breast CSC properties enhancing their invasion and migration potential [36, 152, 181]. Moreover, in metastatic breast cancer cells, exosome-secreted miR-10b, miR-105 and miR-9 are related to enhanced invasiveness through increased endothelial cell migration, angiogenesis and vascular permeability [43]. The miR-200 family found in extracellular vesicles of breast CSCs was related to their metastatic capacity [44]. In glioblastoma, miR-10b is upregulated in CSCs and its inhibition strongly reduces CSCs proliferation and metastasis. OncomiR-138 has also been identified as a prognostic biomarker of GSCs [180, 182]. In ovarian cancer, miR-5703, miR-630, miR-1246 and miR-320b were significantly dysregulated in CSCs compared with primary cancer cells, whereas miR-424-5p level was lower and associated with distant metastasis [183]. Finally, exosomes isolated from oral CSCs displayed nearly consistent downregulation of miR-34 and the up-regulation of miR-21 and miR-155 was related to increased CSC proliferation and migration; therefore, they may be consider indicators of poor prognosis [184]. However, miR-155 overexpression has been correlated with better prognosis and lower metastatic capacity [185, 186] and miR-21 appears down-regulated in CSCs [44]. These differences could be due CSC and inter-patient heterogeneity and to the dual role played by the same miRNA depending on cell state. This encourages further studies to elucidate the role of miRNAs based on this heterogeneity and its relationship with cancer progression. Lastly, miRNAs could be used as prognostic and predictive biomarkers of response to treatment.

Successful metastasis requires a favorable environment for the colonization and growth of tumor cells at the site of the secondary tumor, called the premetastatic niche (PMN) [187]. This PMN requires the recruitment and activation of local resident cells, alteration of the existing vasculature, ECM remodeling, as well as immune system deregulation [187, 188]. Several factors secreted by CSCs are involved in PMN formation. For example, VEGF increases vasculature permeability in the PMN [189] and stimulates MMP-9 expression in premetastatic tissue, thus facilitating tumor cell invasion [190]. Moreover, VEGF enhances the recruitment of bone marrow-derived cells (BMDCs), which are critical for PMN formation [191, 192] and facilitate tumor-promoting microenvironment through CCL9 secretion, induced by TGF-β signaling [193]. In addition, TGF-β and VEGF promote the expression of different inflammatory chemoattractants in the PMN [194]. Similarly, the CXCL12/CXCR4 axis is closely related to angiogenesis, proliferation, invasion and metastasis in most tumors by CXCR4 activation and migration of cells toward CXCL12. In addition, CXCL12 protein levels are highest in organs that are common sites of metastasis [61, 195–197]. CXCL12 induces MMP expression in cancer cells and up-regulates the activity of MMPs in tumor microenvironment, which promotes tumor invasion and metastasis [61]. CSCs express high levels of CXCR4 that have the ability to originate, maintain, disseminate and colonize metastasis sites and PMNs. CXCL12/CXCR4 signaling activation can be indicative of the metastatic CSC population [195, 198]. CSC migration is directed by CXCR4/CXCL12, playing a central role in chemotactic gradient perception. This signaling pathway is related with cell stemness and mobility [199]. Furthermore, intratumor hypoxia at primary site promotes PMN formation in secondary organs through enhancement of the expression of several factors and the involvement of exosomes [200, 201]. The role of exosomes and MVs from the primary tumor in the communication with PMN cells and modification of ECM has also been widely described [202–205]. Therefore, CSCs may play an important role in PMN formation, but further research is needed to clarify the specific role of the CSC secretome.

In summary, CSC secretome promotes metastasis by increasing EMT induction, tumor invasiveness, angiogenesis and CSC self-renewal. In addition, several secreted factors and vesicles are related to PMN formation, which make CSC secretome essential for successful metastasis.

Secretome in chemoresistance
A major challenge in antitumor therapy is to effectively eradicate CSCs, ultimately responsible for tumor relapse after chemotherapy. CSCs show intrinsic resistance to drugs related to ABC transporter overexpression, high ALDH activity, apoptosis evasion mechanisms, enhanced DNA damage repair capacity and activation of key signaling pathways [24, 206–209]. Some of these mechanisms are regulated by CSC-secreted factors. HIF1, secreted by both CSCs and non-SCs, has been described in leukemia
and lung tumors to promote radio and chemoresistance in hypoxic microenvironments by upregulating IGF1 expression and activating IGF1 receptor (IGF1R) [122, 210], which leads to an increase in the SC population and enhances EMT [211].

Furthermore, other interleukins with diverse roles in tumor behavior are also associated with increased drug resistance of CSCs. In colon cancer, the autocrine expression of CSC-secreted IL-4 promotes apoptosis evasion mechanisms, and treatment with IL-4 antibody especially sensitizes this subpopulation, promoting the efficacy of standard chemotherapeutic drugs [76]. Consistent with these studies, the chemoprotective action of IL-4 by increasing anti-apoptotic proteins Bcl-2 and Bcl-xL levels has been proved in other tumor types [212, 213]. The chemoprotective effects of IL-6 have also been described in breast CSCs, where HER2 overexpression increases IL-6 production [214]. Treatment with trastuzumab (target HER2 antibody) in breast cancer results in tumor chemoresistance related to the inactivation of the PTEN tumor suppressor gene. This is mediated by the IL-6 inflammatory loop activation, leading to the expansion of the CSC population. Furthermore, this CSC population secretes IL-6 to a much greater extent than non-SCs, which leads to a feedback loop that can be interrupted by an IL-6 receptor antibody, reducing the CSC population, tumor growth and metastasis [215]. A similar response to treatment with paclitaxel has been described in triple-negative breast cancer (TNBC) through the increase in the CSC population and tumor-initiating capacity in vivo. After treatment, high autocrine TGF-ß signaling and TGF-ß-dependent IL-8 overexpression occur, which promotes the potential of chemotherapy-resistant CSCs; therefore, this resistance can be reduced by pharmacological inhibition of TGF-ß [216]. Indeed, another study focused on blocking the IL-8 receptor CXCR1 using repertaxin, which selectively depleted CSC population in human breast cancer lines [149], confirming the role of this interleukin in maintaining the tumor stem population.

Moreover, the interaction of CSCs with TME cells also promotes a protective environment against chemotherapeutic agents. For example, both CSCs and MSCs secrete SDF-1/CXCL12, which interacts with their receptor CXCR4 overexpressed in CSCs [107, 217, 218]. This interaction contributes to the resistance of the tumor cells to chemotherapy-induced apoptosis [217, 219]. In fact, multiple trials with CXCR4 inhibitors have been conducted in solid tumors with promising anti-tumor effects [220]. Furthermore, another pathway that confers drug resistance to CSCs is their ability to influence cells of the immune system. CSCs from chemoresistant tumors were found to produce multiple proinflammatory cytokines (such as IL-1ß, IL-6, IL-8 or tumor necrosis factor) that also act to generate tumorigenic myeloid cells [221].

In addition to these strategies, the EMT has also been postulated as a therapeutic target against CSCs for its ability to promote chemoresistance through EMT-related signaling pathways and EMT transcription factors such as TGF-ß/Smad4, Hedgehog and Wnt [222, 223]. There are multiple examples of chemoresistance via EMT promotion such as doxorubicin resistance by upregulation of ABC transporters [224–227] or other apoptotic drugs such as cisplatin, paclitaxel and trastuzumab [228–231], which strongly supports the development of new therapies targeting EMT.

The epigenetic control of chemoresistance has been extensively described in CSCs addressing the classic CSC signaling pathways and the expression levels of genes related to chemoresistance and growth factor receptors and others [232]. Unfortunately, there is no research on the epigenetic control of secretome-associated chemoresistance in CSCs or in the total pool of the tumor population. Only one recent publication has related the inhibition of the HSP90 chaperone with a higher sensitivity to chemotherapy and a lower release of various cytokines (IL-8 and others) and, more interestingly, the HSP90 chaperone affected the survival of chemoresistant ALDH cell subpopulations [233]. In addition, there is little information available on oncogenic mutations associated with the influence of chemoresistance on CSC secretome, but p53 mutations have been reported to induce the release of an altered secretome by the tumor pool subpopulations that affects chemoresistance and other tumor processes [234, 235]. For this reason, the epigenetic control of the secretome and oncogenic mutations seems plausible hypotheses related to chemoresistance generated by the secretome, but additional studies are necessary to confirm them.

It is worth discussing how therapeutic treatments can alter the composition and abundance of the tumor secretome, a phenomenon called "therapy-induced tumor cell secretome," which enhances the survival and expansion of CSCs [236]. For example, treatment with paclitaxel or gemcitabine in TNBC increases the production of HIF factors, whose increased activity leads to an increase in the CSC subpopulation through IL-6 and IL-8 signaling and increased expression of multidrug resistance. This suggests that a treatment based on chemotherapeutic agents combined with HIF inhibitors would help overcome chemoresistance [23]. However, chemotherapy is not the only treatment that can alter the tumor secretome. As shown in a previous study by our research team, the dose of radiation therapy administered in vitro and in vivo affects the expression of several MMPs and
their inhibitors [237]. These alterations were related with the molecular profile of breast cancer. The study not only highlights the fundamental role played by the specific characteristics of each tumor and TME in response to treatment, but also that radiotherapy promotes the secretion of matrix remodeling enzymes involved in the dispersion, invasiveness and metastasis of CSCs and in the EMT process [237]. Additionally, Shen et al. demonstrated that chemotherapy induces breast cancer cells to secrete exosome-derived miR-9, miR-195 and miR-203a, which induces CSC phenotypes and expression of stemness-associated genes, thus generating cancer cell communication and self-adaptation to survive treatment [39]. Thereby, miRNAs can also display chemoprotective functions, which means the secretome’s capacity to protect tumors against chemotherapy, as demonstrated by the mentioned studies, in which the released exosomal miRNAs act in response to the treatment favoring the maintenance and expansion of CSCs, avoiding therefore the effect of the treatment and the development of relapses and metastatic processes.

To recapitulate, CSC secretome promotes chemoresistance through different strategies such as inducing the stem phenotype and EMT processes, apoptosis evasion mechanisms and regulation of the immune system. Lastly, chemotherapeutic agents can alter the tumor secretome and consequently tumor cell functions and responses, with a negative effect on treatment outcomes.

**Clinical implications and future trends**

Given the importance of the interplay between CSCs and their niche, the new antitumor therapies focus on simultaneously targeting different communication routes to target TME and starve CSCs (Fig. 3). One of the most recurrent options is to target tumor vasculature, with several FDA-approved angiogenesis inhibitors available (see Table 2) such as bevacizumab (antibody directed against VEGF) or sorafenib and sunitinib, inhibitors of tyrosine kinase receptors (TKRs) that target multiple TKRs, including VEGF receptors (VEGFRs) and PDGF receptors (PDGFRs). The combination of both treatment strategies has increased patient survival in the first months, usually in combination with other chemotherapy approaches; however, in many of these patients the disease will progress [238]. This may be due to a lack of biomarkers to determine which patients will benefit from

![Fig. 3](image-url)  
**Fig. 3** Tumor response to different antitumor strategies. The failure of conventional therapies is due to the tumor and the CSC mechanisms to initiate the carcinogenesis process. For this reason, the new therapies focus on TME, including the CSC secretome. However, CSCs use different pathways to fulfill their functions; therefore, targeting only one of the pathways can lead to tumor relapse. The new therapies are aimed at simultaneously blocking several pathways for better outcomes.
these drugs and the doses required as well as to tumor adaptive resistance mechanisms [239, 240]. This tumor capacity to adapt to therapy by activating other alternative pathways has led to the development of strategies that combine anti-VEGF agents with other drugs targeting different pathways such as VEGFRs, TKRs and epidermal growth factor receptors (EGFRs) inhibitors, with greater or lesser success [241]. Indeed, CSCs can also promote resistance to anti-angiogenic therapy, which leads to intra-tumor hypoxia states resulting in increased HIF-1 and HIF-2 expression and, therefore, increased risk of tumor propagation, CSC self-renewal, drug resistance and even angiogenesis activation [23, 120–122, 125–127]. For example, treatment of breast cancer with sunitinib and bevacizumab increased the CSC population through HIF-1 activation of Wnt pathway [242], and in pancreatic cancer and glioblastoma the use of a VEGFR and TKR inhibitor also increased the risk of invasion and metastasis related to intratumor hypoxic states [243–245]. Nonetheless, when these drugs are used in combination with other cytotoxic drugs, the results are more promising [246, 247], which confirms the idea of using antiangiogenic drugs in conjunction with other therapies for example targeting hypoxia [248] (Fig. 3). Furthermore, antiangiogenic therapy failure has resulted in a different approach involving vascular normalization to improve drug delivery and limit hypoxia [116, 249].

Another widely used approach is to try to block the recruitment or function of stromal cells due to the ability of CSCs to promote their tumor niche. For example, preclinical studies showed that targeting CCL2 or the CCL2 receptor (CCR2) on tumor infiltrating macrophages improved chemotherapeutic efficacy, inhibited metastasis and increased anti-tumor T-cell responses [72, 250, 251]. However, these agents may need to be administered as adjuvant therapy and not as monotherapy [252, 253]. However, the results from clinical trials have not been as promising and a deeper understanding of the underlying mechanisms of the pathways involved is needed in order to success in the clinical translation [254]. A similar result has been observed in treatment with IL-6 inhibitors and its receptor where preclinical trials showed antitumor efficacy against different tumor types, but the clinical trials do not seem to show good results [255]. This suggests

| Drug                          | Target                                                                 | Cancer type                                                                 | References |
|-------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------|------------|
| Abiraterone                   | Androgen deprivation therapy                                          | Prostate cancer                                                             | [309]      |
| Aflibercept                   | Bind VEGF A and B and PGF                                              | Colorectal cancer                                                           | [315]      |
| Axitinib                      | Against VEGR1-3, PDGFRs, c-Kit and FGFRs                              | Advanced renal cell carcinoma and soft tissue sarcoma                        | [316, 317] |
| Bevacizumab                   | Antibody against vascular endothelial growth factor (VEGF)            | Breast, colon and lung cancer                                               | [238]      |
| Cabozantinib                  | MET and VEGFR2 inhibitor                                              | Renal cancer and hepatocellular carcinoma                                   | [318]      |
| Dacomitinib                   | EGFRs inhibitor                                                       | Metastatic NSCLC                                                            | [319]      |
| Enzalutamide                  | Androgen deprivation therapy                                          | Prostate cancer                                                             | [309]      |
| Erdafitinib                   | FGF receptor (FGFR) inhibitor                                        | Urothelial carcinoma                                                        | [320, 321] |
| Erlotinib                     | EGFRs inhibitor                                                       | NSCLC and pancreatic cancer                                                 | [322]      |
| Gefitinib                     | EGFRs inhibitor                                                       | NSCLC                                                                       | [322]      |
| Lapatinib                     | EGFRs inhibitor                                                       | NSCLC                                                                       | [322]      |
| Lenvatinib                    | Against VEGFR1-3, RET, c-kit, and PDGFRs                              | Breast cancer and NSCLC and pancreatic cancer                               | [322]      |
| Mogamulizumab                 | Antibody against CCR4                                                | Thyroid cancer                                                               | [323]      |
| Oncolytic virus               | (talimogene laher-parepvec) Expressing GM-CSF to enhance systemic antitumor immune responses | Skin lymphoma                                                               | [324]      |
| Osimertinib                   | EGFRs inhibitor                                                       | NSCLC                                                                       | [322]      |
| Panitumumab                   | Antibody against endothelial growth factor receptor (EGFR)           | Colorectal carcinoma                                                        | [325]      |
| Pazopanib                     | Against VEGR1-3, PDGFRs, c-Kit, and FGFRs                              | Advanced renal cell carcinoma and soft tissue sarcoma                        | [316, 317] |
| Ramucirumab                   | VEGFR2 inhibitor                                                      | Metastatic gastric and gastro-esophageal junction adenocarcinoma            | [326]      |
| Regorafenib                   | TKRs inhibitor, including VEGFR1-3, FGFRs and PDGFRs                  | Colorectal cancer and hepatocellular carcinoma                              | [327]      |
| Sorafenib                     | Tyrosine kinase receptors (TKRs) inhibitors, that target multiple TKRs, including VEGFRs (VEGFRs) and PDGFR receptors (PDGFRs) | Kidney cancer, renal cell carcinoma and gastrointestinal stromal tumors   | [238]      |
| Sunitinib                     | VEGFR2 and EGFR inhibitor                                             | Medullary thyroid carcinoma                                                 | [328]      |
the need for biomarkers that may help identify target patients and the need for using combined therapies [256–258]. SDF-1/CXCL12 is another protein related to the recruitment and activation of ECs in TME by CSCs [94], to chemoresistance processes, and trials with inhibitors of SDF-1/CXCL12 or its receptor CXCR4 have shown high antitumor potential [220, 259–262] (Fig. 3). In fact, it is already used in the clinical practice [263]. Moreover, there is evidence of its potential as adjuvant therapy by sensitizing the tumor to other therapies [262, 264–268].

Another strategy used has been to directly attack CSC maintenance. For example, IL-8 antagonists such as reparixin in monotherapy or combined with other drugs have shown their efficacy in in vitro and in vivo assays [149, 269–272]. In fact, ongoing clinical trials demonstrate no adverse effects and good prognosis [273, 274]. Other clinical trials targeting TGF-β have been carried out, but its pro-tumorigenic effect depends on the tumor stage and the number of signaling pathways where TGF-β is involved require special attention and robust biomarkers to predict its effect [275].

As previously reported, CSC-secreted factors promote EMT and confer drug resistance, and therefore, many studies have focused on EMT. For example, one of the most studied drugs is salinomycin, which inhibits EMT and sensitizes the tumor to the action of other drugs such as doxorubicin [276–282] (Fig. 3). Metformin also selectively acts against CSCs by targeting EMT, blocking the IL-6/STAT3 axis or decreasing EMT transcriptional factors, and by increasing tumor sensitivity against other current therapies [197, 283–287]; therefore, it has been included in clinical trials [288]. Furthermore, to overcome the resistance acquired by EMT from EGFRs inhibitors, several studies that combine EGFRs inhibitors with drugs targeting other pathways, such as with FGFRs inhibitors, have been performed [289–292]. Despite some good results with some drugs against EMT, their toxicity and ability to promote metastasis still remain a concern [222].

In addition to the previous results, new therapies targeting both the ECM [20, 293] and the MMPs are now emerging [294]. Since one of the main problems is that tumor ECM prevents the correct diffusion of drugs, numerous studies focus on either degrading the matrix, using collagenases, hyaluronidases or hyperthermia [295–300], and on blocking ECM synthesis de novo [301–305], although the latter has not provided positive results in clinical trials [306, 307]. MMPs, which promote angiogenesis, metastasis and the release of factors within the matrix, are also the target of new therapies like andecaliximab and several MMPs antibodies, specific or broad-spectrum MMP inhibitors, which have shown promising results in clinical trials [294] (Fig. 3).

Targeting cancer cell secretome involves not only inhibiting the release or binding with receptors, but rather to stimulate the release of certain factors. For example, talimogene laherparepvec (T-VEC), an oncolytic virus FDA approved for the treatment of advanced melanoma, has been engineered to selectively replicate within tumors and to promote the priming of T cell responses and produce granulocyte–macrophage colony-stimulating factor (GM-CSF) to enhance systemic antitumor immune responses [308]. The role of the tumor secretome in general, and of CSCs in particular, is emerging as an indicator of the response to treatment as shown by the two FDA-approved drugs for androgen deprivation therapy for metastatic prostate cancer, abiraterone and enzalutamide, whose resistance seems to be related to a potentially immunosuppressive tumor microenvironment and whose treatment efficacy can be predicted using IL-6 levels [309].

Finally, a deeper knowledge about the components and dynamics of the CSC secretome has allowed the development of new therapies specifically targeting CSCs through different factors and their overexpressed receptors. In this respect, several clinical trials are conducted to determine the effect of different drugs against CSC secretome and to establish predictive biomarkers for better treatment outcomes (Table 3).

Importantly, as the previous studies have shown, only some patients will effectively benefit from combined therapies while for other patients this type of therapy will be ineffective or even harmful, due to the high heterogeneity between patients. In this respect, several clinical trials are currently conducted to test and validate personalized therapies. A prospective clinical trial that began in 2015 is testing in vitro 73 drugs in different combinations in CSCs from glioblastoma samples to study the effect of personalized therapies [ClinicalTrials.gov identifier: NCT02654964]. Indeed, another prospective clinical trial suggested that personalized antitumor therapies based on molecular profiles of cancer patients had a significant improvement in treatments, compared with current clinical strategies [ClinicalTrials.gov identifier: NCT02534675]. There is increasing evidence that the use of biomarkers would substantially improve clinical practice and patient well-being. Several factors of the CSC secretome have been proposed as biomarkers, like ceruloplasmin identified in pancreatic cancer, which could be used in addition to CA19-9 [310]. Ceruloplasmin was also identified in serum from ovarian cancer patients as a possible prognostic biomarker of chemoresistance [311]. Its overexpression by CSCs in glioma has also been studied, but currently only in vitro and in vivo [312]. In blood samples from breast cancer patients, CSC-secreted programmed death ligand-1 (PD-L1) was related with
Table 3 Clinical trials with CSCs and secretome

| NCT number   | Target | Status       | Drug             | Combined therapy                  | Cancer type                                           |
|--------------|--------|--------------|------------------|-----------------------------------|------------------------------------------------------|
| NCT01861054  | CXCR1  | Completed    | Reparixin        |                                   | Breast cancer                                        |
| NCT02001974  | CXCR1  | Phase 1/completed | Reparixin, Paclitaxel |                                   | Breast cancer                                        |
| NCT01190345  | VEGF   | Phase 2/completed | Bevacizumab, Chemotherapy |                                   | Breast cancer                                        |
| NCT01283945  | VEGFR/FGFR/PDGF | Phase 1/2a completed | Lucitani |                                   | Solid tumor                                           |
| NCT02491840  | CXCR4  | Recruiting   | Prognostic biomarkers |                                   | Gastric and cardia adenocarcinoma                     |
| NCT01955460  | TGF-β  | Phase 1/recruiting | Aldesleukin, Chemotherapy and lymphocytes |                                   | Melanoma                                              |
| NCT01248637  | HIF-1  | Completed    | Pimonidazole hydrochloride |                                   | Pancreatic                                            |
| NCT04137627  | HIF-1  | Phase 3/completed | Melatonin, Adjuvant chemotherapy |                                   | Oral squamous cell carcinoma                          |
| NCT02499458  | HIF-2  | Completed    | Biomarkers        |                                   | Renal cancer                                          |
| NCT03401788  | HIF-2  | Phase 2/not recruiting | PT2977 |                                   | VHL-associated renal cell carcinoma                    |
| NCT03108066  | HIF-2  | Phase 2/not recruiting | PT2385 |                                   | VHL-associated renal cell carcinoma                    |
| NCT01283945  | FGF    | Phase ½ completed | Lucitani |                                   | Solid tumors                                          |
| NCT00657423  | FGF    | Phase 3      | Docetaxel and cisplatin |                                   | Lung neoplasms                                        |
| NCT01440959  | FGF    | Phase 2/completed | Doxitinib |                                   | Gastrointestinal stromal tumors                       |
| NCT00372775  | FGF    | Phase 2/completed | Sunitinib |                                   | Non-small cell lung cancer with brain metastasis      |
| NCT01791985  | FGF    | Phase 1/2 Completed | AZD4547, Anastrozole or letrozole |                                   | Breast cancer                                         |
| NCT01945164  | FGF    | Completed    | XL999 |                                   | Advanced malignancies                                 |
| NCT00021229  | FGF    | Phase 1/2     | Imatinib mesylate | Local irradiation therapy         | Glioma                                                |
| NCT04207086  | FGF    | Phase 2/recruiting | Pembrolizumab, Lenvatini |                                   | Melanoma stage III                                    |
| NCT03303885  | FGF    | Recruiting    | Preclinical biomarkers |                                   | Liposarcoma                                           |
| NCT00216112  | PDGF   | Phase 2/completed | Matinib, mesylate, Docetaxel |                                   | Ovarian cancer                                         |
| NCT03851614  | PDGF   | Phase 2/recruiting | Cedirani, Durvalumab |                                   | Colorectal cancer                                     |
| NCT01372813  | PDGF   | Phase 2/completed | Vandetanib |                                   | Pancreatic adenocarcinoma, Leiomyosarcoma             |
| NCT04042597  | PDGF   | Phase 2/recruiting | Anlotinib hydrochloride |                                   | Renal carcinoma                                       |
| NCT00367679  | PDGF   | Phase 2/completed | Pazopanib |                                   | Chordoma advanced cancer                              |
| NCT00372775  | PDGF   | Phase 2/completed | Sunitinib |                                   | Non-small cell lung cancer                            |
| NCT01105533  | PDGF   | Phase 1/completed | PF-00337210 |                                   | Non-small cell lung cancer                            |
| NCT00600821  | PDGF   | Phase 2/completed | AG-013736 (axitinib), Paclitaxel and carboplatin |                                   | Neoplasm                                              |
| NCT04207086  | PDGF   | Phase 2/completed | Lenvatini, Pembrolizumab |                                   | Non-small cell lung carcinoma                         |
| NCT02178072  | CCL5   | Phase 2/recruiting | 5-Azacitadine |                                   | Melanoma stage III                                    |
| NCT03126630  | CCL5   | Phase 1/2 recruiting | Pembrol Anetumab ravansinezumab |                                   | Head and neck squamous cell carcinoma                  |
| NCT03964337  | CCL5   | Phase 2      | Cabozanitini |                                   | Pleural malignant mesothelioma                        |
| NCT02125344  | CCL5   | Phase 3/completed | Chemotherapy |                                   | Prostate cancer                                       |
| NCT02432378  | CCL5   | Phase ½ recruiting | Cisplatin and DC vaccine, Celecoxib, CKM |                                   | Breast cancer                                          |
| NCT00653250  | PEG2   | Completed    | Celecoxib |                                   | Ovarian cancer                                         |
| NCT03964337  | CCL5   | Phase 2      | Cabozanitini |                                   | Lung cancer                                           |
| NCT02125344  | CCL5   | Phase 3/completed | Chemotherapy |                                   | Breast cancer                                          |
| NCT02432378  | CCL5   | Phase ½ recruiting | Cisplatin and DC vaccine, Celecoxib, CKM |                                   | Ovarian cancer                                         |
| NCT00653250  | PEG2   | Completed    | Celecoxib |                                   | Lung cancer                                           |
metastasis and has been proposed as a potential follow-up biomarker by immune checkpoint blockers \[313\]. However, finding robust markers can be challenging. As for antiangiogenic therapies, numerous biomarkers have been proposed since the approval of bevacizumab, being VEGF-A the most promising. However, in clinical trials its efficacy could not be proven \[314\] and more research is still needed to identify predictive and prognostic biomarkers.

**Conclusion**

The evidence included in this review demonstrates that CSCs regulate multiple tumor hallmarks through the expression of several growth factors, interleukins, cytokines and extracellular vesicles, and that a greater understanding of the pathways that dictate tumor behavior is needed for the development of new antitumor therapies. Furthermore, these therapies must target not only tumor proliferating cells and CSCs, but also they need to be combined with other therapies targeting TME. The interconnected signaling pathways involved in the altered secretome must also be targeted, since tumors can develop evasion mechanisms and have different alternative routes to fulfill their functions. Lastly, robust biomarkers are needed to identify those patients most likely to benefit from these therapies in order to personalize antitumor treatments (Fig. 4).

**Abbreviations**

ALDH: Aldehyde dehydrogenase; BMDCs: Bone marrow-derived cells; CAFs: Cancer-associated fibroblasts; CLC1: Chloride intracellular channel protein 1; CSCs: Cancer stem cells; CTGF: Connective tissue growth factor; ECs: Endothelial cells; ECM: Extracellular matrix; EGF: Epidermal growth factor receptors; EMT: Epithelial–mesenchymal–transition; EPCs: Endothelial progenitor cells; FGF: Fibroblast growth factor; GSCs: Glioblastoma stem-like cells; HGF: Hepatocyte growth factor; Hh: Hedgehog; HIF-1: Hypoxia-inducible factor 1; HIF-2: Hypoxia-inducible factor 1; HDGF: Hepatoma-derived growth factor; IGF: Insulin-like growth factor; IGFR1: Insulin-like growth factor 1 receptor; IL: Interleukin; IL-1β: Interleukin-1β; IL-2: Interleukin-2; IL-4: Interleukin-4; IL-6: Interleukin-6; IL-8: Interleukin-8; IL-10: IL-10; IGF1R: Insulin-like growth factor 1 receptor; LIF: Leukemia inhibitory factor; MIC-1: Macrophage inhibitory cytokine 1; miRNAs: Micro-RNAs; MMPs: Matrix metalloproteinases; mRNAs: Messenger RNAs; MSCs: Mesenchymal stem cells; MVs: Microvesicles; NF-kB: Nuclear factor kappa B; PAR1: Protease-activated receptor 1; PDGF: Platelet-derived growth factor; PDGFRs: Platelet-derived growth factor receptors; PD-L1: Programmed death ligand 1; PGE2: Prostaglandin E2; PMN: Premetastatic niche; SC: Stem cell; SDF-1/CXCL12: Stromal cell-derived factor 1; SHH: Sonic Hedgehog; TAMs: Tumor-associated macrophages; TGF-β: Transforming growth factor β; TKRs: Tyrosine kinase receptors; TME: Tumor microenvironment; TNBC: Triple-negative breast cancer; Treg: Regulatory T lymphocytes; VE-Cadherin: Vascular–endothelial cadherin; VEGF: Vascular–endothelial growth factor; VEGFR: Vascular–endothelial growth factor receptor.

**Acknowledgements**

All figures were created with Biorender.com.

**Authors’ contributions**

GJ and JAM design the study, and JLA and CGL wrote the manuscript. GJ and JAM revised critically the manuscript for important intellectual content. All authors read and approved the final manuscript.

**Funding**

This work was supported by grants from Consejería de Salud y Familias de la Junta de Andalucía (Project Numbers PIN-0224-2019), by the Ministerio de Ciencia, Innovación y Universidades Grant Number RTI2018-101309-B-C22 (FEDER Funds), by the Consejería de Economía, Conocimiento, Empresas y Universidad de la Junta de Andalucía (P18-FR-2470 and SOMM17/0109/UGR, FEDER Funds), by the Ministry of Economy and Competitiveness, Instituto de Salud Carlos III (FEDER funds, Projects Nos. PIE16/00045, DTS19/00143 and DTS17/00087), and from the Chair “Doctors Galera-Requena in cancer stem cell research” (CMC-CTS963).

**Availability of supporting data**

Not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.
Competing interests
None of the authors have competing interest to declare.

Author details
1 Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research (CIBER), University of Granada, 18100 Granada, Spain. 2 Excellence Research Unit “Modeling Nature” (MNat), University of Granada, Granada, Spain. 3 Department of Health Sciences, University of Jaén, 23071 Jaén, Spain. 4 Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, 18016 Granada, Spain.

Received: 12 July 2020 Accepted: 23 September 2020 Published online: 15 October 2020

References
1. Zhang S, Balch C, Chan MW, Lai HC, Matei D, Schilder JM, et al. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Res. 2008;68:4311–20.
2. Al-Haj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003;100:9383–8.
3. Luo M, Clethiou SG, Deol Y, Liu S, Nagrath S, Azizi E, et al. Breast cancer stem cells: current advances and clinical implications. Methods Mol Biol. 2015;1293:1–49.
4. Parada LF, Dirks PB, Wechsler-Reya RJ. Brain tumor stem cells remain in play. J Clin Oncol. 2017;35(21):2428–31.
5. Heng WS, Gosens R, Kruyt FAE. Lung cancer stem cells: origin, features, maintenance mechanisms and therapeutic targeting. Biochem Pharmacol. 2019;160:121–33.
6. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, et al. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Res. 2005;65:9328–37.
7. Wang X, de JKM, Economides KD, Walker O, Yu H, Halli MV, et al. A luminal epithelial stem cell that is a cell of origin for prostate cancer. Nature. 2009;461:495–500.
8. Munro MJ, Wickremesekera SK, Peng L, Tan ST, Itinteang T. Cancer stem cells: origin, features, maintenance mechanisms and therapeutic targeting. Biochem Pharmacol. 2019;160:121–33.
9. Yamashita T, Wang XW. Cancer stem cells in the development of liver cancer. J Clin Invest. 2013;123(5):1911–8.
10. Li W, Ma H, Zhang J, Zhu L, Wang C, Yang Y. Unraveling the roles of CD44/CD24 and ALDH1 as cancer stem cell markers in tumorigenesis and metastasis. Sci Rep. 2017;7(1):13856.
11. Shiraiishi A, Tachi K, Essid N, Tsuibo I, Nagano M, Kato T, et al. Hypoxia promotes the phenotypic change of aldehyde dehydrogenase activity of breast cancer stem cells. Cancer Sci. 2017;108:362–72.
12. Chutahapith S, Erimin J, El-Sheemy M, Erimin O. Breast cancer chemoresistance: emerging importance of cancer stem cells. Surg Oncol. 2010;19(1):27–32.
13. Tirino V, Desiderio V, Paino F, De Rosa A, Papaccio F, La Noce M, et al. Cancer stem cells in solid tumors: an overview and new approaches for their isolation and characterization. FASEB J. 2013;27(1):13–24.
14. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012;21(3):309–22.
15. Wu T, Dai Y. Tumor microenvironment and therapeutic response. Cancer Lett. 2017;387:61–8.
16. Henke E, Nandigama R, Ergun S. Extracellular matrix in the tumor microenvironment and its impact on cancer therapy. Front Mol Biosci. 2020;6:160.
17. Eble JA, Nilsland S. The extracellular matrix in tumor progression and metastasis. Clin Exp Metastasis. 2019;36(5):171–198.
18. Walker C, Mojares E, Del Río HA. Role of extracellular matrix in development and cancer progression. Int J Mol Sci. 2018;19(10):3028.
19. Samanta D, Gilkese DM, Chaturvedia P, Xiang L, Semenza GL. Hypoxia-inducible factors are required for chemotherapy resistance of breast cancer stem cells. Proc Natl Acad Sci U S A. 2014;111(S0):E5429–E54385348.
20. Prieto-Vila M, Takahashi RU, Usuda W, Kohama J, Ochiya T. Drug resistance driven by cancer stem cells and their niche. Int J Mol Sci. 2017;18(12):2574.
21. Capece D, Verzella D, Tesistore A, Alessie E, Capalbo C, Zazzaroni F. Cancer secretome and inflammation: the bright and the dark sides of NF-kB. Semin Cell Dev Biol. 2018;78:51–61.
22. Ye J, Wu D, Wu P, Chen Z, Huang J, The cancer stem cell niche: cross talk between cancer stem cells and their microenvironment. Tumor Biol. 2014;35(5):3945–51.
23. Abels E, Breakefeld X. Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. Cell Mol Neurobiol. 2016;36(3):301–12.
24. Ekström EJ, Bergenfelz C, von Bäckström V, Svennerfors F, Carlmark E, Jonsson G, et al. WNT3A induces release of exosomes containing proangiogenic and immunosuppressive factors from malignant melanoma cells. Mol Cancer. 2014;13:88.
25. Skog J, Würdinger T, van Rijn S, Meijer DH, Ganssche H, Curry WT, et al. Glisoblastoma microvesicles transport RNA and proteins that promote tumor growth and provide diagnostic biomarkers. Nat Cell Biol. 2008;10:1470–6.
26. Taraboletti G, D’Ascenzo S, Giusti I, Marchetti D, Borsotti P, Millimaggi D, et al. Bioavailability of VEGF in tumor-shed vesicles depends on vesicle burst induced by acidic pH 1. Neoplasia. 2006;8:96–103.
27. Chen X, Liang H, Zhang J, Zheng Z, Zhang CY. Secreted microRNAs: a new form of intercellular communication. Trends Cell Biol. 2012;22:125–32.
28. Webber J, Steadman R, Mason MD, Tabi Z, Clayton A. Cancer exosomes trigger fibroblast to myofibroblast differentiation. Cancer Res. 2010;70(23):9621–30.
29. Kim J, Kim TY, Lee MS, Mun JY, Ihm C, Kim SA. Exosome cargo reflects TGF-β1-mediated epithelial-to-mesenchymal transition (EMT) status in AS549 human lung adenocarcinoma cells. Biochem Biophys Res Commun. 2016;478:643–8.
30. Gu J, Qian H, Shen L, Zhang X, Zhu W, Huang L, et al. Gastric cancer exosomes trigger differentiation of umbilical cord derived mesenchymal stem cells to carcinoma-associated fibroblasts through TGF-β1/Smad pathway. PLoS ONE. 2012;7(12):e52465.
31. Aga M, Bentz GL, Raffa S, Tornisi MR, Kondo S, Wakisaka N, et al. Exosomal miRNA-15a/miRNA-181b promotes invasion, metastasis and angiogenesis in A549 human lung adenocarcinoma cells. Oncogene. 2013;32:4613–22.
32. Zhao Y, Dong Q, Li J, Zhang K, Qin J, Zhao J, et al. Targeting cancer stem cell niches and their niche: perspectives for future therapeutic targets and strategies. Semin Cancer Biol. 2018;53:139–55.
33. Najafi M, Mortezaee A, Majidpoor J. Cancer stem cell (CSC) resistance drivers. Life Sci. 2019;234:116781.
34. Hu H, Gatti RA. MicroRNAs: new players in the DNA damage response. J Mol Cell Biol. 2011;3:151–8.
35. Shen M, Dong C, Ruan X, Yan W, Cao M, Pizzio D, et al. Chemotherapy-induced extracellular vesicle microRNAs promote breast cancer stemness by targeting OneCUT2. Cancer Res. 2019;79:3608–21.
36. Yang Q, Diamond MP, Al-Hendy A. The emerging role of extracellular vesicle-derived miRNAs: implication in cancer progression and stem cell related diseases. J Clin Epigenet. 2016;2:110.
37. Patridge JL, Belle L, Khow-Goodall Y. The secretome in cancer progression. Biochim Biophys Acta. 2013;1834:2333–41.
38. Wang M, Yu F, Ding H, Wang Y, Li P, Wang K. Emerging function and clinical values of exosomal microRNAs in cancer. Mol Ther Nucleic Acids. 2019;16:791–804.
39. Kim J, Yao F, Xiao Z, Sun Y, Ma L. MicroRNAs and metastasis: small RNAs play big roles. Cancer Metastasis Rev. 2018;37:5–15.
44. Shoff M, Booker T, Leavitt B, Harmon D, Kingsley K, Howard KM. Differential exosome miRNA expression in oral cancer stem cells. ExRNA. 2020;2:1–9.

45. Grange C, Tapparo M, Collino F, Vitillo L, Deregibus MC, Wang L, Yang G, Zhao D, Wang J, Bai Y, Peng Q, et al. CD103-positive CSC exosome promotes EMT of clear cell renal cell carcinoma: Role of remote MiR-19b-3p. Mol Cancer. 2019;18:1–15.

46. Shirmohamadi M, Eghbali E, Najjary S, Mokhtarzadeh A, Kojabad AB, Hajasgharzadeh K, et al. Regulatory mechanisms of microRNAs in colorectal cancer and colorectal cancer stem cells. J Cell Physiol. 2020;235:776–89.

47. Sharma A. Role of stem cell derived exosomes in tumor biology. Int J Cancer. 2018;142:1086–92.

48. Heiler S, Wang Z, Zöller M. Pancreatic cancer stem cell markers and exosomes—the incentive push. World J Gastroenterol. 2016;22:5971–6007.

50. Ridge SM, Sullivan FJ, Glynn SA. Mesenchymal stem cells: key players in cancer progression. Mol Cancer. 2017;16:31.

51. Jiménez G, Hackenberg M, Catalina P, Boulaiz H, Griñán-Lisón C, Liu S, Ginestier C, Ou SJ, Clouthier SG, Patel SH, Monville F, et al. CXCL7 in liver metastases from colon cancer is correlated to shorter invasion through basement membrane by CXCL7-transfected breast cells. Am J Surg. 2008;196:690–6.

52. Desurmont T, Skrypek N, Duhamel A, Jonckheere N, Millet G, Leteurre E, et al. Overexpression of chemokine receptor CXCR2 and ligand CXCL7 in liver metastases from colon cancer is correlated to shorter disease-free and overall survival. Cancer Sci. 2015;106:262–9.

55. Nomura A, Gupta VK, Dauer P, Sharma NS, Dudeja V, Merchant N, et al. NFκB-mediated invasiveness in CD133+ pancreatic TICs is regulated by autocrine and paracrine activation of IL1 signaling. Mol Cancer Res. 2018;16:162–72.

56. Li Y, Wang L, Pappan L, Gallilher-Beckley A, Shi J. IL-1β promotes stemness and invasiveness of colon cancer cells through Zeb1 activation. Mol Cancer. 2012;11:87.

57. Escober P, Bouclier C, Serret J, Bèche J, Brigitte M, Caicedo A, et al. IL-1β produced by aggressive breast cancer cells is one of the factors that dictate their interactions with mesenchymal stem cells through chemokine production. Oncotarget. 2015;6:29034–47.

58. Malik A, Kanneganti T-D. Function and regulation of IL-1α in inflammatory diseases and cancer. Immunol Rev. 2018;281(1):124–37.

59. Castaño Z, San Juan BP, Spiegel A, Pant A, DieCristo MJ, Laszewski T, et al. IL-1β inflammatory response driven by primary breast cancer prevents metastasis-initiating cell colonization. Nat Cell Biol. 2018;20:1084–97.

60. Pavon LF, Silbov TT, De Souza AV, Da Cruz EF, Malheiro SMF, Cabral FR, et al. Tropism of mesenchymal stem cell toward CD133+ stem cell of glioblastoma in vitro and promote tumor proliferation in vivo. Stem Cell Res Ther. 2018;9(1):310.

61. Meng W, Xue S, Chen Y. The role of CXCL12 in tumor microenvironment. Gene. 2018;641:105–10.

62. Dillenburg-Pilla P, Patel V, Mikels CM, Zárade-Bladés CR, Doçi CL, Amorophimoltham P, et al. SDF-1/CXCL12 induces directional cell migration and spontaneous metastasis via a CXCR4/Gaα120R131 axis. FASEB J. 2015;29(3):1056–68.

63. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2005;7:392–401.

64. Alguacil-Núñez C, Ferrer-Ortiz I, García-Verdú E, López-Pirez P, Llorente-Cortijo JM, Sainz B. Current perspectives on the crossstalk between lung cancer stem cells and cancer-associated fibroblasts. Crit Rev Oncol Hematol. 2018;125:102–10.

65. Lee KW, Yeo SY, Sung CO, Kim SH. Twist1 is a key regulator of cancer-associated fibroblasts. Cancer Res. 2015;75:73–85.

66. Moore-Smith LD, Isayeva T, Lee JH, Frost A, Ponnazhagan S. Silencing of TGF-β1 in tumor cells impacts MMP-9 in tumor microenvironment. Sci Rep. 2017;7:1–10.

67. Tshuyada A, Chow A, Wu J, Somlo G, Chu P, Loera S, et al. CCL2 mediates cross-talk between cancer cells and stromal fibroblasts that regulates breast cancer stem cells. Cancer Res. 2012;72:2768–79.

68. Tan HX, Xiao ZG, Huang T, Fang ZX, Liu Y, Huang ZC. CXCR4/TGF-β1 mediated self-differentiation of human mesenchymal stem cells to carcinoma-associated fibroblasts and promoted colorectal cancer development. Cancer Biol Ther. 2020;21:248–57.

69. Shangguan L, Li X, Krause U, Hui B, Zhao Y, Yang Z, et al. Inhibition of TGF-β1/Smad signaling by RABMI blocks differentiation of human mesenchymal stem cell to carcinoma-associated fibroblasts and abolishes their protrator effects. Stem Cells. 2012;30:2810–9.

70. Valenti G, Quinn HM, Heynjen GJE, Lan L, Holland JD, Vogel R, et al. Cancer stem cells regulate cancer-associated fibroblasts via activation of hedgehog signaling in mammary gland tumors. Cancer Res. 2017;77:2134–47.

71. Zamorano BF, Chen W. Dual roles of immune cells and their factors in cancer development and progression. Int J Biol Sci. 2011;7:651–8.

72. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, Campion LR, et al. CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature. 2011;475:222–5.

73. Fahidian F, Dušek PJG, Safarzaedeh E, Derakshanhi A, Baghbanzadeh A, Baradanar B. Interactions between cancer stem cells, immune system and some environmental components: friends or foes? Immunol Lett. 2019;208:19–29.

74. Yi L, Xiao H, Xu M, Ye X, Hu J, Li F, et al. Glioma-initiating cells: a predominant role in microglia/macrophages tropism to glioma. J Neuroimmunol. 2011;232:75–82.

75. Wu A, Wei J, Kong L-Y, Wang Y, Priebe W, Qiao W, et al. Glioma cancer stem cells induce immunosuppressive macrophages/microgli. Neuro Oncol. 2010;12:1113–25.

76. Todaro M, Alea MP, Di Stefano AB, Cammariere P, Vermeulen L, Iovino F, et al. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. Cell Stem Cell. 2007;1:389–402.

77. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, et al. Molecular definition of breast tumor heterogeneity. Cancer Cell. 2007;11:259–73.

78. Lottaz C, Beier D, Meyer K, Hermann A, Schwarz J, et al. Transcriptional profiles of CD133+ and CD133− glioblastoma-derived cancer stem cell lines suggest different cells of origin. Cancer Res. 2010;70:2030–40.

79. Chen WJ, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, et al. Molecular definition of breast tumor heterogeneity. Cancer Cell. 2011;144:646–74.

80. Liu VC, Wong LY, Jang T, Shah AH, Park I, Yang X, et al. Tumor evasion of stromal cells in glioblastoma in vitro and promote tumor proliferation in vivo. Stem Cell Res Ther. 2018;9(1):310.
Kareva I. Immune evasion through competitive inhibition: the shielding effect of cancer non-stem cells. J Theor Biol. 2015;364:40–8.

Volonté A, Di Tomaso T, Spinelli M, Todaro M, Sanvito F, Albanello L, et al. Cancer-initiating cells from colorectal cancer patients escape from T cell-mediated immunosurveillance in vitro through membrane-bound IL-4. J Immunol. 2014;192:523–32.

Yoshimura A, Muto G. TGF-β function in immune suppression. Curr Top Microbiol Immunol. 2011;350:127–47.

Domenis R, Cesselli D, Tartoflettó B, Bourkoula E, Capannetto F, Manin I, et al. Systemic T cells immunosuppression of glioma stem cell-derived exosomes is mediated by monocytic myeloid-derived suppressor cells. PLoS ONE. 2017;12(1):e0169992.

Grange C, Tapparo M, Trittis S, Deregibus MC, Battaglia A, Gontero P, et al. Role of HLA-G and extracellular vesicles in renal cancer stem cell-induced inhibition of dendritic cell differentiation. BMC Cancer. 2015;15:1009.

Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res. 2005;65:5506–11.

Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, et al. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res. 2008;66:7843–8.

Folkins C, Shaked Y, Man S, Tang T, Lee CR, Zhu Z, et al. Glioma tumor stem-like cells promote tumor angiogenesis and vasculogenesis via vascular endothelial growth factor and stromal-derived factor-1. Cancer Res. 2009;69:7243–51.

Oswald J, Boxbberger S, Joergensen B, Bornhaeuser M, Ehninger G, Werlein J, et al. Endothelial cell-induced inhibition of dendritic cell differentiation. BMC Cancer. 2013;13:136.

Xu C, Wu X, Zhu J. VEGF promotes proliferation of human glioblastoma multiforme stem-like cells through VEGF receptor 2. Sci World J. 2013;2013:417413.

Hamerlik P, Lathia JD, Rasmussen R, Wu Q, Bartkova J, Lee MH, et al. Autocrine VEGF-VEGFR2-Neuropilin-1 signaling promotes glioma stem-like cell viability and tumor growth. J Exp Med. 2012;209:507–20.

Liu X, Hao M, Ouyang Y, Zheng J, Chen D. CD133+ cancer stem cells promoted by VEGF accelerate the recurrence of hepatocellular carcinoma. Sci Rep. 2017;7:41499.

Yoshimura T, Liu M, et al. A multiogenic program mediating breast cancer metastasis to bone. Cancer Cell. 2003;3:537–49.

Petrovaeva L, Kaipainen A, Mustonen T, Orpana A, Ferrara N, Saksela O, et al. Vascular endothelial growth factor is induced in response to transforming growth factor-β in fibroblastic and epithelial cells. J Biol Chem. 1994;269:62971–4.

Thirant C, Galan-Moya EM, Gustavo Dubois L, Pinte S, Chafey P, Broussard C, et al. Differential proteomic analysis of human glioblastoma and neural stem cells reveals HDGF as a novel angiogenic secreted factor. Stem Cells. 2012;30:845–53.

Viallard C, Larivée B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis. 2017;20(4):409–26.

Pertovaara L, Kaipainen A, Mustonen T, Orpana A, Ferrara N, Saksela O, et al. Vascular endothelial growth factor is induced in response to transforming growth factor-β in fibroblastic and epithelial cells. J Biol Chem. 1994;269:62971–4.

Kim JW, Thernhomsov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: A metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 2006;3:177–85.

Holmquist-Mengelberg L, Fredlund E, Lofstedt T, Noguera R, Navaro S, Nilsson H, et al. Recruitment of HIF-1α and HIF-2α to common target genes is differentially regulated in neuroblastoma: HIF-2α promotes an aggressive phenotype. Cancer Cell. 2006;10:413–23.

Soeda A, Park M, Lee D, Mintz A, Androussolis-Theotokis A, McKay RD, et al. Hypoxia promotes expansion of the CD133-positive glioma stem cells through activation of HIF-1α. Oncogene. 2009;28:3949–59.

Wang Y, Liu Y, Malek SN, Zheng P, Liu Y. Targeting HIF-1α eliminates cancer stem cells in hematological malignancies. Cell Stem Cell. 2017;20(4):409–26.

Ng KP, Manieri A, Lee KL, Huang W, Tan SY, Chuah CTH, et al. Physiologic hypoxia promotes maintenance of CML stem cells despite effective BCR-ABL1 inhibition. Blood. 2014;123:3316–26.

Gammon L, Biddle A, Heywood VK, Johannessen AC, Mackenzie IC. Sub-sets of cancer stem cells differ intrinsically in their patterns of oxygen metabolism. PLoS ONE. 2013;8(4):e62493.

Kappler M, Taubert H, Schubert J, Vordermark D, Eckert AW. The real face of HIF-1α in the tumor process. Cell Cycle. 2012;11(21):3932–6.

Kuwai T, Kitadai Y, Tanaka S, Onogawa S, Matsutani N, Kaio E, et al. Expression of hypoxia-inducible factor-1α is associated with tumor vascularization in human colorectal carcinoma. Int J Cancer. 2003;108:399–411.

López de Andrés et al. J Hematol Oncol (2020) 13:136 Page 17 of 22
Morel AP, Lièvre M, Thomas C, Hinkel G, Ansieau S, Puisieux A. Generation of breast cancer stem cells through epithelial-mesenchymal transition. PLoS ONE. 2008;3:e2888.

Bai X, Li YY, Zhang HY, Wang F, He HL, Yao JC, et al. Role of matrix metalloproteinase-9 in transforming growth factor-β1-induced epithelial–mesenchymal transition in esophageal squamous cell carcinoma. Onco Targets Ther. 2017;10:2837–47.

Ren Y, Jia H, Xu Y, Zhou X, Xiao W, Wang Y, et al. Paracrine and epigenetic control of CAF-induced metastasis: the role of HOTAIR stimulated by TGF-β1 secretion. Mol Cancer. 2018;17:5.

Smith AL, Robin TP, Ford HL. Molecular pathways: targeting the TGF-β family pathway for cancer therapy. Clin Cancer Res. 2012;18:4514–21.

Inman GJ. Switching TGFβ from a tumor suppressor to a tumor promoter. Curr Opin Genet Dev. 2011;21(1):93–9.

Xia H, Hui KM. MicroRNAs involved in regulating epithelial-mesenchymal transition and cancer stem cells as molecular targets for cancer therapeutic. Cancer Gene Ther. 2012;19:723–30.

Liu C, Tang DG. MicroRNA regulation of cancer stem cells. Cancer Res. 2011;71:5950–4.

Vahidian F, Mohammadi H, Ali-Hasanzadeh M, Derakhshani A, Mostaan M, Hemmatzadeh M, et al. MicroRNAs and breast cancer stem cells: potential role in breast cancer therapy. J Cell Physiol. 2019;234:3294–306.

Chaudhary UW, Jaincu MJ, Mureithi CM, Ferrer VP, Moura-Neto M. MicroRNAs, hypoxia and the stem-like state as contributors to cancer aggressiveness. Front Genet. 2019;10:1–19.

Cha SY, Choi Y, Hwang S, Jeong JY, An HJ. Clinical impact of microRNAs associated with cancer stem cells as a prognostic factor in ovarian carcinoma. J Cancer. 2017;8(7):3538–47.

Bano N, Yadav M, Mohania D, Das BC. The role of NF-κB and miRNA in breast cancer aggressiveness. Front Genet. 2019;10:1–19.

He XH, Zhu W, Yuan P, Jiang S, Li D, Zhang HW, et al. MiR-155 downregulates ErbB2 and suppresses ErbB2-induced malignant transformation of breast epithelial cells. Oncogene. 2016;35:6015–25.

Jang MH, Kim HJ, Gwak JM, Chung YR, Park SY. Prognostic value of microRNAs associated with cancer stem cells as a predictor of poor clinical outcome. Cell Stem Cell. 2007;1:555–67.

Abdullah LN, Chow TK-H. Mechanisms of chemoresistance in cancer stem cells. Clin Transl Med. 2013;2:1.

Raha D, Wilson TR, Peng J, Peterson D, Yue P, Evangelista M, et al. The cancer stem cell marker aldehyde dehydrogenase is required to maintain a drug-tolerant tumor cell subpopulation. Cancer Res. 2014;74:3579–90.

Murakami A, Takahashi F, Nishiyama F, Kobayashi I, Minakata K, Hashimoto M, et al. Hypoxia increases gefitinib-resistant lung cancer stem cells through the activation of insulin-like growth factor 1 receptor. PLoS ONE. 2014;9:e86459.

Nurwidya F, Andarini S, Takahashi K, Syahrudin E, Takahashi K. Implications of insulin-like growth factor 1 receptor activation in lung cancer. Malays J Med Sci. 2016;23(3):9–21.

Stassi G, Todaro M, Zerilli M, Ricci-Vitiani L, Di Liberto D, Patti M, et al. Thyroid cancer resistance to chemotherapeutic drugs via auto-coronar production of interleukin-4 and interleukin-10. Cancer Res. 2003;63:6784–90.

Conticello C, Pedini F, Zucchi M, Zerilli M, Stassi G, et al. IL-4 protects tumor cells from anti-CD95 and chemotherapeutic agents via up-regulation of antiapoptotic proteins. J Immunol. 2004;172:5467–77.

D’Alessio DC, Chang H, Butcher MD, Harker L, et al. HER2 overexpression elicits a proinflammatory IL-6 autocrine signaling loop that is critical for tumorigenesis. Cancer Res. 2011;71:4380–91.

Korkaya H, Kim GI, Hwang J, Kim D, Yoo N, Seo J, et al. Role of CXCR4 with the novel RCP168 peptide overcomes stroma-mediated chemoresistance in chronic and acute leukemias. Mol Cancer Ther. 2006;5:3113–21.

Barbieri F, Bajetto A, Stamm R, Pattarozi A, Porcile C, Zona G, et al. Overexpression of stromal cell-derived factor 1 and its receptor expression as regulators of cell migration and survival in breast cancer. Mol Cell. 2012;47:570–84.

Duan W, Qian W, Zhou C, Cao J, Qin T, Xiao Y, et al. Metformin suppresses the invasive ability of pancreatic cancer cells by blocking autocrine TGF-β1 signaling. Oncol Rep. 2018;40:1495–502.

Roato I, Ferracci R. Cancer stem cells, bone and tumor microenvironment manipulation: key players in bone metastases. Cancers (Basel). 2018;10(2):56.

Es-haghi M, Softanian S, Dehghani H. Perspective: Cooperation of Nanog, NF-κB, and CXCR4 in a regulatory network for directed migration of cancer stem cells. Tumor Biol. 2016;37(2):1559–655.

Sceneay J, Chow MT, Chen A, Halse HM, Wong CSF, Andrews DM, et al. Primary tumor hypoxia recruits CD11b+Ly6CdMed/Ly6G+ immune suppressor cells and compromises NK cell cytotoxicity in the premetastatic niche. Cancer Res. 2012;72:3906–11.

Deep G, Jain A, Kumar A, Agarwal C, Kim S, Levey WM, et al. Exosomes secreted by prostate cancer cells under hypoxia promote matrix metalloproteinases activity at pre-metastatic niches. Mol Carcinog. 2020;59:323–32.

Yu Z, Zhao S, Ren W, Wang L, Chen Z, Hoffman RM, et al. Pancreatic cancer-derived exosomes promote tumor metastasis and liver pre-metastatic niche formation. Oncotarget. 2017;8:63461–83.

Feng W, Dean DC, Hornickel F, Shi H, Duan Z. Exosomes promote pre-metastatic niche formation in ovarian cancer. Mol Cancer. 2019;18(1):124.

Costa-Silva B, Aielo NM, Ocean AJ, Singh S, Zhang H, Thakur BK, and et al. Pancreatic cancer exosomes initiate premetastatic niche formation in liver cancer. Nat Cell Biol. 2015;17:816–26.

Medeiros B, Goodale D, Penson K, Lowes LE, Kiser P, Hean R, et al. Triple-negative primary breast tumors induce suppressive premetastatic changes in the extracellular matrix and soluble components of the lung microenvironment. Cancers (Basel). 2020;12(1):172.

DeGorter MK, Xia CQ, Yang JJ, Kim RB. Drug transporters in drug efficacy and toxicity. Annu Rev Pharmacol Toxicol. 2012;52:249–73.

Genestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell. 2007;1:555–67.

Abdullah LN, Chow TK-H. Mechanisms of chemoresistance in cancer stem cells. Clin Transl Med. 2013;2:1.

Raha D, Wilson TR, Peng J, Peterson D, Yue P, Evangelista M, et al. The cancer stem cell marker aldehyde dehydrogenase is required to maintain a drug-tolerant tumor cell subpopulation. Cancer Res. 2014;74:3579–90.

Murakami A, Takahashi F, Nishiyama F, Kobayashi I, Minakata K, Hashimoto M, et al. Hypoxia increases gefitinib-resistant lung cancer stem cells through the activation of insulin-like growth factor 1 receptor. PLoS ONE. 2014;9:e86459.

Nurwidya F, Andarini S, Takahashi K, Syahrudin E, Takahashi K. Implications of insulin-like growth factor 1 receptor activation in lung cancer. Malays J Med Sci. 2016;23(3):9–21.

Stassi G, Todaro M, Zerilli M, Ricci-Vitiani L, Di Liberto D, Patti M, et al. Thyroid cancer resistance to chemotherapeutic drugs via auto-coronar production of interleukin-4 and interleukin-10. Cancer Res. 2003;63:6784–90.

Conticello C, Pedini F, Zucchi M, Zerilli M, Stassi G, et al. IL-4 protects tumor cells from anti-CD95 and chemotherapeutic agents via up-regulation of antiapoptotic proteins. J Immunol. 2004;172:5467–77.

D’Alessio DC, Chang H, Butcher MD, Harker L, et al. HER2 overexpression elicits a proinflammatory IL-6 autocrine signaling loop that is critical for tumorigenesis. Cancer Res. 2011;71:4380–91.
et al. J Hematol Oncol          (2020) 13:136

221. Yamashina T, Baghdadi M, Yoneda A, Kinoshita I, Suzu S, Dosaka-Akita H, et al. Cancer stem-like cells derived from chemoresistant tumors have a unique capacity to prime tumor myeloid cells. Cancer Res. 2014;74:2698–709.

222. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules. 2016;21(7):965.

223. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat Rev Clin Oncol. 2017;14(10):611–29.

224. Zhang Y, Lu Y, Zhang C, Huang D, Wu W, Zhang Y, et al. FSCN-1 increases epithelial-mesenchymal transition via upregulation of transforming growth factor β signaling in HCT116 colon cancer cells. Mol Med Rep. 2015;11:192–8.

225. Saxena M, Stephens MA, Pathak H, Rangarajan A. Transcription factors that mediate epithelial-mesenchymal transition lead to multi-drug resistance by upregulating ABC transporters. Cell Death Dis. 2011;2:e179.

226. Oliveras-Ferraros C, Corominas-Faja B, Vazquez-Martin SCA, Martin-Castillo B, Iglesias JM, López-Bonet E, et al. Epithelial-to-mesenchymal transition (EMT) confers primary resistance to trastuzumab (Herceptin). Breast Cancer Res. 2011;2:e179.

227. Xue Li, Mao XB, Ren LL, Chu XY. Inhibition of CXCL12/CXCR4 axis as a potential targeted therapy of advanced gastric carcinoma. Cancer Med. 2017;6(6):1424–36.

228. Yamashina T, Baghdadi M, Yoneda A, Kinoshita I, Suzu S, Dosaka-Akita H, et al. Cancer stem-like cells derived from chemoresistant tumors have a unique capacity to prime tumor myeloid cells. Cancer Res. 2014;74:2698–709.

229. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules. 2016;21(7):965.

230. Zhang Y, Lu Y, Zhang C, Huang D, Wu W, Zhang Y, et al. FSCN-1 increases epithelial-mesenchymal transition via upregulation of transforming growth factor β signaling in HCT116 colon cancer cells. Mol Med Rep. 2015;11:192–8.

231. Xue Li, Mao XB, Ren LL, Chu XY. Inhibition of CXCL12/CXCR4 axis as a potential targeted therapy of advanced gastric carcinoma. Cancer Med. 2017;6(6):1424–36.

232. Yamashina T, Baghdadi M, Yoneda A, Kinoshita I, Suzu S, Dosaka-Akita H, et al. Cancer stem-like cells derived from chemoresistant tumors have a unique capacity to prime tumor myeloid cells. Cancer Res. 2014;74:2698–709.

233. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules. 2016;21(7):965.

234. Zhang Y, Lu Y, Zhang C, Huang D, Wu W, Zhang Y, et al. FSCN-1 increases epithelial-mesenchymal transition via upregulation of transforming growth factor β signaling in HCT116 colon cancer cells. Mol Med Rep. 2015;11:192–8.

235. Xue Li, Mao XB, Ren LL, Chu XY. Inhibition of CXCL12/CXCR4 axis as a potential targeted therapy of advanced gastric carcinoma. Cancer Med. 2017;6(6):1424–36.

236. Yamashina T, Baghdadi M, Yoneda A, Kinoshita I, Suzu S, Dosaka-Akita H, et al. Cancer stem-like cells derived from chemoresistant tumors have a unique capacity to prime tumor myeloid cells. Cancer Res. 2014;74:2698–709.

237. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules. 2016;21(7):965.

238. Zhang Y, Lu Y, Zhang C, Huang D, Wu W, Zhang Y, et al. FSCN-1 increases epithelial-mesenchymal transition via upregulation of transforming growth factor β signaling in HCT116 colon cancer cells. Mol Med Rep. 2015;11:192–8.

239. Xue Li, Mao XB, Ren LL, Chu XY. Inhibition of CXCL12/CXCR4 axis as a potential targeted therapy of advanced gastric carcinoma. Cancer Med. 2017;6(6):1424–36.

240. Yamashina T, Baghdadi M, Yoneda A, Kinoshita I, Suzu S, Dosaka-Akita H, et al. Cancer stem-like cells derived from chemoresistant tumors have a unique capacity to prime tumor myeloid cells. Cancer Res. 2014;74:2698–709.

241. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules. 2016;21(7):965.

242. Zhang Y, Lu Y, Zhang C, Huang D, Wu W, Zhang Y, et al. FSCN-1 increases epithelial-mesenchymal transition via upregulation of transforming growth factor β signaling in HCT116 colon cancer cells. Mol Med Rep. 2015;11:192–8.

243. Xue Li, Mao XB, Ren LL, Chu XY. Inhibition of CXCL12/CXCR4 axis as a potential targeted therapy of advanced gastric carcinoma. Cancer Med. 2017;6(6):1424–36.

244. Yamashina T, Baghdadi M, Yoneda A, Kinoshita I, Suzu S, Dosaka-Akita H, et al. Cancer stem-like cells derived from chemoresistant tumors have a unique capacity to prime tumor myeloid cells. Cancer Res. 2014;74:2698–709.

245. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules. 2016;21(7):965.
261. Benedicto A, Romayor I, Artea B. CXCR4 receptor blockade reduces the contribution of tumor and stromal cells to the metastatic growth in the liver. Oncol Rep. 2018;39:2022–30.

262. Gravina GT, Mancini A, Marampoline F, Colapietro A, Delle Monache S, Serra R, et al. The brain-penetrating CXCR4 antagonist, PX77561, increases the antitumor effects of bevacizumab and sunitinib in preclinical models of human glioblastoma. J Hematol Oncol. 2017;10:1–16.

263. Zhou Y, Cao HB, Li WJ, Zhao L. The CXCL12 (SDF-1)/CXCR4 chemokine axis: oncogenic properties, molecular targeting, and synthetic and natural product CXCR4 inhibitors for cancer therapy. Chin J Nat Med. 2018;16(11):801–10.

264. Duda DG, Kozin SV, Kirkpatrick ND, Xu L, Fukumura D, Jain RK. CXCL12 (SDF-1)/CXCR4/CXCR7 pathway inhibition: an emerging sensitizer for anticancer therapies? Clin Cancer Res. 2011;17:2074–80.

265. Zhou KK, Xie LH, Peng X, Guo QM, Wu QY, Wang WH, et al. CXCR4 antagonist AMD3100 enhances the response of MDA-MB-231 triple-negative breast cancer cells to ionizing radiation. Cancer Lett. 2018;418:196–203.

266. Richert MM, Vaidya KS, Mills CN, Wong D, Korz W, Hurst DR, et al. Inhibition of CXCR4 by CTCE-9908 inhibits breast cancer metastasis to lung and bone. Oncol Rep. 2009;21:761–7.

267. Wu A, Maxwell R, Xia Y, Cardarelli P, Oyasu M, Belcaid Z, et al. Combinatorial CXCR4 antagonist FRX177561 increases the antitumor effects of bevacizumab and sunitinib in preclinical models of human glioblastoma. J Neurooncol. 2019;143:241–9.

268. Yu Z, Cheng H, Zhu H, Cao M, Lu C, Bao S, et al. Salinomycin enhances sensitivity of glioblastoma multiforme to cetuximab treatment via downregulating Wnt/β-catenin pathway. Mol Cancer. 2017;16:8:35946–61.

269. Li L, Han R, Xiao H, Lin C, Wang Y, Liu H, et al. Metformin sensitizes EGFR-TKI-resistant human lung cancer cells in vitro and in vivo through inhibition of IL-6 signaling and EMT reversal. Clin Cancer Res. 2014;20:2714–26.

270. Yin X, Wei Z, Song C, Tang C, Xu W, Wang Y, et al. Metformin sensitizes hypoxia-induced gefitinib treatment resistance of HNSCC via cell cycle regulation and EMT reversal. Cancer Manag Res. 2018;10:5785–98.

271. Mao Z, Wu Y, Zhou J, Xing C. Salinomycin reduces epithelial-mesenchymal transition in triple-negative breast cancer cells. Oncogene. 2019;38:63599–413.

272. Chae YK, Aya N, Malecek MK, Shin DS, Carneiro B, Chandra S, et al. Repurposing metformin for cancer treatment: current clinical studies. Onco Targets. 2016;7:40767–80.

273. Raoof S, Mulfoid LF, Frisco-Cabanos H, Nangia V, Timonina D, Labrot E, et al. Targeting FGFR overcomes EMT-mediated resistance in EGFR mutant non-small cell lung cancer. Oncogene. 2019;38:63599–413.

274. Schott AF, Goldstein LJ, Cristofanilli M, Ruffini PA, McCanna S, Reuben Porvaznik S, Sakamoto N, Kusmartsev S, Eruslanov E, Kim WJ, Cao W, Fu S, Chen X, Lin HJ, Lin J. Inhibition of interleukin 8/C-X-C chemokine receptor 1/2 signaling reduces malignant features in human pancreatic cancer cells. Int J Oncol. 2018;53:349–57.

275. Li L, Han R, Xiao H, Lin C, Wang Y, Liu H, et al. Metformin inhibits thyroid cancer cell growth, migration, and EMT through the mTOR pathway. Tumor Biol. 2015;36:36205–304.

276. Kolosnjaj-Tabi J, Marangon I, Nicolas-Boluda A, Silva AKA, Gazeau F. Targeting the EMT transcription factor TWIST1 overcomes resistance to EGFR inhibitors in EGFR-mutant non-small-cell lung cancer. Oncogene. 2019;38:63599–413.

277. Insua-Rodríguez J, Oskarsson T. The extracellular matrix in breast cancer. Adv Drug Deliv Rev. 2016;97:41–55.

278. Torigoe H, Shien K, Takeda T, Yoshioaka T, Namba K, Sato H, et al. Therapeutic strategies for dasatinib-resistant lung cancer harboring HER2 alterations. Cancer Sci. 2018;109:1493–502.

279. Yochum ZA, Cades J, Wang H, Chattejee S, Simons BW, O'Brien JP, et al. Targeting the EMT transcription factor TWIST1 overcomes resistance to EGFR inhibitors in EGFR-mutant non-small-cell lung cancer. Oncogene. 2019;38:6656–70.

280. Sesumi Y, Suda K, Mizuuchi H, Kobayashi Y, Sato K, Chiba M, et al. Effect of dasatinib on EMT-mediated resistance of EGFR inhibitors in lung cancer cells. Lung Cancer. 2017;104:85–90.

281. Zhang C, Lu Y, Li Q, Mao J, Hou Z, Yu X, et al. Salinomycin suppresses TGF-β1-induced epithelial-to-mesenchymal transition in MCF-7 human breast cancer cells. Chem Biol Interact. 2016;248:74–81.

282. Hermawan A, Wagner E, Roild A. Concurrent salinomycin treatment reduces doxorubicin resistance of breast tumor cells by diminishing drug efflux pump expression and activity. Oncol Rep. 2016;35:1732–40.

283. Tong D, Liu Q, Li G, Xu J, Lan W, Yang J, et al. Metformin inhibits castration-induced EMT in prostate cancer by repressing COX2/PG2/STAT3 axis. Cancer Lett. 2017;389:23–32.

284. Liu Q, Tong D, Liu G, Xu J, Dk K, Geary K, et al. Metformin reverses prostate cancer resistance to enzalutamide by targeting TGF-β1/STAT3-axis-regulated EMT. Cell Death Dis. 2017;8:e3007.

285. Diop-Frimpong B, Chauhan VP, Krane S, Boucher Y, Jain RK. Losartan inhibits collagen I synthesis and improves the distribution and EMT reversal. Cancer Manag Res. 2018;10:5785–98.

286. Winer A, Adams S, Mignatti P. Matrix metalloproteinase inhibitors in lung cancer therapy: turning past failures into future successes. Mol Cancer Ther. 2018;17(6):1147–55.

287. Pan A, Wang Z, Chen B, Dai W, Zhang H, He B, et al. Localized co-delivery of collagenase and trastuzumab by thermosensitive hydrogels for enhanced antitumor efficacy in human breast xenograft. Drug Deliv. 2019;26:1405–93.

288. Wang X, Luo J, He L, Cheng X, Yan G, Wang J, et al. Hybrid pH-sensitive nanogels surface-functionalized with collagenase for enhanced tumor penetration. J Colloid Interface Sci. 2018;525:269–81.

289. Kolosnjaj-Tabi J, Marangon I, Nicolas-Boluda A, Silva AKK, Gazeau F. Nanoparticle-based hyperthermia, a local treatment modulating the tumor extracellular matrix. Pharmacol Res. 2017;126:123–37.

290. Lichtenberg MA, Chan DS, Neesse A, Bepiro TE, Cook N, Frese K, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. Gut. 2013;62:112–20.

291. Su X, Lee F, Gao S, Tan MH, Kuriyama M. Hyaluronidase-incorporated hyaluronic acid-tyramine hydrogels for the sustained release of trastuzumab. J Control Release. 2015;216:47–55.

292. Nagase H, Kudo D, Sato A, Yoshida E, Sato S, Negishi M, et al. 4-methylumbelliferone suppresses hyaluronan synthesis and tumor progression in human breast cancer. J Biol Res. 2017;50:e6147.

293. Kolosnjaj-Tabi J, Marangon I, Nicolas-Boluda A, Silva AKK, Gazeau F. Nanoparticle-based hyperthermia, a local treatment modulating the tumor extracellular matrix. Pharmacol Res. 2017;126:123–37.

294. Lichtenberg MA, Chan DS, Neesse A, Bepiro TE, Cook N, Frese K, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. Gut. 2013;62:112–20.
in SCID mice intra-abdominally inoculated with pancreatic cancer cells. Pancreas. 2017;46:190–7.

303. Yoshida E, Kudo D, Nagase H, Suto A, Shimoda H, Suto S, et al. 4-Methylumbelliferone decreases the hyaluronan-rich extracellular matrix and increases the effectiveness of 5-fluorouracil. Anticancer Res. 2018;38:799–804.

304. Lokman NA, Price ZK, Hawkins EK, Macpherson AM, Oehler MK, Ricciardelli C. 4-methylumbelliferone inhibits cancer stem cell activation and overcomes chemoresistance in ovarian cancer. Cancers (Basel). 2019;11(8):1187.

305. Cheng XB, Sato N, Kohi S, Koga A, Hirata K. 4-methylumbelliferone inhibits enhanced hyaluronan synthesis and cell migration in pancreatic cancer cells in response to tumor-stromal interactions. Oncol Lett. 2018;15:6297–301.

306. Hecht JR, Benson AB, Vyushkov D, Yang Y, Bendell J, Verma U. A phase II, randomized, double-blind, placebo-controlled study of simtuzumab in combination with FOLFIRI for the second-line treatment of metastatic KRAS mutant colorectal adenocarcinoma. Oncologist. 2017;22:243.

307. Benson AB, Wainberg ZA, Hecht JR, Vyushkov D, Dong H, Bendell J, et al. A phase II, randomized, double-blind, placebo-controlled study of simtuzumab or placebo in combination with gemcitabine for the first-line treatment of pancreatic adenocarcinoma. Oncologist. 2017;22:241.

308. Bommareddy PK, Patel A, Hossain S, Kaufman HL. Talmogene laherparepvec (TVEC) and other oncolytic viruses for the treatment of melanoma. Am J Clin Dermatol. 2017;18(1):1–15.

309. Pal SK, Moreira D, Won H, White SW, Duttagupta P, Lucia M, et al. Reduced T-cell numbers and elevated levels of immunomodulatory cytokines in metastatic prostate cancer patients de novo resistant to abiraterone and/or enzalutamide therapy. Int J Mol Sci. 2019;20(8):1831.

310. Brandi J, Pozza ED, Dando I, Biondani G, Robotti E, Jenkins R, et al. Secretome protein signature of human pancreatic cancer stem-like cells. J Proteomics. 2016;136:1–12.

311. Huang H, Li Y, Liu J, Zheng M, Feng Y, Hu K, et al. Screening and identification of biomarkers in ascites related to intrinsic chemoresistance of serous epithelial ovarian cancers. PLoS ONE. 2012;7(1):1–5.

312. Tye SL, Gilg AC, Tolliver LB, Wheeler WG, Toole BB, Maria BL. Hyaluronan regulates ceruloplasmin production by gliomas and their treatment-resistant multipotent progenitors. J Child Neurol. 2008;23(10):1221–300.

313. Mazel M, Jacot W, Pantel K, Bartkowiak K, Topart D, Cayrefourcq L, et al. Frequent expression of PD-L1 on circulating breast cancer cells. Mol Oncol. 2015;9(1773):622.

314. Hegde PS, Wallin JJ, Mancao C. Predictive markers of anti-VEGF and emerging role of angiogenesis inhibitors as immunotherapeutics. Semin Cancer Biol. 2018;52(2):117–24.