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Molecular Markers and Networks for Cancer and Stem Cells

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Review Article

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Abstract

Stem cell differentiation and self-renewal are regulated by several factors, including molecules that each cell expresses both inside and on its surface. Cancer stem cells (CSCs) exist in some populations of cancer cells, however, the origin and characteristics of CSCs remain incompletely understood; thus, a deeper analysis of the essence of CSCs is required. Since the CSCs exhibit the properties to initiate tumor and be resistant to anti-cancer drugs, inquiries into the molecular mechanisms in CSCs may lead to the discovery of novel therapeutic targets for cancer. Epithelial-mesenchymal transition (EMT), in which cells transit from epithelial-like into mesenchymal-like cell features, is an important phenotype of CSCs and cancer metastasis. In this review article, the molecules and signaling pathways involved in CSCs, with a focus on molecules so-called CD antigens, of which combinations represent cancer types and CSCs, are summarized and described for further investigation of CSCs as well as the stem cell properties of cancer. Considering that CSCs and stem cells may have similar properties, and cancer and stem cells exhibit similar signaling pathway activation in self-renewing, the phenotypes of CSCs including EMT may confer tumorigenic properties to the stem cells. From overviewing the literatures, it is suggested that CSCs are defined with combinations of several markers, and investigation of EMT network is important.

Keywords: Cancer stem cell; Epithelial-mesenchymal transition; Gene; Genome; Stem cell

Abbreviations: CSCs- Cancer Stem Cells; NOD- Nonobese Diabetic; SCID- Severe Combined Immunodeficient; EMT- Epithelial-Mesenchymal Transition; ALDH- Aldehyde Dehydrogenase

Introduction

It is important to investigate the properties of cancer stem cells (CSCs) for cancer treatment. CSCs can be defined as the cells in cancer that have self-renewal potential and differentiate into cancer cell lineages [1-5]. The concept of CSCs are very complicating and still need to be investigated, since the CSCs initiate tumors and are resistant for conventional cancer therapy [1]. Furthermore, cancer and stem cells share similar mechanisms of signaling pathways such as Wnt pathway, Hedgehog pathway, and Notch pathway [1]. Several agents have targeted self-renewal signaling pathways or CSC surface markers, indicating the importance of investigation in CSCs [1]. CSCs have tumorigenic capacity when transplanted into nonobese diabetic (NOD)/severe combined immunodeficient (SCID) mice and have drug

resistance and metastatic properties [2,5-8]. CSCs exhibit epithelial-mesenchymal transition (EMT) characteristics and the cellular phenotypic changes accompanied by EMT promoting the metastatic property of the cells [9]. The cells exhibiting the EMT phenotype are suggested to transform into CSC-like cells [10,11]. Although evidence indicating the close link between CSCs and the EMT mechanism has accumulated, the combination of CSC markers related to the phenotype, malignancy and genetic alteration of cancer are not fully revealed, which emphasizes the significance of elucidating the pathological role of CSC markers and the relationship between cancer grade and CSC markers [12]. The CSC model is also implicated as a cancer-initiating cancer model, and CSCs have potential to initiate tumors [13,14]. Plasticity of CSCs should be investigated to reveal the cancer malignancy and EMT correlation [15]. In this review article, markers for CSCs and the signaling networks of CSCs and EMT are described.

Definitions of CSCs

CSCs are defined as cells that have capacity to generate tumors and self-renewal [1,6,16]. CSCs are probably originated from cancer cells in niche by transiting the phenotypes from differentiated cells into un-differentiated cells [1,16]. The CSCs reside in side population (SP) fraction defined by the Hoechst-33342 dye exports can also be used to identify CSCs [16-18]. SP cells with faint staining of Hoechst-33342 dye are sub-population of stem cells in cancer, which have capacity of self-renewal and differentiation [19]. CSCs are identified with tumor sphere formation, which includes dilution assay of neurospheres from brain to evaluate self-renewal capacity and proliferation [20].

CSC Markers

The Riddle of CSC Markers and the Significance of their Combination

Abundant studies have investigated CSC markers in cancer. $CD44^+$ and $CD24^{-/low}$ cells have demonstrated the capacity to induce tumors in NOD/SCID mice by transplantation [16]. CD133 (also known as PROM1, a pentaspantransmembrane glycoprotein maintaining stem cell properties), CD44 (a receptor for hyaluronic acid), Sca1 (stem cell antigen-1) and CD90 (also known as Thy1, a cell surface glycoprotein involved in cell adhesion and cell communication) are often used for identification of stem cells [16]. However, these markers are also expressed in normal tissues, and they are quite controversial dependent on the literatures [16]. The exact CSC markers are not fully understood and need to be elucidated. The combination of the markers is significant to solve the riddle of CSCs. The example of several CSC marker candidates studied in various cancers is shown in Table 1.

Cancer type	CSC marker candidates	Reference
gastric cancer	$ALDH^+$	Nishikawa S et al.[23]
	$CD90^+$	Jiang J et al. [24]
	$CD44^+CD166^+$	Nguyen PH et al. [26]
	$CD44^+CD26^+$	Nishikawa S et al. [28]
	$CD133^+$	Konishi H et al. [32]
	$CD44^+$	Takaishi S et al. [29]
	$CD44^+EpCAM^+$	Han ME et al.[31]
	$CD44^+CD24^+$	Zhang C et al. [34]
pancreatic cancer	$CXCR4^+CD133^+$	Polireddy K et al. [35]
		Hermann PC et al. [36]
	$CD24^+CD44^+ESA^+$	Wang H et al. [37]
		Li C et al. [38]
	$CD24^+CD44^+EpCAM^+CD133^+$	Skoda J et al. [39]
	$CD133^+$	Miyazaki Y et al. [40]
	$YY1^{low}SOX2^{low}OCT4^{hi}BMI1^{hi}$	Kaufhold S et al. [41]
	$CD24^+CD44^+$	Liu L et al. [42]
breast cancer	$CD44^+CD24^{-/low}$	Clarke MF et al. [16]

	$CD44^+CD24^{-/low}$, $ALDH^+$	Dai M et al. [43]
		Habib JG et al. [45]
	$CD44^+CD24^-$	Horimoto Y et al. [44]
	$CD44^+CD24^{-/low}$, $SOX2^+$, $KLF4^+$, $ABCG2^+$	Park SJ et al. [91]
liver cancer	$CD133^+CD49f^+$	Rountree CB et al. [48]
	$CD44^+CD90^+$	Yang ZF et al. [50]
colorectal cancer	$CD133^+$	Ricci-Vitiani et al. [52]
	$CD133^+CD54^+CD44^+$	Fang C et al. [54]
gioblastomamultiforme	$GFAP^+SOX2^+$	Bradshaw AR et al. [60]
prostate cancer	$CD44^+CD24^-$	Sharpe B et al. [64]

Table 1: Cancer stem cell markers in various cancers.

CSC Markers in Gastric Cancer

Tumor-initiating cells are enriched in SP cells characterized by the high expression of the ABC transporter genes, ATP binding cassette subfamily B member 1 (*ABCB1*, also known as *MDR1*) and *ABCG2* (also known as *BCRP1*), in gastric cancer [21]. These tumor-initiating cells are considered as tumor therapeutic targets [21]. Several driver mutations within tumor protein p53 (*TP53*), AT-rich interaction domain 1A (*ARID1A*), cadherin 1 (*CDH1*), mucin 6, oligomeric mucus/gel-forming (*MUC6*), catenin alpha 2 (*CTNNA2*), GLI family zinc finger 3 (*GLI3*), ring finger protein 43 (*RNF43*) and ras homolog family member A (*RHOA*) genes exist in gastric cancer [22]. The combination of the specific molecular alterations may distinguish different cancer types [22]. Aldehyde dehydrogenase (ALDH) is one of the causes for the chemoresistance in cancer cells, and it may be a CSC marker [23]. CD44 and epithelial cell adhesion molecule (EPCAM) are expressed in gastric cancer cell lines, whereas CD90, CD133, and CD117 (also known as KIT, a proto-oncogene receptor tyrosine kinase) were undetectable in gastric cancer cell lines [23]. A gastric cell line expresses CD13 (also known as ANPEP, alanylaminopeptidase), and 4 out of 6 gastric cancer cell lines express CD26 (also known as DPP4, dipeptidyl peptidase 4) [23]. CSCs are characterized by the presence of the CD90 surface marker in primary gastric tumor models [24]. On the other hand, a report has shown that CD90 was down-regulated in ovarian cancer, and patients with higher CD90 expression had better prognosis compared to patients with lower CD90 [25]. These results suggest that CD90 may be differentially expressed in different types of cancer. Considering that CD90 was not expressed in gastric cancer cell lines and expressed in the primary gastric tumor model, the cellular stemness phenotype may change from primary tumor to cell lines, and the degree of stemness may contribute to the cancer phenotype and resistance.

A subpopulation of gastric cancer cells expressing CD44 together with EPCAM, CD133, and CD166 (also known as ALCAM, an activated leukocyte cell adhesion molecule) contains CSCs that have high ALDH activity [26]. Abnormal spindle microtubule assembly (Aspm) was identified as a novel possible oxyntic stem/progenitor cell marker [27]. Both CD44 and CD26 were suggested as potential markers for human gastric CSCs [28]. CD44-positive gastric cancer cells possessed stem cell properties and chemotherapy or radiation resistance [29]. The signal transducer and activator of transcription-3 (STAT3) activation is a possible marker for gastric CSCs [30]. The gastric cancer cells expressing both EPCAM and CD44 showed resistance to chemotherapy [31]. In the diffuse-type gastric cancer, Notch1 signaling was involved in the induction of CD133 expression [32]. The runt related transcription factor 1 (RUNX1) enhancer element is promoted in the isthmus stem cells of the stomach corpus, and it may provide a clue to gastric carcinogenesis [33]. It has been reported that a subpopulation of $CD44^+$ and $CD24^+$ cells was identified as gastric CSCs [34].

CSC Markers in Pancreatic Cancer

The combination of the markers CD133 and CXCR4 is considered essential for pancreatic cancer metastasis in the liver [35,36]. CD24 (a sialoglycoprotein), CD44, and epithelial specific antigen (ESA) are used to identify pancreatic CSCs [37,38]. The pancreatic CSCs and the expression of CD24 and ESA were inhibited by bufalin, a toad poison ligand and a potential anticancer agent, possibly via the Hedgehog (Hh) signaling pathway [37]. The population of $CD24^+CD44^+EPCAM^+CD133^+$ cells in pancreatic ductal adenocarcinoma had enriched pro-tumorigenic properties [39]. It has been reported that

pancreatic CSC-like cells expressing the CSC marker CD133 are affected by the inhibition of Hh/GLI and mTOR signaling [40]. The Yin Yang 1 (YY1) transcription factor is involved in CSC properties and in the regulation of SRY-box 2 (SOX2), POU class 5 homeobox 1 (POU5F1, also known as OCT4) and BMI1 proto-oncogene, polycomb ring finger (BMI1) [41]. The classification of cancers using molecular markers include $YY1^{lo}SOX2^{hi}BMI1^{hi}OCT4^{hi}$ for prostate, lung, cervical, endometrial, ovarian and glioma cancers, $YY1^{hi}SOX2^{lo}BMI1^{hi}OCT4^{hi}$ for skin, testis and breast cancers, $YY1^{lo}SOX2^{lo}BMI1^{hi}OCT4^{hi}$ for liver, stomach, renal, pancreatic and urothelial cancers, and $YY1^{hi}SOX2^{hi}BMI1^{lo}OCT4^{hi}$ for colorectal cancer, lymphoma and melanoma [41]. The inhibition of the WNT/β-catenin pathway with FH535 down-regulated the gene expression of CD24 and CD44 in pancreatic cancer [42].

CSC Markers in Breast Cancer

CSCs in triple negative breast cancers (TNBCs) exhibiting a high expression of CD44 and a low expression of CD13 are targeted for treatment [43]. In TNBCs, cyclin-dependent kinase 4 (CDK4) regulates CSCs, and the suppression of CDK4 changes the mesenchymal phenotype of TNBCs expressing CD44, CDK4 and vimentin into the epithelial-like phenotype of the cells expressing CD24 and E-cadherin [43]. Another study has demonstrated that $CD44^{+}CD24^{-}$ cells denote CSCs in TNBCs [44]. The Hh pathway is implicated in TNBC and CSC reprogramming, since the pathway has a role in stem cell renewal [45]. Sonic Hedgehog (Shh) was up-regulated in TNBCs, which suggests the possibility of targeting the Sonic Hh (Shh) pathway in cancer treatment [46]. Plumbagin, a naphthoquinone, selectively reduces the population of ALDH1-positive cells in basal-like BRCA1-defective breast cancer cells, which suggests that CSCs are selectively targeted by the reactive oxygen species inducer plumbagin [47]. Plumbagin represses the expression of stemness and EMT markers in cancer xenografts [47].

CSC Markers in Liver Cancer

The $CD133^{+}CD49f^{+}$ cells had tumorigenic potential and were identified as CSCs in liver cancer [48]. The hepatocellular carcinoma CSCs positive for CD133 were resistant to IFN-γ-induced autophagy [49]. The $CD44^{+}CD90^{+}$ cells were also identified as CSCs in human liver cancer [50]. CD90 protein level may be a cancer-type specific marker such as liver cancer, and the combination with other markers may be a hallmark for liver CSCs. At least, it seems like CD90 expression in combination with CD44 demonstrate the tumorigenic capacity in liver cancer [50]. The growth of hepatic cancer can be inhibited by WM130, a novel derivative of matrine that is a major active alkaloid of the Chinese herbal medicine *SophoraflavescensAit* [51]. It has been suggested that its interference with cancer growth is mediated by the inhibition of glycogen synthase kinase 3β (GSK3β /β-catenin signaling in hepatic CSCs [51].

CSC Markers in Colorectal Cancer

CD133 was identified as a CSC marker for human colon cancer [52,53]. The $CD133^{+}CD54^{+}CD44^{+}$ circulating tumor cells are suggested to be prediction markers for liver metastasis in colorectal cancer patients [54]. BMI1 was demonstrated as a therapeutic target for cancer stemness, and BMI1 inhibition resulted in colorectal cancer initiating cell loss [55]. BMI1, CD44, CD133 and EpCAM were reported to be expressed in colorectal CSCs, whereas CD29, leucine rich repeat containing G protein-coupled receptor 5 (Lgr5) and musashi RNA binding protein 1 (MSI1) were expressed in both normal colorectal stem cells and colorectal CSC [56-59].

CSC Markers in Glioblastoma multiforme

The $SOX2^{+}$ CSC population in glioblastomamulti forme expresses (pro)renin receptor (PRR), angiotensin II receptor 1 (ATIIR1) and ATIIR2, which suggests the presence of a relationship between the renin-angiotensin system and CSCs [60]. Glioblastoma multiforme CSCs are positive for GFAP and SOX2, which may be candidates for the therapeutic targeting of glioblastoma multiforme [60].

CSC Markers in Prostate Cancer

The tumor-initiating stem-like cells in prostate cancer are characterized by co-expression of the human pluripotent stem cell markers TRA-1-60, CD151 and CD166 [61]. ELL-associated factor 2 (EAF2)-knockout mice had increased prostate microvessel density and prostatic intraepithelial neoplasia [62]. Methylation silencing of the Nrf2 promoter, resulting in reduced Nrf2 expression, was associated with the progression of murine prostate cancer TRAMP cells, which is modulated by radix *Angelicae Sinensis*, which has an anti-cancer effect [63]. $CD44^{+}/CD24^{-}$ cells in prostate cancer DU145 cell lines were identified as CSC markers [64]. ALDH1 and EZH2 were shown to be potential CSC markers in prostate cancer [65].

EMT and CSCs

EMT

EMT is defined by cellular phenotypic change from epithelial-like cells into mesenchymal-like cells, and the

induction of EMT confers stem cell like properties on epithelial cells [66]. Cells express CSC markers such as $CD44^{high}/CD24^{low}$ have up-regulated expression of EMT markers including twist family bHLH transcription factor 1 (*TWIST1*), vimentin (*VIM*), snail family transcriptional repressor 2 (*SNAI2*), *TWIST2* and fibronectin 1 (*FN1*), compared to $CD44^{low}/CD24^{high}$ cells [66]. The EMT-induction with tamoxifen using a vector expressing tamoxifen-activatable form of either the Snail or the Twist transcription factors resulted in promotion of CSC generation indicated with increase in tumor sphere formation [66]. EMT itself is a phenotypic transition occurs during embryonic development, in which epithelial cells transit into mesenchymal-like cells that have migration capacity with loose intra-cell connections [67]. EMT is induced with TGFβ1 treatment, or expression of Snail or TWIST, resulting in cellular transition into mesenchymal cells with $CD44^{high}/CD24^{low}$ expression pattern [67]. It is still in discussion whether EMT-induced cancer cells equal to CSCs, or what mechanism underlies EMT and CSCs. The models suggest that cancer stem-like cells are implicated with autophagy of EMT-induced mesenchymal tumor cells [68]. It has also been reported that LGR5-expressed human gastric adenocarcinoma MGC803 tumor sphere cells exhibit tumorigenic CSC phenotype as inoculated in nude mice [69]. The MGC803 sphere cells have higher expression in LGR5 and NANOG, a stemness marker, compared to MGC803 adherent cells [69]. MET, an inverse mechanism of EMT is dysregulated in cancer, in which Wnt/β–catenin signaling pathway is activated to promote stemness and invasion [70]. Phenotype transition and plasticity in cancer and stem cells are still needs to be investigated and revealed.

EMT in Stem Cells

The plasticity through transitional states during EMT and mesenchymal-epithelial transition (MET) has been reported [71]. The cellular transition between epithelial and mesenchymal phenotypes consists of several states, not only the distinct mesenchymal or epithelial states but also intermediate states called EM states [71]. In these states, different combinations of molecules are expressed [71]. EMT induced by PDGF-D is linked with stem cell signatures such as Nanog and Sox2 expression [10]. In embryonic stem cells, RUNX1 regulates EMT and cell differentiation [72]. RUNX1 is up-regulated in the early stages of human ES cell mesendodermal differentiation [72]. *CDH1*, an epithelial marker, was up-regulated, whereas *CDH2*, a mesenchymal marker, was down-regulated in diffuse-type gastric cancer compared to MSCs [73]. Catenin beta 1 (*CTNNB1*), an EMT-related gene, is up-regulated in the diffuse-type gastric cancer compared to MSCs [74]. Wnt/β-catenin signaling is important in EMT and in the progression of cancer [73-76]. MSCs play a dual role as tumor supportive effects, including transition to tumor-associated fibroblasts and the stimulation of EMT, and tumor suppressive effects, including the down-regulation of the Wnt/β-catenin pathway and the PI3K/AKT pathway in tumors [77]. It is still controversial whether epithelial plasticity renders stemness, as MET initiates and is required for the reprogramming of fibroblasts into induced pluripotent stem cells [71,78]. There might be transition between mesenchymal and epithelial phenotypes, even in stem cells.

EMT in CSCs

The early EMT program with epithelial plasticity correlates with CSCs [79]. The cells exhibiting an EMT program enter the CSC state with transient expression of EMT transcription factors [80]. The EMT phenotype is exhibited in transformed cells, and FOXA1 and FOXA2 transcription factors are down-regulated in transformed cells [81]. EMT is associated with IGF1R activation via IGF1 [82]. IGF1R also mediates the transforming growth factor β1 (TGFβ1)-induced EMT [82,83]. In the acquisition of the EMT phenotype, the lipogenic enzyme fatty acid synthase (FASN), lysyl oxidase like 2 (LOXL2), integrin subunit alpha 6 (ITGA6), and Dickkopf-1 (DKK1) are reported to promote EMT and CSC-like phenotypes [84-87]. E-cadherin down-regulation and VIM up-regulation, which are indicators of the EMT phenotype,are observed in residual nasopharyngeal carcinoma cells with high radioresistance and cross-resistance to paclitaxel and cisplatin [88]. High expression of Lgr5 and c-myc, which are markers of the CSC phenotype, was also demonstrated in residual nasopharyngeal carcinoma [88]. CD44, a receptor for hyaluronic acid, promotes EMT and CSC expansion [79]. Etoposide, a topoisomerase II, is reported to revert the EMT signature correlated with the expression of CD44 in breast cancer cells [89]. The EMT phenotype is also induced by 4-methylnitrosamino-1-3-pyridyl-1-butanone, which is a major risk factor for cancers and may be involved in CSC induction and chemoresistance [90]. Stimuli such as 17β-estradiol, TGFβ1 and hypoxia promote EMT and CSC phenotypes in breast cancer [91]. EMT, associated with CSC formation, induces DNA accessibility in regions distant from transcription start sites and enriched with chromatin enhancer marks [92]. FOXN2 and FOXQ1 regulate the CSC phenotype [92].

Hh signaling induces EMT and ABCG2 up-regulation, leading to resistance to EGFR tyrosine kinase inhibitor in

primary and secondary resistant non-small-cell lung cancer cells [93]. TWIST1 and BMI1 are involved in EMT-related CSC proliferation, cancer metastasis and chemoresistance [94]. A histone demethylase named retinoblastoma-binding protein 2 (RBP2) promotes EMT in renal cell carcinoma and may be an epigenetic regulator initiating CSC phenotype via EMT [95]. Galectin-3 (Gal3) is reported to be associated with CSC characteristics, and the knockdown of Gal3 leads to EMT, increased sphere-formation ability and drug resistance [96]. Hepatocellular carcinoma may follow the phenotype plasticity model, in which the bidirectional conversion between cancer cells and CSCs undergoing EMT may lead to cancer development [97]. EMT is the driver of hepatic CSC plasticity, and the EMT phenotype is linked with CSC biology [97]. The Z-cad dual sensor determines the epithelial and mesenchymal state of carcinoma cells [98]. The EMT/MET plasticity response to various stimuli can be detected with the Z-cad dual fluorescent sensor [98]. Numb-like (NumbL), a protein involved in cell development, adhesion and migration, down-regulates the EMT and CSC-related transcripts and CSC-like phenotypes [99]. NumbL is reported as an independent tumor suppressor inhibiting the Notch pathway [99]. CD44 and CD24 are related to the reprogramming of nasopharyngeal carcinoma cells into CSC phenotype via STAT3 activation, which suggests that the combination of CD44/CD24/STAT3 could be a potential therapeutic target [100]. CSC signaling pathways were found to control key driver genes regulating parallel signal transduction in the quiescence, survival and maintenance of stemness in CSCs [101]. The plasticity of the CSC phenotype and the EMT/MET mechanisms provide the therapeutic targets for oral squamous cell carcinoma [102]. It is known that the $CD44^{high}CD24^{low}$ signature determines the CSCs and EMT phenotype [103]. DNA methyltransferase 1 (DNMT1) promotes prostate cancer metastasis via EMT and CSC regulation [104]. The Hippo pathway has been reported to be involved in breast tumor cell invasion promoted by Twist-mediated EMT [105]. In human hepatocellular carcinoma, SOX9 was reported as a CSC marker regulating Wnt/β-catenin signaling and osteopontin [106]. Janus kinase 2 (JAK2) expression is dysregulated by TrkC, which induces EMT in metastatic breast cancer [107]. EMT in breast cancers is suppressed by 3,6-dihydroxyflavone via the inhibition of the Notch signaling pathway [108]. In pancreatic carcinogenesis, it was reported that α-mangostin-encapsulated poly(D, L-lactic-co-glycolic acid) (PLGA) nanoparticles (Mang-NPs) inhibit CSCs and EMT associated with the down-regulation of pluripotency maintaining factors, stem cell markers, EMT-related molecules and components of the Shh pathway [109].

Signaling Pathways in CSCs and EMT

Many signaling pathways regulate cancer transformation, which includes Wnt signaling pathway started by Wnt ligand binding to the receptors Frizzled (Fz) and low-density lipoprotein receptor-related protein 5 (LRP5) and LRP6, leading to the release of β-catenin from the complex consists of adenomatous polyposis coli protein (APC), axis inhibition protein (Axin), GSK3β and casein kinase 1α (CK1α), Hedgehog (Hh) signaling pathway initiated by binding of a Hh ligand to protein patched homologue (PTCH), and Notch signaling activated by interaction between the Notch ligand and Notch receptor [110]. It has been reported that activation of Wnt/β-catenin signaling in combination with laminins leads to increase in gene expression of ISL1, OCT4, KDR and NKX2.5, markers for cardiac progenitors and multipotent stem cells [111]. EMT is induced by several signaling pathways, including the Hh, TGFβ, receptor tyrosine kinase, Wnt, Notch and Hh, matrix and hypoxia signaling pathways [85,86,112]. Shh pathway molecules, such as Gli1, Gli2, Ptch1/2 and Smo, are down-regulated by Mang-NPs, which are related to CSCs and EMT inhibition in pancreatic carcinogenesis [109]. The Hh pathway is activated by pro-inflammatory cytokines, TNF-α and IL-1β through the up-regulation of the expression of GLI1, an important gene in EMT, in pancreatic ductal adenocarcinoma [113].

Galectin-1, a β–galactoside-binding protein expressed in activated cancer-associated fibroblasts, induces EMT, GC cell migration and invasion through Gli1 up-regulation [114]. Galectin-1 also induces EMT via the non-canonical Hh pathway, leading to the increased transcription of Gli-1 in an SMO-independent manner [115]. PI3K/AKT/mTOR and Shh pathways inhibit CSC characteristics and tumor growth in pancreatic cancer [116]. Inhibitors of PI3K/mTOR (NVP-LDE-225) and SMO (NVP-BEZ-235) were shown to inhibit EMT by regulating cadherin, vimentin, Snail, Slug and Zeb1 [116]. Several long non-coding RNAs (lncRNAs) associated with the Hh pathway are dysregulated in Twist-positive breast cancer and regulate CSC maintenance via growth arrest specific 1 (GAS1), an enhancer of Hh signaling, SOX2 and OCT4 [117]. The demethylation of trimethylated histone H3 lysine 27 (H3K27me3) increases under hypoxic conditions in the MCF7 human mammary adenocarcinoma cell line and is reversed upon re-oxygenation [118]. The H3K27me3 may be implicated with CSC signaling, considering that repeated hypoxia and re-oxygenation are known to promote tumor stem cell properties, and stem cell niches are hypoxic [118].

Targeting CSC Phenotype

The cells positive for CD36, a fatty acid receptor, and CD44 isolated from primary oral orthotopic tumors are considered to be metastasis-initiating cells that exhibit a correlation with poor cancer prognosis [119]. The HER family molecules c-MET, ALK, and IGF-IR are targets for cytotoxic drugs [120]. Pan tyrosine kinase inhibitors (TKIs) (canertinib, neratinib and afatinib) are effective in the inhibition of ovarian cancer cell growth and attenuation of phosphorylation of EGFR, HER2, AKT and MAPK in ovarian cancer cell lines [120].

MicroRNAs (miRNAs) are potential biomarkers for the early detection of pancreatic cancer [121]. It is suggested that the up-regulation of miR-106a and miR-27a and the down-regulation of miR-219-13p are associated with EMT, whereas the down-regulation of miR-17-92 is related to chemoresistance in pancreatic cancer [121]. It is also known that miR-139-5p is down-regulated in colorectal carcinoma cells and in the multiple drug resistant CSC model comprising a $CD44^{+}CD133^{+}$ cell population [122]. NOTCH1 is a direct target of miR-139-5p, and the expression of NOTCH1 and miR-139-5p is inversely correlated and regulates the CSC drug-resistant phenotype [122].

The expression of the tumor-suppressive miRNAs miR-200b, miR-200c, miR-122 and miR-145 is correlated with DEAD-box helicase 3, X-linked (DDX3), and the reduction of DDX3 promotes DNA methyltransferase 3A (DNMT3A), indicating that DDX3 prevents CSC formation via the epigenetic regulation of tumor-suppressive miRNAs [123]. Adult T-cell leukemia/lymphoma (ATL)-derived exosomes containing miR-21 and miR-155 have been reported to modulate MSCs via the activation of the NF-κB pathway and IκB-α phosphorylation [124]. MSCs were reported to induce metastasis of 3D-cultured hepatocellular carcinoma cells through TGFβ-induced EMT [125]. In upper urinary tract urothelial cell carcinoma cells, androgen receptors (ARs) contribute to CSC expansion upon alteration of the CSC-related miRNA profile, suggesting the possibility of targeting AR in cancer therapeutics [126]. It has been reported that the decrease in the lncRNA HOTAIR inhibits human colorectal CSCs, which indicates the possibility of targeting HOTAIR expression in $CD133^{+}$ CSCs in colorectal cancer therapeutics [127].

Conclusion

The research on CSC markers has revealed an abundant number of targets for use in the therapy for multiple drug resistant cancers. The combination of molecules and the pathways involved in CSCs need to be elucidated. EMT is involved in CSC formation, and the phenotypic change via EMT is an important factor for cancer metastasis and drug resistance in CSCs. The CSCs in the SP of cancer cells exhibiting EMT contribute to tumor formation. Further investigation is needed to understand the whole picture of CSCs and EMT.

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