Review

Interplay Between Epithelial-Mesenchymal Transition and Cancer Stem Cells: Unveiling Potential Therapeutic Avenues

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Interplay between Epithelial-Mesenchymal Transition and Cancer Stem Cells: Unveiling Potential Therapeutic Avenues

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Simple Summary: Regardless of significant advancements in cancer therapies, complete cancer eradication remains elusive. Cancer stem-like cells (CSCs) and epithelial-mesenchymal transition (EMT) are key components of various cancer hallmarks, enhancing cancer cell survival. This review explores the interplay between EMT and CSCs, and the regulation of associated signaling pathways. Understanding the link between EMT and CSCs is critical for developing new chemotherapeutic strategies and identifying novel therapeutic targets, which could lead to improved cancer treatments by targeting these processes.

Abstract: The global prevalence of cancer is on the rise, and while targeted therapies, early diagnosis, immunotherapies, and conventional personalized chemotherapy have significantly upgraded outcomes for cancer patients, there is still a significant likelihood that cancer cannot be fully eradicated. Cancer stem-like cells (CSCs) and Epithelial-mesenchymal transition (EMT) exhibit pivotal roles in cancer cell metastasis, tumor recurrence, chemotherapy resistance, and cancer cell death, among other processes. This review aims to elucidate the molecular mechanisms underlying EMT and CSCs, their interplay, associated signaling pathways, and regulation of the EMT process and the initiation of CSCs. EMT induction confers mesenchymal properties and the potential of cancer cells to adopt the CSC state. These processes are intricately associated with the regulation of Notch and Wnt signaling pathways, transforming growth factor-β (TGF-β), as well as the expression of miRNAs. Further exploring the relationship between EMT and CSCs is essential for the development of new chemotherapeutic strategies and the identification of novel therapeutic targets, and hence, the development of therapeutic strategies targeting EMT, or CSCs holds promise for improving cancer therapy.

Keywords: Epithelial-mesenchymal transition (EMT); Cancer stem cells (CSCs); Hypoxia; Plasticity; Cancer signaling pathways; miRNA; Therapeutic avenues

1. Introduction

Epithelial-to-mesenchymal transition (EMT) is the process by which epithelial cells no longer have their defining characteristics and transform into mesenchymal cells under certain conditions [1]. This transformation is crucial in normal embryonic development, tissue fibrogenesis, and tumor progression [2]. Cancer stem/stem-like cells (CSCs) exhibit the ability to self-renew and differentiate without limitation, contributing to metastasis. These cells have a decisive role in tumorigenesis, metastasis, recurrence, drug-efflux, and resistance to drugs [3]. During EMT, epithelial cells lose their expression markers such as E-cadherin and obtain mesenchymal phenotypes by showing the expressions of N-cadherin, fibronectin, and vimentin, facilitating detachment from the primary tumor and promoting invasiveness [4]. Cells undergoing EMT acquire stemness or stem cell-like features, while cancer cells with stem cell characteristics display EMT biomarkers [5]. EMT, as a prime regulator of stemness phenotype of cancer cells, influences on the biological behavior of CSCs.

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Additionally, EMT also plays an important role in resistance to apoptosis and immune evasion, making it a key contributor to cancer aggressiveness and poor prognosis [4]. Various cancer, including pancreatic, colorectal, and breast cancers, show EMT-driven progression, revealing its universal role across malignancies.

Conventional cancer therapies, such as chemotherapy, radiotherapy, and surgical resection, etc. primarily target rapidly dividing tumor cells. While these treatments can eliminate some cancer cells, they fail to eradicate CSCs persuaded by EMT, which ultimately leads to persistent tumor recurrence and metastasis [6]. EMT-induced cancer cells are associated with cancer invasion, and EMT-induced CSCs aid to tumor recurrence and resistance to chemoradiotherapy. Therefore, targeting both CSCs and EMT is essential for improving the existing cancer treatments. Additionally, exploring the relationship between EMT and CSC could lead to the development of novel cancer therapeutic strategies.

2. CSCs and Cancer

CSCs were first hinted at in Rudolf Virchow's cytopathological study [7,8]. Later, Paget proposed that cancer cells have an innate propensity to metastasize to distant organs [9]. The CSC hypothesis suggests the self-renew potentiality of certain cancer cells, which assists the sustenance of tumor growth and differentiation into multiple cell types [10–12]. Both genetic and epigenetic alterations endow these cells with immense proliferative ability, self-renewal capability, and the potential for multidirectional differentiation, which make them a determining factor in cancer development, metastasis, and further recurrence. Dick's team first discovered CSCs [13], identifying that a small subset of leukemia cells expressing $CD34^+$ and $CD38^+$, could influence heterogeneity in acute myeloid leukemia when transplanted into mice [14–16]. Ponti et al. further revealed that breast cancer cells with $CD44^+/CD24^-$ markers were endowed with self-renewal proficiency and could form new tumors *in vitro*, impersonating the progression of normal breast stem cells [17]. Gene expression profiling of $CD44^+/CD24^-$ breast cancer cells revealed invasive signatures compared to normal breast epithelial cells [18], which further implies the CSCs-mediated tumor invasiveness. Breast stem cells with this phenotype are indicative for cancer cell spread [19]. In addition, $ALDH^+$ breast CSCs exhibit self-renewal potentiality, with as few as $CD44^+/CD24^-$ and $ALDH^+$ cells able to form new tumors [20]. Thus, generation of CSC is a prime factor in metastasis.

CSCs are pivotal in cancer recurrence after treatment [21], their self-renewal capacity [22] significantly contributes to tumor relapse [23]. Distant CSCs can elicit the regrowth of metastatic tumors [24,25], stipulating that tumors with higher CSC numbers have poorer prognosis. Traditional chemotherapy and radiotherapy kill apoptosis-sensitive cancer cells, however, often fail to banish the tumor-resistant CSCs, which ultimately leads to the tumor recurrence [26,27]. The resistance of CSCs against existing cancer treatments arises from both intrinsic and extrinsic factors. There is an intrinsic connection of EMT to the stemness of CSCs [28], empowering immune evasion and resistance to chemotherapy and radiotherapy [29]. EMT programs can be prompted in non-CSCs, modifying them into apoptosis-resistant CSCs. Hypoxic conditions within tumors moreover potentiate CSC resistance to drugs [30], and reduce their responsiveness to both radiotherapy and chemotherapy [31]. Hypoxia not only triggers CSC self-renewal [30] but also fosters late-stage migration and invasion. CSCs also display highly efficient DNA repair mechanisms [32], aiding them foil DNA damage from reactive oxygen species accumulation [13]. These robust repair systems enable CSCs to dodge apoptotic signals, contributing to their resistance to radiotherapy [33]. Moreover, CSCs were reported to be augmenting ATP-binding cassette (ABC) transport proteins, which actively pump chemotherapy drugs out of the cell, taking the edge off drug efficacy and concentration [12]. For example, the ABC-G2 transport protein exhibits an essential role in the development of multidrug resistance (MDR) in tumor cells [34]. Furthermore, tumor microenvironment (TME) or tumor immune microenvironment (TIME) is another extrinsic factor governing CSCs-mediated drug resistance. TIME encompasses tumor cells, cytokines, immune cells, and other components, and their interplay shape the antitumor immunity [35]. These various constituents showed an impact on CSC characteristics, with the tumor-stroma interlinkage being important in developing chemoresistance in CSCs. Additionally,

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conditions such as hypoxia, acid-base imbalances, and nutrient deprivation within the TIME can provoke adaptive responses in CSCs [36].

3. EMT and Cancer

EMT is vitally important for various biological processes. Initially proposed by Hay [37], EMT was first noted during embryogenesis. Epithelial cells, which are usually considered fully differentiated and closely connected, can lose their epithelial characteristics under certain factors, allowing them to migrate and invade, thereby transforming into mesenchymal-like cells [2,38]. This transformation implies a shift from a polygonal to a spindle-shaped morphology, loss of cell polarity and contacts, elevated resistance to apoptosis, and increased migratory and invasive capabilities [39–41]. EMT can be triggered by specific extracellular stimuli during cancer progression [42]. Growth factors such as TGF-β, epidermal growth factor, and fibroblast growth factors, released in the TME during hypoxic conditions, can induce the expression of transcription factors like zinc-finger homologs, basic helix-loop-helix transcription factors, and the ZEB family [43]. These transcription factors were reported to suppress E-cadherin expression, hence promoting EMT and facilitating tumor progression [42].

EMT is crucial for the normal embryonic development, such as the formation of the primitive mesoderm and the differentiation of neural structures into somites, muscles, and bones [44,45]. However, abnormal EMT activation can expedite pathological conditions, including organ fibrosis, wound healing, and cancer progression. In cancer, EMT entitles tumor cells to sneak into surrounding tissues and metastasize to distant sites, empowering tumor spread, recurrence, and chemotherapy resistance. Epithelial-origin tumors comprise the majority of cancer types. During transformation, cancer cells express adhesion molecules and create structures that impound them to the primary site [43]. Still, cells that are undergoing EMT near the tumor gain mesenchymal traits, diminishing cell adhesion and promoting motility and extracellular matrix degradation, which eases local and distant invasion (metastasis) [43]. Mesenchymal-epithelial transition (MET) also displays a vital role in tumor metastasis, facilitating cancer cells to go back to an epithelial phenotype at distant sites, enabling metastatic tumor growth [46].

The precise mechanisms by which EMT promotes chemotherapy resistance are still unclear. Transcriptional regulators of EMT (such as Snail, Twist, and Slug, etc.) uplift tumor invasiveness and survival in unfavorable microenvironments by resisting apoptosis [43]. In addition, EMT is associated with resistance to targeted therapies, as reported in non-small cell lung cancer (NSCC) studies showing reduced sensitivity to erlotinib in EMT-like phenotypes [10,47]. Table 1 summarizes the importance of the EMT process in the onset and progression of cancer.

Table 1. An overview of the association between EMT and different tumorigenic processes. The table briefly outlines the interconnection of EMT with key aspects of cancer progression, including cancer stemness, angiogenesis, metastasis, CTCs, cytokine involvement, stromal tumor cell interactions, immune evasion, inflammation, tumor dormancy, chemoresistance, and senescence, emphasizing the role of EMT in uplifting tumor cell motility, invasion, survival, immune evasion, and even resistance to therapeutic strategies.

EMT association	Findings	References
Stemness	EMT activation is closely linked to the generation of CSCs, contributing to tumorigenesis, metastasis, drug resistance, and relapse. EMT transcription factors, such as Zeb1, suppress epithelial differentiation and facilitate stemness, while signaling pathways like TGF-β, Snail1/Twist1, and Notch promote the acquisition of stem-like traits.	[48–64]
Tumor Angiogenesis	Angiogenesis and EMT are unified; VEGF, EGF, NECTIN-4 pathways promote EMT and are associated with increased tumor cell motility and invasion.	[65–71]

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Metastasis	EMT is also linked with early metastatic processes, which includes cell invasion, cytoskeletal reorganization, and MMPs-mediated basement membrane degradation.	[38,72–76]
Circulating Tumor Cells (CTCs)	CTCs show incomplete EMT, express both epithelial and mesenchymal markers, and are involved in metastasis and poor patient prognosis.	[77,78]
Cytokine involvement	Cytokines like HGF, FGF, EGF, IL-6, IL-8, TGF-β, TNF-α, and IL-27 play important roles in stimulating or regulating EMT in different cancer types.	[79–86]
Stromal Tumor Cells	Cytokines and growth factors from tumor stroma (EGF, HGF, TGF-β, PDGF etc.) activate transcription factors (Snail, Slug, ZEB1, Twist) that induce EMT.	[87–94]
Immune interactions	EMT also contributes to immune evasion; a strong association exists between high EMT activity in tumors and the presence of inflammatory cytokines and immune checkpoints (e.g., PD-1, PD-L1).	[95–103]
Inflammation	Inflammatory mediators (e.g., TNF-α and IL-8) promote EMT in cancer cells, upregulating tumor progression and metastasis, particularly in inflammatory breast cancer.	[104–111]
Tumor dormancy	EMT aids in tumor dormancy, with Snail and LOXL2 involved in persevering the mesenchymal phenotype and CSC-like traits.	[112–115]
Chemoresistance	EMT contributes to cancer drug resistance by influencing cell survival, cell fate transition, elevating the drug-resistance-involved genes, promoting stemness, dysregulating transcription factors, and immune suppression.	[116–128]
Senescence	Senescence and EMT are interconnected; EMT can prohibit senescence, promoting tumor progression and invasion.	[129–134]

4. EMT-CSCs Nexus

The EMT-CSCs association is firmly established, with many researchers exploring how EMT contributes to the initiation of CSCs and the progression of cancer metastasis. The seminal works by Hanahan and Weinberg provide a comprehensive framework for understanding cancer mechanisms. Their reports outline key hallmarks and emerging aspects of cancer biology, which are important for placing EMT and CSCs in the broader context of cancer research [135,136]. Becoming resistant to chemotherapy and adjuvant drugs after EMT is quite common, which ultimately leads to tumor relapse. This chemotherapy resistance is a hallmark of CSCs, as documented by $CD44^{+}/CD24^{-}$ breast CSCs undergoing EMT [49]. EMT markers have been identified in stem cells from mice and human mammary glands, and breast epithelial cells underwent EMT expressed elevated efficiency in forming tumors and soft agar colonies [49]. Therefore, EMT and CSCs are closely linked; EMT entitles cancer cells to gain mesenchymal properties and stem-cell-related traits, leading to more aggressive and metastatic tumor cell types [137,138].

For tumor cells to metastasize, they trigger the EMT program, transforming into metastatic CSCs (MCSCs). These MCSCs enter the bloodstream, disseminate, extravasate, and colonize distant sites, accomplishing the metastatic process [139]. The level of EMT transcription factors is hyper activated in cancers such as urinary liver cancer, bladder carcinoma, and lung cancer, stipulating their omnipresent role in cancer cell metastasis and in triggering the differentiation of cancer stem cells into more aggressive and treatment-resistant traits. Major EMT signaling pathways, including TGF-β, Wnt, and Notch, collectively initiate the EMT program in CSCs through a series of cell signaling mechanisms [140]. Nevertheless, EMT can be served both an instigator and a consequence of CSCs induction. EMT can facilitate the acquisition of CSC-like properties in cancer cells, potentiating their migratory and invasive properties through the activation of prime signaling pathways (such as TGF-

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β and Wnt), which are known to ultimately promote stemness [2,49]. On the other hand, CSCs can also induce EMT as an adaptive response to their environment cues, forming a feedback loop that increases tumor progression and resistance to therapeutics [141,142].

4.1. Mechanisms Governing EMT-CSC Pathways

Both EMT and CSC are modulated at the genetic level through various pathways such as TGFβ-SMAD, MAPK/ERK, JAK/STAT, WNT/β-catenin, and PI3K-AKT-NFκB [143]. Figure 1 provides a pictorial representation of the EMT-CSC linkage. Numerous genes contribute to cancer phenotypes and heterogeneity, elucidating breast cancer subtypes [144]. EMT and CSCs impact immune modulation, which is vital for cancer immunity in response to immunotherapy [145–147]. EMT and CSC traits also favor the resistance against cytotoxic T lymphocytes [148]. Tumor-associated macrophages (TAMs) in the TME foster EMT characteristics [149]. EMT and CSCs are also related to cellular senescence, which can be earmarked to address stemness in cancer therapy [150,151]. CSC-allied senescence is a notable factor in anticancer treatments that impede cell division [150,151]. Tumor dormancy, governed by CSCs, contributes to resistance against chemotherapy [152]. Dormant tumor cells with EMT traits may elevate the metastatic proliferation [115]. Cytoskeletal changes programmed by TGFβ signaling are pivotal for cell metastasis [153]. EMT phenotype cells display unclasped tight junctions and decreased cell-to-cell adhesion, which are involved in facilitating migration [153,154].

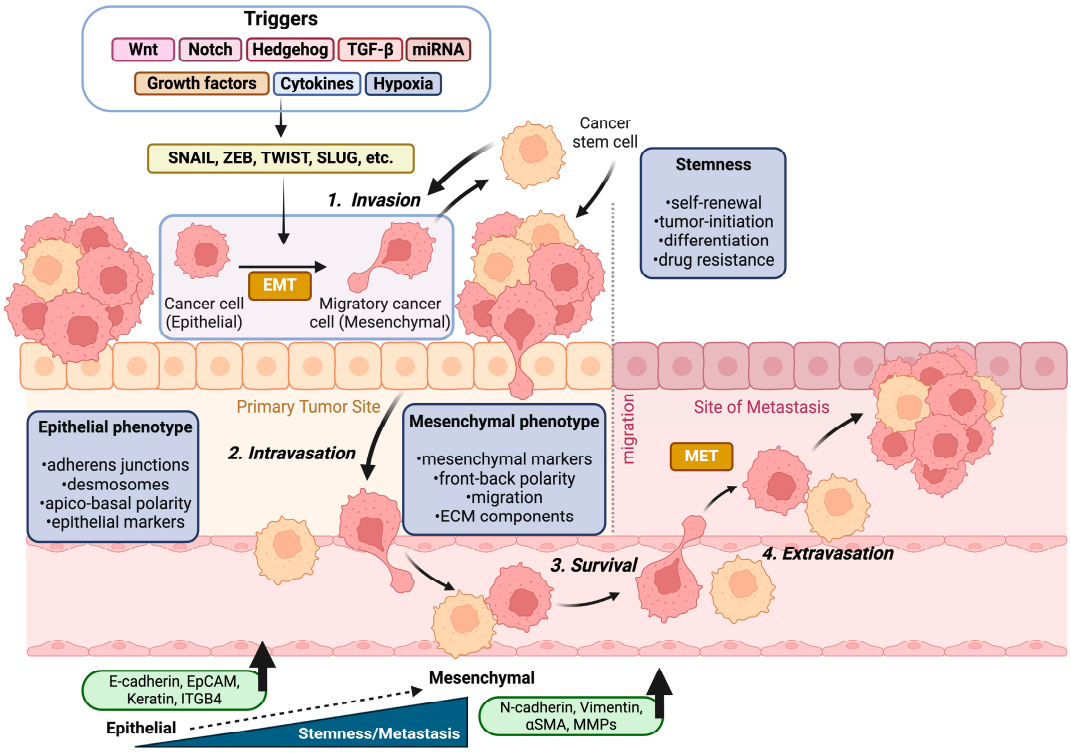

Figure 1. This schematic model illustrates the linkage between EMT and the CSCs concept in the context of tumor progression. EMT induction in carcinoma cells leads to the acquisition of stem-like properties, characterized by the expression of mesenchymal markers and the loss of epithelial markers. Additionally, CSC subpopulations exhibit EMT phenotypes, with EMT and CSC pathways being regulated at the gene level through various signaling pathways. Cancer cells detach from the basement membrane, intravasate into nearby blood vessels through EMT. Once the cells exit the bloodstream and/or lymphatic system (extravasation), they enter a new microenvironment (secondary site) where they undergo a MET. This transition supports them to colonize the surrounding tissue and initiate the formation of secondary tumors.

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4.2. Involvement of EMT and CSCs in Hypoxia

Hypoxic condition triggers the formation and aggressiveness of solid tumors, with hypoxia-inducible factor (HIF) modulating hypoxia-responsive genes that boost proliferation, survival, invasion, angiogenesis, metastasis, and therapy resistance. EMT and CSCs are linked to hypoxia, maintaining the CSC phenotype. *In vitro* studies show that hypoxia induces EMT in human carcinoma cell lines through HIF-1 activation [155–157]. HIF-1 promotes EMT in carcinoma cells, suppressing E-cadherin via ZEB1, ZEB2, and E12/E47 induction [158,159]. Recently, the cell-cell adhesion molecule NECTIN-4 has been identified as a marker for both cancer cells and cancer stem cells, playing a role in EMT promotion [70]. Moreover, reports suggest that NECTIN-4 is activated by HIF-1α [71,72], stipulating a strong connection between hypoxia, EMT, and CSCs. Collectively, these findings put a spotlight towards the complexity of factors driving EMT in carcinoma cells, posing challenges for therapeutic approaches.

4.3. Plasticity in EMT and CSCs

CSCs exhibit substantial heterogeneity, both intra- and intertumorally [160,161], complicating targeted therapy development. CSC heterogeneity arises from a combination of genetic and epigenetic alterations, mutations, and changes in microenvironmental, including different factors like cytokines, cell-cell interactions, hypoxia, etc. [160,161]. Data shows CSCs exist in specialized tumor niches supporting their survival [162,163]. CSCs rely on niche signals across different tumors [162,164] and can modulate their niche, using signaling pathways to maintain homeostasis, including inflammation, hypoxia, EMT, and angiogenesis [162,164]. These niches adapt with tumor progression and treatment, potentially drifting back non-tumorigenic cells into CSCs via EMT, elevating tumor invasion and metastasis [11,162,164,165]. This dynamic interchange revealed that therapies targeting CSCs alone may result in tumor relapse if residual cancer cells repopulate CSC niches. Cells with a mesenchymal phenotype get into circulation as CTCs, potentially undergoing MET to express EPCAM, an epithelial cell adhesion molecule in blood. The relationship between high EPCAM level in CTCs and survival remains debated [166], and not to be mentioned, CTCs also have diverse subpopulations [167].

4.4. EMT and CSCs and Their Involvement in Chemoresistance

EMT has been reported to be strongly associated with cancer drug resistance, especially multidrug resistance and radioresistance, likely due to elevated cancer cell survival, cell fate transitions, and hyperactivation of drug resistance-related genes [116,117]. SPARC levels help in identifying aggressive breast cancers with increased EMT, treatment resistance, and poor outcomes. Amino-bisphosphonates may reverse EMT by blocking the suppressive activity of certain stem cells [123]. EMT also bridges the CSC phenotype to resistance to traditional therapeutics across various carcinomas, with EMT programs upregulating genes associated with resistance in breast cancer [124] and shifting cell dependency from EGFR to AXL in NSCLC and ovarian cancer, which ultimately, develops resistance to EGFR-targeted therapies [125,126]. Additionally, EMT and CSCs also regulate ATP-binding cassette (ABC) transporters, leading to the drug-efflux-mediated development of chemoresistance [168,169]. Furthermore, EMT has a contribution in immunosuppressive tumor microenvironment, further influencing resistance to immunotherapies [127,128].

5. Signaling Pathways in CSCs and EMT

The signaling pathways involved in CSCs are thoroughly detailed by Sinha et al. [170], elucidating pathways crucial for cancer stem cell growth, survival, differentiation, and so on. This current work, however, briefly describes the signaling cascades associated with both CSCs and EMT.

5.1. Wnt Signaling

Wnt/β-catenin signaling, a major signaling for stem cell proliferation, activates EMT in tumor cells [171]. Upregulation of Six1 induces EMT in mouse breast tumor cells, initiates Wnt signaling,

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and promotes stem-like traits *in vivo* [172]. Lipoprotein receptor-related protein 6 downregulates Wnt/β-catenin signaling in breast carcinoma, inhibiting Slug and Twist expression and prohibiting cancer cell self-renewal [173]. In breast carcinoma, upregulated HER2 leads to Wnt-3 hyperactivation, triggering Wnt/β-catenin signaling and EMT, with elevated β-catenin nuclear expression and N-cadherin, Twist, and Slug levels [174]. Consequently, in breast cancer cells, expressing higher level of HER2, exhibit resistance to trastuzumab [174]. miR-1 reduces proliferation, stemness, and migration of breast CSCs by aiming at Frizzled-7 and tankyrase-2, downregulating the Wnt/β-catenin pathway [175]. In colorectal cancer (CRC) patients, miR-146a reduces Numb expression, which leads to inhibition of β-catenin, and moreover, repressing Wnt signaling, and cancer stemness [176,177].

5.2. Notch Signaling

Increasing evidence have established an important association between Notch-regulated transcription factors and the pathways that regulate stem cells, pinpointing that Notch serves as a common signaling pathway linking tumor EMT to CSCs [72]. In breast cancer cells, Notch signaling potentiates EMT, invasion, and migration by activating Slug expression [56]. In addition, Notch1 stimulates the phosphorylation of transcription activator 3, facilitating the acquisition of EMT and CSC traits [73]. In the same study, quercetin-3-methyl ether was shown to prohibit sphere formation (three-dimensional clusters of cancer cells that are enriched for CSCs) and the expression of stemness-related genes such as SRY-box2 and Nanog, while also downregulating Notch1 expression in breast cancer patients [74]. Moreover, doxorubicin stimulates the Notch pathway, with a marked expression of target genes observed at cytostatic doses [75]. Thus, doxorubicin may induce stemness in osteosarcoma cells through Notch pathway activation. Taken together, this evidence underscores the relationship between EMT and CSCs, indicating that Notch signaling potentiates CSC stemness, thereby contributing to cancer metastasis and treatment resistance.

5.3. Hedgehog Signaling

Hedgehog signaling is pivotal in CSCs formation and EMT process [171]. In pancreatic cancer, silencing the smoothened gene impedes Hedgehog signaling, which in turn hinders EMT and the invasion of pancreatic CSCs. As a result, Hedgehog signaling modulates the stemness and chemoresistance of pancreatic CSCs, stimulating carcinogenesis and metastasis [178]. Similarly, Hedgehog signaling inhibition through cyclopamine treatment diminishes Snail expression while elevating E-cadherin expression, thereby repressing stemness and EMT induction, eventually bringing down the pancreatic cancer metastasis [179]. Moreover, Hedgehog signaling is associated with the self-renewal of breast stem cells [180,181]. Stimulation of the RAS-mitogen-activated protein kinase pathway in a breast tumor progression model leads to the differentiation of human mammary epithelial cells into CSCs and the induction of EMT, which eases the acquisition of stem cell properties [55]. In PTEN-deficient cells, RAS signaling activation elicits EMT, endowing these cells with CSC characteristics [182]. These signaling pathways illuminate the correlation between EMT and CSCs, specifying that cells undergoing EMT are more susceptible to invasion and the acquisition of stem cell-like traits.

5.4. TGF-β Signaling

TGF-β, a multifunctional cytokine, primarily contributes to tumor metastasis [183,184]. Supporting this, van der Horst et al. reported that the tumor-initiating stem cell (TISC) properties in mesenchymal liver cancers were associated with EMT [185]. TGF-β aids EMT and TISC traits by uplifting Snail and Nanog expressions [186]. In hepatoma cells, miR-148A weakens cancer stemness traits by suppressing TGF-β/Smad2 signaling [187,188]. Obstructing EMT in breast cancer averts the progression of breast CSC characteristics [189,190]. Report also suggests that TGF-β type I receptor kinase inhibitor, EW-719, downregulates Snail expression, hence, reducing EMT and breast CSC characteristics. Similarly, miR-190 hinders breast cancer metastasis by targeting the TGF-β pathway and Smad2 expression [187]. Inhibition of miR-138-5p triggers TGF-β1, potentiating EMT and CSC

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traits, and declining lung cancer cell resistance to chemoradiotherapy [191]. Moreover, miR-495 suppresses TGF-β signaling; its hyperactivation backpedals CSC formation and EMT in oral squamous cell carcinoma, restricting the tumor growth *in vivo* [192]. Thus, TGF-β signaling plays a key role in cancer initiation, migration, and the EMT and CSC transformation processes.

5.5. MicroRNAs

MicroRNAs (miRNAs) also play a central role in modulating EMT-induced CSCs. These small, non-coding RNA molecules foil translation or lessen the firmness of their target mRNAs by binding to their 3′-untranslated regions [193]. Findings indicate that certain miRNAs directly modulate EMT transcription factors, influencing the EMT process and enduring CSC functionality, thereby assisting tumorigenesis. The miR-200 family, highly conserved and widely studied, is known for its role in modulating EMT and stemness properties by tempering the expression of Bmi1 and Notch1 [194]. Members of the miR-200 family and their targets form a regulatory loop that directs EMT [140]. This loop involves in negative regulation of ZEB1 and ZEB2, which leads to downregulation of Notch signaling components such as Jag1, Maml2, and Maml3. By targeting ZEB1 and ZEB2, miR-200 family members bring down their expression, thereby maintaining stemness by forbidding the expression of the miR-200 family [183]. In addition, miR-200 stamps out BMI1 expression, which in turn suppresses the proliferation and oncogenicity of breast cancer cells [183].

6. EMT as Therapeutic Target against CSCs

The obligatory role of EMT in acquiring CSC characteristics indicates that targeting EMT could be a potent strategy to hinder CSC growth. For instance, targeting EMT-associated pathways to combat CSCs represents an encouraging therapeutic strategy. Wang et al. developed nanoparticle drug delivery systems containing salinomycin, which targeted ZEB1 and ZEB2, significantly reducing their expression and repressing the EMT process, thereby inhibiting CSC formation [195,196]. Chatterjee and Kundu also utilized nano-formulated quinacrine in highly metastatic cervical cancer stem cells to battle against NECTIN-4, which directly associated with EMT and CSCs [197]. Hyperactivation of miR-495 hinders homeobox C6-mediated TGF-β signaling, therefore holding up the EMT progression and repressing the proliferation, metastasis, and invasion of oral squamous CSCs [192]. Metformin interrupts the mixed E/M stem-like state by enhancing Notch-Jagged signaling, which negatively regulates EMT and CSC invasiveness [187,198]. MiR-612 modulates EMT-linked stem cell-like traits via the Wnt pathway, with its elevation resulting in declined tumor sphere size [199]. Curcumin impedes epithelial transformation and CSC property progression in bladder cancer by inactivating Wnt/β-catenin signaling [200].

In addition, targeting the lncRNA HOX transcript antisense intergenic RNA can hinder CSC formation in CRC by regulating the EMT process, marked by enhanced E-cadherin and lessen vimentin expression [201]. Again, knocking down TROY can resist gefitinib resistance and moderately reverse EMT and CSC formation in NSCC [202]. Further, downregulating miR-21 expression reverses EMT and CSC phenotypes by disabling the Akt and extracellular-regulated kinase 1/2 pathways and targeting PTEN [203]. Furthermore, the miRNA-200 family was found to be involved in inhibiting EMT and CSC formation. Liu et al. reported that nanoparticles co-delivering miR-200c and docetaxel promote docetaxel's cytotoxicity and repress CSC expression [204]. Nevertheless, hindering miR-429 modulates its downstream target ZEB1, therefore reducing EMT as well as the tumorigenicity of osteosarcoma stem cells [205–207].

7. Conclusions

The surge in cancer incidence poses a significant threat to humanity, with tumors striking various organs and tissues. While advancements in therapies have extended the lives of cancer patients, complete eradication of tumors remains grueling. Conventional treatments often fail to eliminate cancer cells in their activated state, particularly those that have undergone EMT and acquired cancer stemness traits. Thus, understanding the contributions of EMT and CSCs in tumor

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biology has shed light on the mechanisms underlying treatment resistance. The gene expression profiles in EMT and CSCs are interrelated, stipulating a major connection. This study highlighted the interaction between EMT and CSCs through various signaling pathways, confirming their complex association. EMT can promote CSC formation, endowing them with elevated metastatic potential due to their mesenchymal phenotype. The exact mechanism by which CSCs arise from the primary tumor and expedite metastasis remains elusive. A deeper understanding of this mechanism could lead to develop therapeutics not only to prevent tumor growth and recurrence but also to inhibit metastasis. Targeting the signaling pathways associated with EMT and CSCs holds promise for upgrading cancer therapy, with miRNAs appearing as potential therapeutic targets due to their regulatory effects on EMT and CSC phenotype. Future research endeavors should delve into the reciprocation between EMT and CSCs to illustrate the mechanisms driving CSC stemness and strengthen treatment strategies. Therefore, advancing tailored therapies that target EMT or CSCs could revolutionize cancer treatment and pave the way for novel therapeutic interventions.

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