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REVIEW

Targeting cancer stem cell-specific markers and/or associated signaling pathways for overcoming cancer drug resistance

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Abstract Cancer stem cells (CSCs) are a small subpopulation of tumor cells with capabilities of self-renewal, de-differentiation, tumorigenicity, and inherent chemo-and-radio therapy resistance. Tumor resistance is believed to be caused by CSCs that are intrinsically challenging to common treatments. A number of CSC markers including CD44, CD133, receptor tyrosine kinase, aldehyde dehydrogenases, epithelial cell adhesion molecule/epithelial specific antigen, and ATP-binding cassette subfamily G member 2 have been proved as the useful targets for defining CSC population in solid tumors. Furthermore, targeting CSC markers through new therapeutic strategies will ultimately improve treatments and overcome cancer drug resistance. Therefore, the identification of novel strategies to increase sensitivity of CSC markers has major clinical implications. This review will focus on the innovative treatment methods such as nano-, immuno-, gene-, and chemotherapy approaches for targeting CSC-specific markers and/or their associated signaling pathways.

Keywords Cancer stem cell · Drug resistance · Nanotechnology · Gene therapy · Immunotherapy · Review

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Abbreviations

ABCG2	ATP-binding cassette subfamily G member 2
ABCB5	ATP-binding cassette transporter B5
ADR	Adriamycin
ALDH1	Aldehyde dehydrogenase 1
AML	Acute myeloid leukemia
ANT2	Adenine nucleotide translocase 2
ATRA	All-trans retinoic acid
AuNRs	Gold nanorods
BRAC1	Breast cancer type 1
CAF	Carcinoma-associated fibroblast
Chk1	Checkpoint kinase
CSF-1	Colony stimulating factor 1
CSCs	Cancer stem cells
dCD133KDEL	Deimmunized *Pseudomonas* exotoxin fused to anti-CD133 scFv with a KDEL terminus
DEAB	4-(Diethylamino)benzaldehyde
DLBCL	Diffuse large B cell lymphoma
DNR	Daunorubicin
DPAGT1	Dolichyl-phosphate *N*-acetylglucosamine phosphotransferase 1
DOX	Doxorubicin
EGFR	Epidermal growth factor receptor
EpCAM	Epithelial cell adhesion molecule
ERK	Extracellular-signal-regulated kinases
ESA	Epithelial specific antigen
FA	Folate
FAHAC18	Folate hyaluronic acidoctadecyl

FLT3	FMS-like receptor tyrosine kinase 3
GBM	Glioblastoma multiforme
GRP78	78 kDa glucose-regulated protein
GSK3β	Glycogen synthase kinase 3 beta
HA	Hyaluronan
HAC18	Hyaluronic acidoctadecyl
HAPEI/HA	HA poly(ethyleneimine)/HA
Hh	Hedgehog
ISL	Isoliquiritigenin
PEG	Poly(ethylene glycol)
HDAC6	Histone deacetylase 6
HER2	Human epidermal growth factor receptor 2
HGF	Hepatocyte growth factor
HSP90	Heat shock protein 90
JAK	Janus family kinases
JNK	Jun N-terminal kinase
LA	Lipoic acid
pDNA	Plasmid DNA
MAPK	Mitogen-activated protein kinase
METF	Metformin
mAb	Monoclonal antibody
MDR1	Multidrug resistance protein 1
MM	Multiple myeloma
mPEG-PLGA-PLL, PEAL	Monomethoxy polyethylene glycol–polylactic acid/glycolic acid–poly(L-lysine) triblock copolymer
MT	Microtubules
NPs	Nanoparticles
NSCLC	Non-small cell lung cancer
PEG	Poly(ethylene glycol)
PEO	Poly(ethylene oxide)
PDGF	Platelet-derived growth factor
PI3	Phosphoinositide 3
PPI	Polypropylenimine
PTEN	Phosphatase and tensin homologue deleted on chromosome 10
PTPRK	Receptor-type protein tyrosine phosphatase k
PTX	Paclitaxel
P-gp	Permeability glycoprotein
PZ-39	*N*-(4-chlorophenyl)-2-[(6-{[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl] amino}-1,3-benzothiazol-2-yl; sulfanyl] acetamide)
ROR1	Type I receptor tyrosine kinase-like orphan receptor
RTK	Receptor tyrosine kinase
SAL	Salinomycin
SCF	Stem cell factor
shRNA	Short hairpin RNA
STAT	Signal transducers and activators of transcription
SSCs	Somatic stem cells
TKI	Tyrosine kinase inhibitors
Topo	Topoisomerase

Introduction

Tumors generally develop significant resistance to repeated treatment with one/many kinds of anticancer agents, and cancer drug resistance can be a major obstacle in the efficacy of chemotherapy agents [1]. In this regard, most of the cancers are resistant to chemotherapeutic drugs due to cancer stem cells' (CSCs) population which ultimately results in tumor relapse [2]. CSCs are a small subpopulation of tumor cells with capabilities of self-renewal, dedifferentiation, tumorigenicity, and inherent chemo- and radiotherapy resistance [3]. Therefore, a successful cancer treatment will likely need to eliminate CSCs. At present, conventional anticancer therapies such as chemotherapy, radiotherapy, and immunotherapy can rapidly suppress the growth of differentiated tumor cells; however, they potentially remain behind cancer-initiating cells [4, 5]. Herein, CSC markers are attractive targets for novel cancer-targeting therapy since the high expression of these markers has been observed in most human tumors [6, 7]. Surface markers of CSCs are generally shared by somatic stem cells (SSCs). However, slight surface antigen differences as well as signaling pathways and metabolic alterations can hopefully distinguish between CSCs and SSCs and may be exploited for the selective tumor-targeted therapies [8]. In this regard, a number of CSC markers including CD44, CD133, receptor tyrosine kinase (RTK), aldehyde dehydrogenases (ALDH), epithelial cell adhesion molecule/epithelial specific antigen (EpCAM/ESA), and ATP-binding cassette subfamily G member 2 (ABCG2) have been proved as the useful targets for defining CSC population in solid tumors (Table 1) [9, 10]. Therefore, selective tumor targeting of these CSC markers with new therapeutic strategies will ultimately improve cancer treatments via overcoming drug resistance (Fig. 1). There are many CSC drug resistance agents including ATP-binding cassette (ABC), permeability glycoprotein (P-gp), microtubules (MTs) alteration, topoisomerase (Topo), P53, breast cancer type 1 (BRAC1), and human epidermal growth factor receptor 2 (HER2) [11]. Currently, new treatment methods such as targeting of antibody and nanoparticle (NP)-based CSC-specific markers or the related signaling pathways are available or under investigation.

This review will focus on the association between CSC markers and several related signaling pathways in regulating tumor cell survival and multi-drug resistance. This new

Table 1 Surface markers used for the identification of CSCs [12]

Markers	Expression in normal tissues	Expression in tumor tissues
CD19	Broadly on B lymphocytes	B cell malignancies
CD20	Broadly on B lymphocytes	Melanoma
CD24	Broadly on B cells; neuroblasts	Pancreas/lung cancer, negative on breast cancer
CD34	Hematopoietic and endothelial progenitors	Hematopoietic malignancies
CD38	Multiple stages of B and T cells	Negative on AML
CD44	Broadly on many tissues	Breast/liver/head and neck/pancreas cancer
CD90	T cells, neurons	Liver cancer
CD133	Proliferative cells in multiple organs	Brain/colorectal/lung/liver cancer
EpCAM/ESA	Panepithelial marker	Colorectal cancer, pancreatic cancer
ABCB5	Keratinocyte progenitors	Melanoma

See the main text for abbreviation definitions

knowledge can serve as groundwork for the future development of new drug targets for inhibiting CSC markers in order to overcome drug resistance in the progression of cancers.

CD44

CD44 is a major surface hyaluronan (HA) receptor which is implicated in the progression of some cancers including melanoma, breast, ovarian, and head and neck [13]. It can upregulate in a broad range of malignant tumors, and its high expression may correlate with poor prognosis of some cancers. In fact, it is one of the important surface markers on CSCs [14–16]. Interestingly, recent studies have indicated that both HA and CD44 involve in chemotherapeutic resistance in many cancers [17]. Specifically, HA binding is capable of stimulating multidrug resistance protein 1 expression and drug resistance in tumor cells [18]. Moreover, CD44 can interact with P-gp to promote cell migration and invasion of tumor cells [9]. Furthermore, HA/CD44-mediated ErbB2 signaling and phosphoinositide 3-kinase (PI3k)/AKT-related survival pathways may involve in cancer drug resistance [19]. In addition, activation of several HA/CD44-mediated oncogenic signaling pathways, e.g., intracellular Ca^{2+} mobilization, epidermal growth factor receptor (EGFR)-mediated extracellular signal-regulated kinases (ERK), and topoisomerase

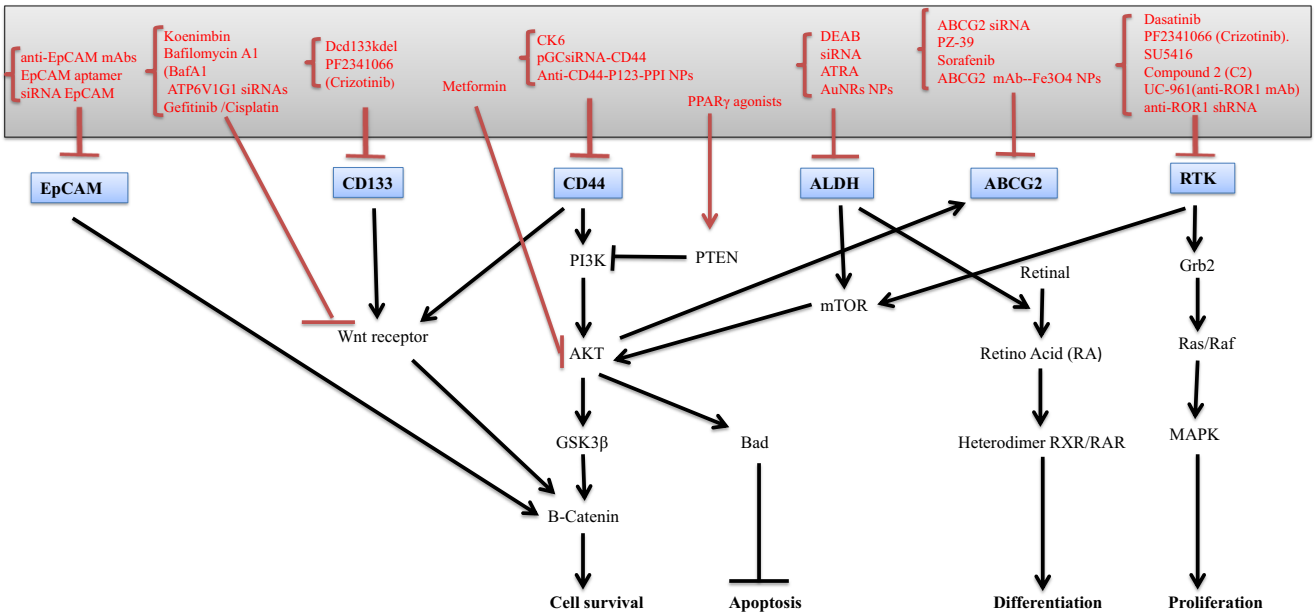

Fig. 1 Various approaches applied for targeting CSC-specific markers and/or the related signaling pathways to overcome drug resistance in CSCs. A number of CSC markers including CD44, CD133, RTK, ALDH, EpCAM/ESA, and ABCG2 and/or their signaling pathways have been proved as useful targets for defining CSCs population in solid tumors. The innovative treatment methods such as nano-, immuno-, gene-, and chemotherapy approaches applied for targeting CSC-specific markers and/or pathways associated with their markers. See the main text for more details and abbreviations

activation, lead to multidrug resistance in head and neck cancers [20]. In another study, Miletti-Gonzalez et al. in 2005 have suggested that there is an association between CD44 and ABCB1 in their transcriptional mechanism [21]. Moreover, the expression of Nanog (a transcription factor critically involved in self-renewal of undifferentiated embryonic stem cells) and its interaction with HA and CD44 can also lead to the appearance of stem cell regulators such as Rex1 and Sox2 in ovarian and breast tumors. The formation of Nanog and Stat-3 complex can induce MDR1 gene expression and activation by HA-CD44 interaction that may persuade multidrug resistance [22]. Accordingly, evaluation and targeting of CD44 and its related signaling pathways can be helpful for overcoming cancer drug resistance.

The cell surface membrane-bound proteins are interesting objects for enriching, isolating, and identifying CSCs, and consequently cancer therapy. Delivering cytotoxic drugs to CSCs via specific markers can be a useful method in cancer therapy. Furthermore, the use of inhibitors of drug-detoxify enzymes, drug-efflux pumps, and/or transcription factors of CSCs can represent a potential approach to target CSCs, and consequently reduction of cancer recurrence and metastasis [3, 7]. Gu et al. in 2015 applied the gene- and chemotherapy via application of anti-CD44 antibody-conjugated pluronic P123-PPI (anti-CD44-P123-PPI)-based nanocarrier to deliver selectively pDNA-iMDR1-shRNA into MCF-7/ADR cells. The results indicated that the anti-CD44-P123-PPI/pDNA-iMDR1-shRNA nanocomplexes can specifically silence MDR1 and inhibit P-gp expression in MCF-7/ADR cells. These results also demonstrated that the sensitivity of MCF-7/ADR cells to doxorubicin (DOX) was significantly enhanced after transfection with pDNA-iMDR1-shRNA [14]. Yang et al. in 2015 indicated that the expression of MDR1 and functional activity of P-gp were decreased via delivering MDR1 siRNA with HA poly(ethyleneimine)/HA poly(ethylene glycol) (HAPEI/HAPEG) nanoparticle into SKOV3TR and OVCAR8TR (MDR ovarian cancer lines). Moreover, increasing cell sensitivity to paclitaxel (PTX) was observed in this method. Thus, CD44 can be a potential therapeutic target for antibody-drug conjugates, and anti-CD44 antibody-drug conjugates may be a therapeutic agent for elimination of $CD44^+$ tumors [23]. Currently, Zhiyuan Zhong et al. in 2015 have found that HA-Lys-LA nanoparticle conjugates (Lys, L-lysine methyl ester; LA, lipoic acid) can be a multifunctional platform for delivery of active CD44-targeting DOX, and consequently overcoming drug resistance in in vitro and in vivo studies. These HA NPs with excellent CD44-targetability have appeared as novel potent platforms for CD44-targeted chemotherapy with effective overcoming approach to cancer drug resistance [24]. Furthermore, Liu et al. in 2014 demonstrated that the cytotoxicity of PTX loaded in hyaluronic acidoctadecyl (HAC18) and folate hyaluronic acidoctadecyl (FAHAC18) micelles can increase intracellular delivery of PTX by active receptor-mediated endocytosis. It was concluded that the dual targeting of FAHAC18 micelles can demonstrate an excellent MDR overcoming ability and can provide a novel effective nanoplatform for anticancer drug delivery in cancer chemotherapy [25]. Additionally, objective construction of RNA interference vectors targeting CD44 could specifically target CD44 gene and down-regulate its expression. These can inhibit K562/A02 cell proliferation and induce apoptosis and may effectively reverse the multidrug resistance [26].

In summary, the collected data demonstrate that CD44 is one of the most important CSC markers associated with drug resistance agents such as P-gp as well as its related signaling pathways such as Wnt/β-catenin and PI3-kinase/AKT pathways [18, 19]. Thus, targeting CD44 has beneficial effects on overcoming cancer drug resistance (Table 2). In this regard, applying nanotechnological approaches has gained an immense popularity for targeting CD44 in the recent years due to their potential capabilities to improve therapeutic effects. NPs as non-viral vectors can avoid viral vector disadvantages and can also be conjugated with tumor targeting or other drug molecules, siRNA, and CD44 antibodies for the purpose of combination therapies in order to overcome multidrug resistance [11]. Moreover, the combination of nanotechnological approaches with gene- and chemotherapy agents has been applied for targeting CD44 and overcoming drug resistance due to P-gp. The results of pre-clinical studies displayed that using nanocarrier for delivery of MDR1 SiRNA and pDNA-iMDR1-shRNA can have beneficial effects on cancer drug resistance via inhibition of P-gp expression at mRNA and protein levels [14, 23, 26]. In addition, PTX-loaded HA-C18 and FA-HA-C18 micelles and DOX-loaded-HA-Lys-LA caused high drug accumulation in the tumor as well as drug leakage inhibition and apoptosis in in vitro and in vivo studies [24, 25]. Moreover, applying MDR siRNA and shRNA among gene therapy methods attained great attention in comparison with other genes and can suppress MDR1 expression [14, 23, 26]. Yet, overcoming cancer-drug-resistant agents and their related signaling pathways has not vastly been used in clinical trials via targeting CD44. In this regard, it seems that the combination of nano-, gene-, and chemotherapy approaches can have a great potential to be adopted by clinical trials in the future.

CD133

CD133, a member of prominin family, consists of five transmembrane single-chain glycoproteins [29] and is initially identified in the CD34-positive hematopoietic stem cells [30, 31]. It consists of two extracellular domains with a potential glycosylation, affected by alternative splicing, which can produce various epitopes [32]. The localization of CD133 in highly curved plasma membrane protrusions seems to be

Table 2 Various approaches to overcoming drug resistance in $CD44^+$ CSCs

Drugs and methods	Study type	Study model	Endpoints	References
Paclitaxel NPs for delivery of MDR1 SiRNA (HA-PEI/HA-PEG/MDR1 siRNA)	In vitro	SKOV-3TR	Suppressed growth of ovarian cancer Down-regulation of MDR1 and P-gp expression	[23]
ADR P123-conjugated polypropylenimine (PPI) dendrimer (P123-PPI) for carrier of pDNA-iMDR1-shRNA	In vitro	MCF-7/ADR	Suppression of P-gp expression at the mRNA and protein levels Improved the internalization and cytotoxicity of ADR	[14]
DOX -HA-Lys-LA	In vitro	MCF-7 human breast cancer cells	Superior antitumor activity High drug accumulation in the tumor	[24]
DOX siRNA plasmid vector specifically targeting CD44 gene(pGCsiRNA-CD44)	In vitro	K562/A02	Down-regulated the expression of CD44 gene Inhibited cell proliferation and induced apoptosis Decreased expression of MDR1, BCL2 and CD44 mRNA	[26]
PTX Methotrexate SU9516(a specific cyclin-dependent kinase 2 (Cdk2) inhibitor)	In vitro	Stem-like $CD44^+/CD24^{-}$/low subpopulation isolated from SUM149PT cells	Induced a stronger cytotoxic effect of drug Activation of apoptosis Inhibited cyclin E/Cdk2 oncogenic signaling pathway	[27]
Koenimbin (isolated from *Murraya koenigii* Spreng)	in vitro	MCF7 breast cancer stem cells ($CD44^+/CD24^{-}$/low)	Induced apoptosis with related pathway Suppressed Wnt/β-catenin signaling pathway	[28]
PTX NPs for delivery of MDR1 SiRNA (HA-PEI/HA-PEG/MDR1 siRNA)	In vivo	SKOV-3TR Xenograft mice model	Induced inhibition of tumor growth Decreased P-gp expression Increased apoptosis	[23]
ADR Anti-CD44-P123-PPI/pDNA-iMDR1-shRNA nanocomplexes	In vivo	MCF-7/ADR xenografts BALB/c nude mice model	Inhibited tumor growth Enhanced therapeutic efficacy of ADR Increased accumulation of ADR	[14]
DOX HA-Lys-LA	In vivo	MCF-7/ADR tumor xenografts in nude mice	High drug accumulation in the tumor Inhibited drug leakage and prolonged blood circulation time	[24]
PTX Taxol HA-C18 and FA-HA-C18 PTX-loaded micelles	In vivo	MCF7 tumor-bearing mice	Demonstrated excellent MDR overcoming ability Improved tumor distribution	[25]

See the main text for abbreviation definitions

essential for scaffolding membrane glycoprotein [30, 33, 34]. Among crucial CSC biomarkers, CD133 can play a dominant role in drug resistance [35]. CD133 can confer drug resistance via various signaling pathways. In this regard, CD133 can enhance the activity of histone deacetylase 6 (HDAC6) via Wnt/β-catenin signaling pathway and subsequently leads to degradation and activation of β-catenin signaling targets [36]. In concordance with molecular features of cancer stem cells, $CD133^+$ cells express high levels of cell surface receptors such as the cytokine receptor CXCR4 which is necessary for paracrine signaling axis. In addition, Celia Chao et al. in 2012 showed that CD133 can trigger the paracrine signaling pathways with carcinoma-associated fibroblasts (CAFs) into the tumor microenvironment [37]. Recent studies have revealed that the receptor-type protein tyrosine phosphatase kappa (PTPRK) as a novel binding partner of CD133 has the ability to dephosphorylate CD133 [38]. A previous study demonstrated that there is an association between the phosphorylation level of CD133 and the human glioma progression [39]. Consistent with these observations, tyrosine phosphorylation of CD133 via subsequent activation of AKT/β-catenin oncogenic pathway was observed as a prominent role of CD133 in colon carcinogenesis. Moreover, phosphorylation of the tyrosine-828 residue in CD133 C-terminal cytoplasmic domain contributes to preferential activation of the PI3K-Akt pathway in $CD133^+$ glioma cells via direct interaction between CD133 and the PI3K regulatory subunit (p85) which can result in MDR1 activation through the PI3K/Akt/NF-kB pathway. Furthermore, high levels of CD133 in MDR cells were correlated in resistance to chemotherapy [40, 41]. Another study determined that the high expression level of DNA-dependent protein kinase (DNA-PK) in chemoresistance can prevent cancer cells from undergoing apoptosis following chemotherapy [42]. In support of this notion, it has been shown that CD133 and DNA-PK independently contribute in regulation of MDR1 expression via PI3K or Akt/NF-kB cascades. Moreover, regarding to the biological properties of CD133, in contrast to normal cell, CD133-positive cells exhibit stronger activation of ataxia telangiectasia- and Rad3-related (ATR) dependent DNA-damage response (DDR) pathway on treatment with inter-strand crosslinking (ICL agents) and subsequently cause phosphorylation of checkpoint kinase 1 (CHK1) [43]. It has been previously elucidated that CD133-expressing tumor cells may preferentially activate DNA damage checkpoints in response to radiation and can repair radiation-induced DNA damage more effectively than CD133-negative tumor cells. These findings suggested ATR/CHK1-dependent DNA damage as a likely mechanism of CSC drug- and irradiation-resistance in several tumors [44]. Therefore, CD133 can play a dominant role in establishment of resistance to many curative options. In this regard, many studies attempted to suggest solutions in order to overcome the resistance.

Some studies have been performed in order to overcome drug resistance via targeting CD133 and/or related signaling pathways (Table 3). Chi-Tai Yeh et al. in 2012 revealed that trifluoperazine combined with gefitinib or cisplatin can be a potential anti-CSC agent for non-small cell lung cancer (NSCLC) cell line. Trifluoperazine anticancer properties concord with its ability to suppress CD44/CD133 lung spheroids formation which can down-regulate Wnt/β-catenin signaling pathway. Additionally, trifluoperazine can suppress the stemness-associated expressions such as CD133, c-Myc, and β-catenin, while modulating apoptotic factors including Bax, Bad, Bcl-2, and caspases. It can also cause a significant reduction in cell viability, ALDH-1 activity, and self-renewal of NSCLC spheroids [45]. According to the research conducted by Gallmeier et al. in 2011, the inhibition of ATR function through caffeine and RNA interference may impoverish the tumorigenicity in $CD133^+$ human and xenograft-derived primary colon cancer cells. Likewise, caffeine induced depletion of $CD133^+$ cells that was mediated through direct inhibition of ATR by indirect inhibition of its main effector kinase CHK1 [43]. Additionally, in vitro and in vivo applying of dCD133KDEL (deimmunized *Pseudomonas* exotoxin fused to anti-CD133 scFv with a KDEL terminus) showed a promise for ovarian cancer therapy [46].

In summary, CD133 and preferential activation of many related signaling cascades in CSCs can play a dominant role in establishment of resistance to many curative options. In this regard, many studies attempted to suggest solutions in order to overcome the resistance. In most of preclinical studies, targeting CD133-related signaling pathways has been used for overcoming drug resistance in $CD133^+$ CSCs. In this context, targeting Wnt/β-catenin, ATR-dependent DDR, Notch, mTOR, and PI3K/Akt signaling pathways and also their intermediate molecules led to suppression or down-regulation of these signaling pathways and/or their intermediate molecules (Table 3). So far, considerable combination therapy in preclinical studies and single therapy strategies in clinical trials have not been vastly used for overcoming drug resistance with $CD133^+$ CSCs. Thus, it seems that the combination of nano-, gene-, and chemotherapy approaches can have great potentials to be adopted by preclinical and clinical trials in the future.

Receptor tyrosine kinases

Among the various targets in cancer inhibition, receptor tyrosine kinases (RTKs) have attractive features. Structurally, RTKs consist of an extracellular domain that serves as the ligand-binding region; traverse the cell plasma membrane and transduce extracellular signals into the intracellular sections. Overexpression and mutations of RTKs are known to be involved in pathophysiology of several kinds of cancers [51]. They have been classified into different classes such as

Table 3 Several approaches to overcome $CD133^+$ CSCs

Drugs and methods	Study type	Study model	Endpoint	References
Trifluoperazine	In vitro	NSCLC	Inhibited the tumor growth	[45]
Gefitinib/Cisplatin			Down-regulated Wnt/b-catenin signaling pathway	
Caffeine	In vitro	Human colon cancer cell lines: DLD1, Colo320, RKO and COGA-12 cell	Overcome drug resistance through ATR/CHK1-signaling pathway	[43]
			Activation of the ATR-dependent DNA direct repair	
GSI-18(γ-secretase inhibitor)	In vitro	Medulloblastoma cell lines (DAOY, PFSK, D283Med, and D425Med cell lines)	Suppressed proliferation and inducing apoptosis or differentiation via Notch blockade	[47]
			Blocked xenograft formation with no apparent side effects	
Bispecific antibodies (BiAbs)	In vitro	CD133high colorectal cancer cells	Demonstrated longer and stronger cytotoxic activity	[48]
			Inhibited tumor growth	
Bafilomycin A1 (BafA1)	In vitro	SW1088, T98G and LN229 glioma cell lines	Inhibited V-ATPase	[49]
ATP6V1G1 siRNAs			Targeting Notch, Wnt, or mTOR signaling could represent an attractive novel therapeutic	
dCD133KDEL(an anti-CD133 targeted toxin)	In vitro	NIH:OVCAR5, SKOV3, A2780-s, and MA148 ovarian cancer cell lines	Inhibited the growth of tumor	[46]
Metformin	In vitro	Human hepatocellular carcinoma cell line (Huh-7 cells)	Induced apoptosis	[50]
			Down-regulated PI3K/Akt signal pathway via phosphorylation of Akt	
			Selectively targeting CD133(+) cells	
Caffeine	In vivo	DLD1 xenograft nude mice	Activation of the ATR-dependent DNA direct repair	[43]
			Overcome drug resistance through ATR/CHK1-signaling	
Trifluoperazine	In vivo	NOD/SCID mice	Down-regulated Wnt/b-catenin signaling	[45]
Gefitinib/Cisplatin			Inhibited the tumor growth	
GSI-18(γ-secretase inhibitor)	In vivo	Tumor xenografts in nude mice	Suppressed proliferation and inducing apoptosis via notch blockade	[47]
Bispecific antibodies (BiAbs)	In vivo	NOD/SCID mice	Demonstrated longer and stronger cytotoxic activity	[48]
			Inhibited tumor growth	
dCD133KDEL(an anti-CD133 targeted toxin)	In vivo	NIH:OVCAR5 cells OVCAR5 subline in nude mice	Decreased in ovarian cancer tumor growth	[46]

See the main text for abbreviation definitions

epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and colony stimulating factor-1 receptor (CSF-1R). These have been demonstrated to increase phosphorylation of both Janus family kinases (JAK) and signal transducers and activators of transcription (STAT). JAK/STAT pathway is related to stem cell factor (SCF) signal transduction, but there is little knowledge about it [52]. FMS-like receptor tyrosine kinase 3 (FLT3) is the class 3 of RTK, and its mutations can be the most frequent genetic alterations reported in acute myeloid leukemia (AML). FLT3 mutations may cause some problems in chemotherapy and stem cell transplantation in AML patients [51]. Moreover, targeting other receptor tyrosine kinases including MET and/or its ligand, hepatocyte growth factor (HGF), will be a new strategy to inhibit proliferation and angiogenesis in the high-grade gliomas [53]. Other important signaling pathways involving in CSC drug resistance include PI3K/AKT/mTOR, PI3K/Akt and MAPK, AKT-ERK1/2 and p70 s6k, and EGFR/mTOR pathways.

Dysregulation of tyrosine kinases expression often leads to cell transformation, which is observed in a wide variety of malignancies. Therefore, targeting RTK signaling pathways by tyrosine kinase inhibitors (TKIs) is an intensive challenge for scientists and clinicians (Table 4). Numerous TKIs are being clinically developed to target RTKs, MAP kinase, and PI3K/AKT pathways. Both dual blockade of extra- and intracellular parts of RTKs and/or targeting more RTKs using mAbs and TKIs may decrease drug resistance rate and improve cancer treatment. A combination of trastuzumab and lapatinib in xenografted mice with HER2 over-expressing cells has displayed a considerable prevention from tumor growth and survival rate [54]. Using cetuximab and gefitinib for dual targeting of EGFR has shown the synergic effects on suppression of tumor proliferation and inhibition of drug resistance in colon cancer cells [55]. Furthermore, a combination of trastuzumab and lapatinib had a more significant clinical response [56, 57]. Zhiguang Xiao et al. in 2014 showed a possible molecular mechanism underlying the inhibitory activity of METF/SAL through EGFR targeting that can lead to inhibit EGFR-mediated pro-survival and anti-apoptotic signals by MAPK/ERK and PI3K/AKT pathways in NSCLC cells [58]. Rongxin Dengand et al. in 2013 have currently found a new gamboge derivative, compound 2 (C2), which had suppressive effects on CSCs via EGF/EGFR signaling pathways. In this study, EGF failed to initiate the phosphorylation of EGFR and led to significantly and non-significantly decreased expression of phospho-Akt and phospho-Erk1/2, respectively [59]. Furthermore, Cedric Dos Santos et al. in 2013 demonstrated that the combination of dasatinib with daunorubicin (DNR) inhibited MAPK phosphorylation in AML progenitors and increased p53 activity through PI3K/Akt and MAPK signaling pathways [60].

Concisely, several RTK–TKIs and other inhibitors have been developed, but only a few TKIs have been used in clinical trials or have been approved by authorities for cancer treatment. In this regard, SU5416 (inhibitor of VEGF receptors, c-kit, and FLT3) was applied in AML patients in phase II clinical trial [61]. Moreover, targeting the other members of receptor tyrosine kinases family and/or molecules of related signaling pathways was used in pre-clinical studies using mAb and siRNA (Table 4) [27, 62, 63]. Furthermore, most TKIs are multi-targeted drugs and applying them has several disadvantages such as side effects and the complication of the results interpretation. Therefore, developing more specific/selective TKIs is essential because of current problems. Understanding the effects and characteristics of each TKI in preclinical studies using different animal models and deeper understanding of CSC biology are required for developing specific RTK–TKIs in order to target CSCs and overcome drug resistance.

ABCG2

ABCG2 belongs to ABC transporter family as one of the most common drug resistance mechanism and consists of four domains; two nucleotide-binding and two transmembrane [69, 70]. ABCG2 has an important physiological function in tissue protection against toxins and xenobiotics through drug elimination [71]; however, its expression increases in many types of solid tumors, particularly GI (gastrointestinal), endometrium, and melanoma tumors [71, 72]. Moreover, one of the plausible arguments for drug resistance is the high levels of ABCG2 transporter [73–75] that extrude the therapeutic drugs out of cells. ABCG2 is also associated with chemo-resistance through several critical regulatory pathways such as hedgehog (Hh), PI3K/Akt, and β-catenin/ABCG2 signaling pathways. Therefore, many studies have designed some strategies in order to evade MDR by targeting ABCG2-related signaling pathways and/or employing ABCG2 inhibitors/modulators [76, 77]. Accordingly, Hh signaling pathway has shown to be involved in maintaining high expression levels of MDR1 and ABCG2 in some epithelial cancers [78]. The abnormal expression of Hh signaling-related proteins such as sonic hedgehog and GLI1 can positively enhance ABCG2 up-regulation. Applying cyclopamine-KAAD showed a therapeutic rate in overcoming chemo-resistance in diffuse large B cell lymphoma (DLBCL) via inhibition of Hh signaling pathway [79, 80]. Moreover, β-catenin signaling pathway showed a positive correlation with chemo-resistance and ABCG2 expression. Furthermore, the usage of isoliquiritigenin (ISL) as an ABC inhibitor resulted in inhibition of β-catenin/ABCG2 signaling via direct targeting of 78-kDa glucose-regulated protein (GRP78) and activating of proteasome

Table 4 Targeting RTK and/or molecular of related signaling pathways to overcoming drug resistance in CSCs

Drugs and methods	Study type	Study model	Endpoints	References
Combination of METF and SAL	In vitro	HCC4006, NCI-H1975, and HCC95 (human NSCLC cell lines)	Significant reduction of sphere formation Promoted cell death Suppressed the EGFR signaling pathway accompanied by inhibition of AKT and ERK1/2 phosphorylation	[58]
PTX SU9516, a specific cyclin-dependent kinase 2 (Cdk2) inhibitor	In vitro	Stem-like CD44+/CD24−/low subpopulation isolated from SUM149PT	Restored chemosensitivity Delayed recurrence of inflammatory breast cancer Activation of apoptosis Probably effect on cyclin E/Cdk2 oncogenic signaling	[27]
Dasatinib (Src family tyrosine kinase and c-KIT inhibitor) DNR(daunorubicin) siRNA	In vitro	AML CD34+ cells	Increased the elimination of AML stem cells Increased p53 activity through PI3K/Akt and MAPK signaling pathways	[60]
IKVAV (isoleucine-lysine-valine-alanine-valine) epitope of PA (peptide amphiphile) nanofiber	In vitro	Glioma stem-like cells isolated from primary human gliomas and maintained in stem cell conditions	Induced striking apoptosis via FAK inhibition Increased immobilized b1-integrin at the cell membrane Targeting b1-integrin signaling through FAK	[64]
Anti-RON antibody Zt/c9-directing doxorubicin-immunoliposomes (Zt/c9-Dox-IL) Lapatinib, sunitinib, or dasatinib	In vitro	Triple-positive pancreatic CSCs (CSCs+24/44/ESA)	Reduction the viability of CSCs(+24/44/ESA) RON is a suitable target for CSCs	[65]
UC-961) (ROR1) an anti-ROR1 mAb shRNA silencing of ROR1	In vivo	ROR1-positive (ROR1+) cells isolated from primary tumor-derived xenografts (PDXs)	Impair the self-renewal capacity of CSC cells in vivo Down-modulated ROR1	[62]
c-Met kinase inhibitor PF2341066 (crizotinib) Anti-hepatocyte growth factor (HGF) monoclonal antibody L2G7	In vivo	Tumor-propagating stem-like cells in human GBM xenografts	c-Met pathway inhibitor therapy Tumor growth inhibition Depletion of tumors with sphere-forming cells Inhibition of tumor expression of stem cell markers CD133, Sox2, Nanog, and Musashi	[66]
CK6 (a fully human IgG1 monoclonal antibody)	In vivo	Human xenograft tumor growth in NCI-H526 SCLC and Malme-3M melanoma models	Down-regulated the expression of CD44 gene Inhibited cell proliferation and induce apoptosis Decreased expression of MDR1, BCL2 and CD44 Block c-Kit signaling pathway such as MAPK and AKT	[63]
Compound 2 (C2)(a new gamboge derivative)	In vivo	HNSCC in BALB/C nude mice	Down-regulated of multiple CSC-related molecules Suppressed EGFR tyrosine phosphorylation and blocking the activation of EGF/EGFR signal pathway	[59]
SU5416 (inhibitor of VEGF receptors, c-Kit and FLT3)	Phase II clinical trial	45 patients with myeloproliferative disorders	Minimal clinical activity in patients	[67]
SU5416	Phase II clinical trial	33 patients with AML	Modulated RTK phosphorylation in some patient Decreased FTL3 level	[61]
Sunitinib malate (SM) Figitumumab	Phase I clinical trial	11 patients with progressive metastatic solitary fibrous tumor	Antitumor activity in SFT, possibly through a PDGFRB-mediated mechanism	[68]

See the main text for abbreviation definitions

degradation in CSCs. In addition, ISL could down-regulate the β-catenin/ABCG2 pathway through activation of glycogen synthase kinase 3 beta (GSK3β) and inhibition of Akt, and consequently led to increase β-catenin phosphorylation and proteasome degradation [77]. As aforementioned, PI3K/Akt signaling activation has been considered as one of the main causative factors underlying cancer progression. A recent study showed that peroxisome proliferator-activated receptor (PPAR) agonists can be described as a potential agent for overcoming drug resistance through up-regulating of PTEN and inhibition of the PI3K/Akt pathway. PPAR agonists may also play a role in driving the internalization of ABCG2 to cell cytoplasm and may inhibit MDR [76]. PTEN inactivation has been found in various cancers and has been observed to be accompanied by tumor progression [81]. According to another study, tunicamycin combined with cisplatin was used in hepatocellular carcinomas to overcome drug resistance via targeting dolichyl-phosphate *N*-acetyl glucosamine phosphotransferase 1 (DPAGT1), Akt, and ABCG2 pathways. Additionally, blockage of drug efflux employing ABCG2 inhibitors or modulators seems to be the feasible strategy to restore drug sensitivity in MDR cancer cells [47]. A previous study demonstrated that the suppression of adenine nucleotide translocase 2 (ANT2) by short hairpin RNA (shRNA) can inhibit the migration and the invasion of SK-BR3 (HER2/neu-overexpressing human breast cancer cells) through suppression of HSP90's (heat shock protein 90) function and inhibition of PI3K/Akt signaling pathway [82]. Furthermore, Jang et al. in 2012 revealed that knockdown of ANT2 by adeno-shRNA virus is a useful strategy to induce cell death and the chemosensitivity of MCF7, MDA-MB-231, and MCF10A stem-like cells to doxorubicin by down-regulation of ABCG2 [83]. Moreover, ABCG2 mAb plus PTX-loaded Fe_3O_4 NPs induced the therapeutic response in multiple myeloma in non-obese-diabetic/severe-combined-immunodeficiency (NOD/SCID) mouse model [84]. Furthermore, a novel specific ABCG2 inhibitor, PZ-39 (*N*-(4-chlorophenyl)-2-[(6-{[4,6-di(4-morpholinyl)-1,3,5-triazin-2-yl] amino}-1,3-benzothiazol-2-yl) sulfanyl] acetamide), showed a beneficial treatment effect by inhibiting ABCG2 activity and accelerating lysosome-dependent degradation in cancer cells over-expressing ABCG2 [85].

Briefly, ABCG2 can play a key role in MDR and protection of CSCs. Thus, ABCG2 can be an ideal target for development of chemo-sensitizing agents for better treatment of drug-resistant cancers and helping eradicate CSCs. Accordingly, many studies have designed the strategies in order to suppress MDR through targeting ABCG2 related signaling pathways such as Hh, PI3K/Akt, and β-catenin/ABCG2 and/or employing ABCG2 inhibitors/modulators (Table 5). In this regard, ISL and PPARγ agonists were used to overcome drug resistance via targeting β-catenin/ABCG2 and DPAGT1/Akt/ABCG2 signaling pathways, respectively [76, 77]. Moreover, the new specific ABCG2 inhibitors (e.g., PZ-39), gene therapy approaches, NPs, and ABCG2 mAb combination were used in preclinical studies (Table 5). In this context, knockdown of ABCG2 and ANT2 was applied in preclinical studies for ABCG2 inhibition as gene therapy methods through ABCG2 siRNA and adeno-shRNA virus [83, 86]. In addition, the combination of NPs-based approaches with gene- and chemotherapy agents was applied for targeting ABCG2 and overcoming drug resistance [84, 87]. However, overcoming cancer-drug-resistant agents and their signaling pathways via targeting ABCG2 has not been vastly used in clinical trials. It seems that the combination of nano-, gene-, and chemotherapy approaches can have a great potential to be adopted by clinical trials in the future.

Aldehyde dehydrogenase

Aldehyde dehydrogenase (ALDH), a multifunctional enzyme with 11 families and 4 subfamilies, is widely distributed in tissues and can catalyze the oxidation of endogenous and exogenous aldehydes to their equivalent carboxylic acids [92, 93]. Among ALDH superfamily, two isoenzymes (ALDH1A1 and ALDH3A1) have been shown to be eligible markers for identification between normal cells and CSCs. A previous study demonstrated that in comparison with differentiated cells, human hematopoietic stem cells are characterized by high levels of ALDH1 expression [94]. High ALDH1A1 or ALDH3A1 activity was also reported to be attributable to CSCs' aggressiveness through conferring drug resistance [95]. Moreover, high activity of ALDH1 was detected in various cancer types that assumed to be responsible for lower overall survival in patients with ALDH1-positive tumors compared to the ALDH1-low patients [95, 96]. ALDH via retinoid signaling pathways can play a key role in regulation of gene expression and cell differentiation. It is one of four retinoid dehydrogenases involved in the process of retinoid acid synthesis which mediates transcription of different sets of genes controlling growth and development in CSCs [97, 98]. In addition, ALDH1 is transcriptionally activated in a c-Jun-dependent manner through a pathway consisting of RhoA, MAP kinase-kinase-4, and Jun N-terminal kinase (JNK) [99]. Furthermore, application of specific ALDH1 inhibitors such as 4-(diethylamino)benzaldehyde (DEAB) or siRNA can abate the intrinsic retinoic acid by decreasing cEBPε (RAR-specific response gene) that can be a promising curative option, especially in patients with high ALDH activity [94, 96]. In this regard, treatment of Lovo-1- and K1-resistant cells with ALDH1A3 siRNAs or DEAB showed to be an effective strategy for sensitizing these cells to Y15 [an inhibitor of focal

Table 5 Various approaches for targeting ABCG2 to overcome drug resistance in CSCs

Drugs and methods	Study type	Study model	Endpoints	References
PPARγ agonists (telmisartan, pioglitazone, and rosiglitazone)	In vitro	MCF-7	Inhibited the PI3K/Akt pathway Upregulated PTEN	[76]
ABCG2 siRNA PEAL NPs	In vitro	MCF-7/ADR and MCF-7/S	Induced the therapeutic response via silencing ABCG2	[87]
Dox Adeno-shRNA virus	In vitro	MCF7, MDA-MB-231, and breast epithelial cells (MCF10A)	Induced cell death Increased the chemosensitivity of Dox Down-regulation of ABCG2 and knock-down of ANT	[83]
Isoliquiritigenin (ISL)	In vitro	MCF-7, MDA-MB-231, and BT-549 cell lines	Down-regulated β-catenin pathway Inhibited β-catenin/ABCG2 signaling by activating the proteasome degradation pathway	[77]
PZ-39	In vitro	MCF7 cells	Inhibition of ABCG2 activity Acceleration of lysosome-dependent degradation	[85]
DOX PTX Cisplatin, −ABCG2 siRNA	In vitro	MCF7 cells	Enhanced chemosensitivity via Targeting ABCB1 and ABCG2	[86]
Sorafenib	In vitro	K562/A02 cell line	Inhibition of ABCG2 Accelerated ABCG2 lysosome-dependent degradation	[88]
Tunicamycin/Cisplatin	In vivo	MHCC-97L and Huh7 cells xenografts in mice	Targeting the DPAGT1/Akt/ABCG2 pathway Reduced the expression levels of several CSC markers Suppressed the tumorigenicity	[89]
Isoliquiritigenin (ISL)	In vivo	MDA-MB-231 xenograft NOD/SCID mice	Chemosensitize breast CSCs via the GRP78/β-catenin/ABCG2 pathway	[77]
PTX ABCG2 mAb–Fe_3O_4 NPs	In vivo	Xenograft MM CSCs NOD/SCID mouse model	Induced the therapeutic response	[84]
Pioglitazone Rofecoxib Trofosfamide	Phase I clinical trial	Malignant vascular tumors (angiosarcomas and hemangioendothelioma)	Median progression-free survival was 7.7 months Side effects were mild	[90]
GW572016 (lapatinib)	phase I/II clinical trial	Patient with multiform glioblastoma	No significant activity in patients –	[91]

See the main text for abbreviation definitions

adhesion kinase (FAK) and ALDH1A3 up-regulator] and consequently inhibition of cancer cell growth [100]. Moreover, an in vivo study demonstrated that applying copolymer of poly(-ethylene glycol) with poly (D,L-lactide) ($MPEG_{5K}PLA_{11K}$) NPs loaded with low-dose decitabine (DAC) combined with $MPEG_{5K}PLA_{11K}$ NPs and DOX had a promising outcome in tumor suppression and growth inhibition of MDA-MB-231 cells ($ALDH^{hi}$ CSC) [101]. Co-treatment with UCN-01, a checkpoint kinase 1 (Chk1) inhibitor, and all-trans retinoic acid (ATRA) as ALDH inhibitor represented a promising pharmacological targeted strategy that can significantly sensitize CSCs to photon or carbon ion radiation and induce cell death in SQ20B (radio-resistant head and neck squamous cell carcinoma) [102]. Furthermore, thermo-chemotherapy platform with salinomycin-loaded gold nanorods (AuNRs) revealed a competent strategy for reduction of MCF-7 ($ALDH^+$) cells subpopulation [103].

In summary, several studies have been designed to overcome drug resistance in ALDH-positive cells due to ALDH1 multiple functional roles in normal cells and CSCs. Targeting ALDH as an important CSC marker can be promising in cancer treatment using pharmacological agents (e.g., DEAB), molecular targeting (e.g., siRNA), and also nanotechnology approaches (Table 6). In preclinical studies, applying ALDH inhibitors such as DEAB and ATRA have beneficial effects on $ALDH^+$ CSC treatment. Moreover, $MPEG_{5K}PLA_{11K}$ and AuNRs NPs were used as nanocarriers and showed a promising outcome in tumor suppression and growth inhibition of $ALDH^+$ CSCs. Additionally, new thermo-chemotherapy platform with NPs provides a new combinatorial strategy for effective inhibition of radio-resistant $ALDH^+$ CSCs [103]. Among these approaches, ATRA in combination with cyclophosphamide have been applied for treatment of patients with acute promyelocytic leukemia in clinical trial and relatively

Table 6 Several approaches to overcome drug resistance in ALDH positive cells

Drugs and methods	Study type	Study model	Endpoints	References
Salinomycin	In vitro	Breast cancer stem cells (BCSCs)	Down-regulated cyclin D1 and increased p27(kip1) nuclear accumulation Down-regulated the transcription factors Nanog, Oct4 and Sox2	[106]
Histone deacetylase inhibitor (LBH589)	In vitro	ER-positive aromatase-overexpressing MCF-7 and T47D cell lines	Induced apoptosis Arresting the cell cycle G2/M Regulating epithelial-mesenchymal transition (EMT) Reduced the level of NF-kB1	[107]
Dickkopf-1 (Dkk-1)	In vitro	Osteochondral sarcoma line MOS-J	Inhibited tumor growth and expression of stem cell markers Probably effect on Jun-mediated Wnt pathways	[99]
Ellipticine PTX	In vitro	MCF7 cell line and SUM159 cell line	Reduced the proliferation and self-renewal ability Reduced the formation of mammospheres	[108]
Salinomycin TAE684 ZSTK474(PI3K inhibitor) BH3 mimetic ABT-263	In vitro	H3122 cells and H2228 NSCLC lines	Inhibited expression of the CSC marker ALDH1 Blocked the development of EGFR and/or HER2 Effect on PI3K-AKT-mTOR signaling pathway	[109]
LBH589	In vivo	MCF7aro xenograft Balb/c models	Reduced the level of NF-kB1 Inhibited tumor growth	[107]
Salinomycin TAE684 ZSTK474(PI3K inhibitor) BH3 mimetic ABT-263	In vivo	H3122 Luc cells xenografts NOD mice	Improved treatment with modulating drug resistance	[109]
SAL	In vivo	MDA-MB-231-xenografts in mice	Reduction in tumor growth via down-regulation of ALDH1 and CD44 levels	[106]
Decitabine ATRA Valpronic acid	Phase II clinical trial	Elderly patients with AML	Improved overall response and survival	[110]

See the main text for abbreviation definitions

improved disease-free and overall survival in the patients [104]. Moreover, combination of tamoxifen with ATRA demonstrated an acceptable toxicity in a small phase I/II trial of an advanced breast cancer patient [105]. Thus, it seems that understanding of the pathways associated with ALDH family in CSCs and designing new approaches for overcoming drug resistance in CSCs will improve the current cancer treatment.

Epithelial cell adhesion molecule

Epithelial cell adhesion molecule (EpCAM; also called CD326, ESA, EGP-2, or TROP-1) belongs to type 1 membrane glycoprotein family which is an important CSC marker [111]. It consists of an extracellular (EpEx), a transmembrane (EpTM), and an intracellular (EpICD) domain. EpCAM is a tumor-associated antigen and its over-expression has been reported in epithelial tumors such as breast cancer and retinoblastoma [112, 113]. Moreover, EpCAM is involved in metastasis of adenocarcinoma and breast cancer and can play an essential role in oncogenic signaling pathways through its proteolysis and EpICD translocation into the nucleus [114, 115]. Proteolysis of EpCAM causes the complex formation of EpICD with FHL2 and β-catenin that can consequently modulate transcription of their target genes [114]. Moreover, EpICD may positively regulate SOX2, OCT4, and NANOG transcription factors which may contribute to self-renewal and pluripotency of cancer cells [116]. Therefore, it seems that EpCAM can be as an immunotherapeutic target for the treatment of most abundant cancers (Table 7). Various anti-EpCAM therapeutic antibodies as single or in combination therapies have been developed in (pre)clinical studies over the past 30 years [117, 118]. In this regard, a previous study showed that a bispecific EpCAMxCD3 antibody (BxPC-3)

Table 7 Various approaches for targeting EpCAM to overcome drug resistance in CSCs

Drugs and methods	Study type	Study model	Endpoints	References
RNA aptamer	In vitro	MCF-7, SW480 T47D HT-29, MDA-MB-231	Improved treatment and molecular imaging of tumor	[10]
EpCAM aptamer siRNA EpCAM chimera siEpCAM chimera (EpApt-siEp)	In vitro	WERI-Rb1-RB cell l ine and MCF7-breast cancer cell line	Reduced cellular proliferation Induced cytotoxicity Repression SOX2, OCT4, NANOG, and CD133 by knockdown EpCAM	[122]
3-17I (a novel human EpCAM-targeting monoclonal antibody) Saporin Photosensitizer TPCS2a (Amphinex)	In vitro	EpCAM-positive human cancer cell lines MCF7 (breast), BxPC-3 (pancreas), WiDr (colon), and the EpCAM-negative COLO320DM (colon)	Reduced strongly cell viability, proliferation, and colony-forming capacity	[118]
EpApt-siEp	In vivo	MCF7 xenograft mice model	Tumor growth regression without any toxicity in animals Down-regulation of EpCAM, MRP1, ABCG2, stathmin and survivin Caused apoptosis by intrinsic pathway with minor alteration in cytokines Up-regulation of ATM	[122]
RNA aptamer	In vivo	MCF-7/Adr xenograft mice	Induced apoptosis Suppressed tumor growth Prolonged survival	[123]
EpCAMxCD3 (bispecific EpCAMxCD3 antibody)	In vivo	BxPC-3 pancreatic carcinoma xenografts NOD SCID mice	Decreased growth of tumor Inhibited cell proliferation Induced apoptosis	[119]

See the main text for abbreviation definitions

can improve the immune response and treatment outcome in in vitro and in vivo pancreatic cancer [119]. Another anti-EpCAM antibody, MOC31, has extensively been studied for tumor targeting in various drug delivery systems [120]. Suggested mechanisms of anti-EpCAM therapeutic antibodies can involve in antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated cytotoxicity (CDC). So far, a few anti-EpCAM mAbs such as MT201 (adecatumumab), adecatumumab, ING-1, 3622W94, and chimeric edrecolomab have been demonstrated under clinical investigations in patients with prostate and breast cancer [111, 117]. ING-1 and 3622W94 have also demonstrated a much higher affinity in comparison with adecatumumab and edrecolomab [111, 117]. Moreover, these antibodies have increased lysis of EpCAM-expressing cancer cells via both ADCC and CDC. In addition, the chimeric version of edrecolomab with a human Fcγ1 domain was more potent in ADCC compared to murine IgG2a version. Furthermore, among these anti-EpCAM mAbs, only adecatumumab displayed a significant prevention from MCF-7 proliferation in the absence of complement proteins and the other immune cells [111, 117]. Besides mAb, aptamers as synthetic oligonucleotides (RNA/ssDNA) or peptide molecules are applied to overcome drug resistance in EpCAM-expressing CSCs. These molecules bind to a specific target (e.g., EpCAM) with high affinity due to their three-dimensional structures [121, 122]. Several aptamer–siRNA chimerization strategies were applied for targeting EpCAM in CSCs [10]. For example, EpCAM RNA aptamer–EpCAM siRNA chimera (EpApt-siEp) showed a high antitumor activity without any toxicity in EpCAM-positive cells and xenograft model with MCF7 cells [122]. So, applying of anti-EpCAM mAbs and aptamers can be a useful method to overcome cancer drug resistance in EpCAM-positive CSCs.

Conclusions

Tumors generally develop a significant resistance to repeated treatments with one/many kinds of anticancer agents, and so, cancer drug resistance can be a major obstacle in the efficacy of chemotherapy agents [124]. Here, most of the cancers are resistant to the chemotherapeutic drugs due to CSC population which ultimately result in tumor relapse [2]. CSCs are a small subpopulation of cells within multiple malignancies with capabilities of self-renewal, dedifferentiation, tumorigenicity and inherent chemo-and-radio therapy resistance [3]. A number of CSC markers including CD44, CD133, RTK, ALDH, EpCAM/ESA, and ABCG2 have been proved as useful targets for defining CSC population in solid tumors (Table 1) [9,

10]. Therefore, selective tumor targeting by these CSC markers with new therapeutic strategies will ultimately improve most malignancies. Currently, the new treatment methods such as antibody-directed therapies and NPs-based targeting of CSC-specific markers or their signaling pathways are available or under investigation (Fig. 1). In the present study, we focused on the association between CSC markers and several related signaling pathways in tumor cell survival and multi-drug resistance. Applying nanotechnological approaches has gained an immense popularity for targeting CD44, ABCG2, and ALDH due to their potential therapeutic capabilities. NPs can also be conjugated with tumor-targeting molecules, siRNA, and antibodies with the purpose of combination therapies in order to overcome multidrug resistance [11, 125]. Moreover, knock-down of CD44, CD133, ALDH, and ABCG2 was applied in pre-clinical studies through siRNA and adeno-shRNA viruses as the gene therapy methods [83, 86]. Furthermore, mAbs were applied to overcome drug resistance for CSC markers including CD133, EpCAM/ESA, and ABCG2. Among mAbs applications, few anti-EpCAM mAbs such as MT201 (adecatumumab), ING-1, 3622W94, and chimeric edrecolomab have demonstrated clinical potential and are currently under clinical investigation in patients with prostate and breast cancer [111, 117]. Among treatment methods, combination of nano-, immuno-, gene-, and chemotherapy approaches seem to have a great potential to be adopted by preclinical and clinical trials in the future. Moreover, understanding of the pathways associated with CSC markers can be helpful for designing new approaches for overcoming drug resistance in CSCs in the future.

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Compliance with ethical standards

Conflicts of interest The author(s) report no conflicts of interest and are responsible for the content of the paper.

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