Nanomedicine in Cancer Stem Cell Therapy

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Piyush Kumar Gupta, Gunasekaran Dharanivasan, Ranjita Misra, Santosh Gupta, and Rama Shanker Verma

Abstract

It is now well established that most of the tumors are heterogeneous in nature that comprise a population of cancer stem cells (CSCs) and differentiated cancer cells. Like normal stem cells, CSCs have also self-renewal, proliferation, and differentiation capacities that are responsible for the development of drug resistance and relapse. Therefore, targeting CSCs is essential for the elimination of tumor recurrence condition. Although several anti-CSC therapeutics have been used in clinics, they are found to have limited efficacy due to poor solubility, lesser stability, and short circulation time in the blood. Therefore, tools in nanomedicines are being used to tackle these limitations. Recently, nanodrug carriers have been used to target CSCs and somewhat eliminate drug resistance by targeting CSC metabolism, inhibiting drug transporters, disturbing CSC survival pathways, etc. Even with these progress, the challenges for targeting CSCs by nanomedicines still remain and open up plenty of space for further development and improvement in synthesizing drug carriers with higher efficacy. In this chapter, we summarize about CSCs and their biological characterization toward resistance, then discuss several anti-CSC therapeutic approaches based on nanomedicines in the current state of research and development, and finally overview their future directions.

Keywords

Nanotherapy · Cancer therapy · Biomarkers · Drug resistance · Stem cells

P. K. Gupta · G. Dharanivasan · R. Misra · S. Gupta · R. S. Verma (✉)
Stem cell and Molecular Biology Laboratory, Bhupat and Jyoti Mehta school of Biosciences, Department of Biotechnology, Indian Institute of Technology-Madras, Chennai, Tamilnadu, India
e-mail: vermars@iitm.ac.in

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4.1 Introduction

Many historical studies have established that malignant cancers contain a subpopulation of rare cells that display self-renewal, proliferation, and differentiation capacities in new cancer cells, which are called cancer stem cells (CSCs) or tumor-initiating cells. These cells are responsible for metastasis, drug resistance, and tumor recurrence condition (Gupta et al. 2009). Like multidrug-resistant cells, CSCs also display the same phenotypic feature including overexpression of ABC transporters, metabolism reprogramming, and activation of survival pathways (Dean et al. 2005). As the presence of CSCs has been seen in many malignant tissues like the breast, brain, lung, colon, pancreatic, etc., that was concluded with the xenotransplantation of primary tumor into mice. Also, the treatment of tumor with conventional methods like chemotherapy, radiotherapy, and targeted therapy results in an increase of CSC fraction by which tumor cells survive and lead to the metastasis at distant sites (Ma et al. 2008). During the treatment cycles of chemotherapy, tumor recurrence condition is observed due to the presence of resistant CSCs. If these CSCs are targeted with different therapeutic modalities, then tumor-relapsed condition can be eliminated. Generally, tumors exhibit plasticity that means two types of tumor cell population, i.e., CSCs and non-CSCs. If CSCs are eliminated without killing non-CSCs, then complete cure cannot be seen. Therefore, there is a need for more preclinical and clinical studies to understand the CSC response during therapy.

Currently, several effective therapeutic agents are available to target and kill CSCs. Most of them are chemo- or radiotherapy drugs, therapeutic nucleic acids, targeted monoclonal antibodies, or small molecular inhibitors. In clinic, the therapeutic efficacy of these agents has decreased due to several limitations like lesser stability, poor water solubility, nonspecific biodistribution, short circulation time, or off-target effects (Chen 2010). Therefore, nanotechnology-assisted drug delivery systems, i.e., nanomedicine, have gained the significant attention to overcome these limitations (Davis et al. 2008; Rink et al. 2013). Usually, nanomedicines can be loaded with high payload of single or multiple drugs by controlling their size and surface properties. As a result, the pharmacokinetic and pharmacodynamic properties of nanomedicines have improved by reducing their side effects on healthy cells. In current state, the clinically approved anticancerous nanomedicines are Doxil (doxorubicin-encapsulated liposomes), Oncaspar (PEG-L-asparaginase), and Abraxane (albumin-paclitaxel conjugate). In current settings, several multifunctional nanoparticles have designed their cancer theranostic applications under the special consideration of CSC targeting (Sun et al. 2014a). Further, several proofs of concept studies have been designed to tackle CSC's associated challenges. Some of them have displayed inspiring results previously. For example, codelivery of both doxorubicin (Dox) and all-trans retinoic acid (ATRA) was carried out using polymeric nanoparticles for eliminating human breast CSCs with drug-resistant cancer cells and exhibited improved anticancer therapy compared to free agents (Sun et al. 2015). Also, SignPath Pharmaceutical Company developed curcumin-loaded nanodrug carriers called NanoCurc™ which significantly inhibited the growth of glioblastoma by reduction of CD133+ CSCs (Lim et al. 2011). In this setting, several CSC-targeting

nanomedicines have been developed, and their efficacy was evaluated in various preclinical studies. However, many clinical challenges have to be addressed before their use in clinics. In this chapter, we briefly described about CSCs and their biological processes in the background of drug resistance, followed by brief discussion on CSC-targeting nanomedicine approaches in the context of delivery of different types of therapeutic agents. We also emphasized the future directions of anti-CSC nanomedicines including the consideration of most innovative therapeutic strategies and the development of highly efficient nanodrug carriers.

4.2 CSCs and Drug Resistance

4.2.1 CSCs and How Does It Lead to Drug Resistance or Tumor Recurrence Condition??

CSCs are well known to have many distinct properties such as self-renewal capacity, proliferative capability, and resistance to apoptosis (Vinogradov and Wei 2012). Furthermore, several studies have already proved that CSCs are associated with high invasiveness and metastasized tumorigenic potential leading to drug-resistant condition for the current conventional therapies in clinic (Liu et al. 2010). Therefore, CSCs have become an important target for the success of potential therapeutic approach in translational cancer research.

Currently available treatment modalities are able to kill the cancer cells only but are unable to eliminate the critical CSCs that are present in tumor cell population which escape by pertaining some specific resistance mechanisms. This survival of CSCs leads to disease relapse by developing tumors which are more malignant, highly invasive, and resistant to chemo- and radiotherapy. According to the traditional view, cancer cells lead to the development of a small population of drug-resistant cancer cells with repeated chemotherapeutic treatment that could result to the inactivation of drugs, alterations in drug targets, and reduced drug accumulation inside the cancer cells (Niero et al. 2014).

However, the current CSC concept states that the relapse condition is mainly contributed by the intrinsic and acquired resistance mechanisms of the CSC population, present in cancer cell mass. Moreover, the CSCs are drug resistant due to several other factors such as the tumor microenvironment which normally contains different kinds of proteins including growth factors and cytokines that could help in the activation of CSC survival pathways and the possible role of chemo- or radiotherapy to enhance the stemness property by converting cancer cells into CSCs. Recently, it was reported that the irradiation of breast cancer cells leads to increased number of CSC population and also found that some noncancerous cells attained the CSC phenotype (Atena et al. 2014). Studies have shown that human gastric cancer cell lines with 5-fluorouracil (5-FU) treatment become resistant in longer time and acquire the stem cell features such as stemness, tumorigenicity, and self-renewal capacity (Xu et al. 2015a). In this background, the involvement of CSCs in metastasis, tumor progression, drug resistance, and relapse was shown in Fig. 4.1.

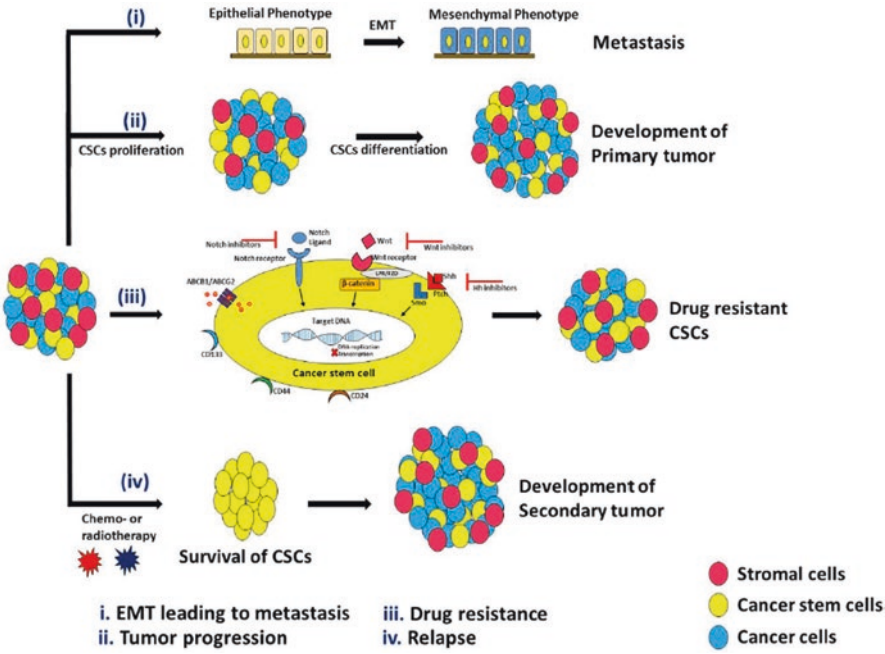

Fig. 4.1 Involvement of CSCs in metastasis, tumor progression, drug resistance, and relapse. **(i)** CSCs generally led to the local and distant metastasis via epithelial-mesenchymal transition (EMT) program; **(ii)** CSCs drive tumor progression using self-renewal, proliferation, and differentiation properties; **(iii)** CSCs develop multiple drug-resistant mechanisms to protect itself from conventional cancer treatment, leading to enrichment of CSCs within tumors; and **(iv)** CSCs have the capability to proliferate and differentiate after the success of initial treatment, leading to the development of relapsed condition

4.2.2 CSC Isolation and Characterization

It is well known that CSCs have different functions and phenotypic features from non-stem cancer cells (Abbaszadegan et al. 2017). These diverse features led to the development of several assays to isolate and characterize the CSCs. The CSCs can be identified by their fundamental properties such as self-renewal and lineage capacity. Moreover, these CSCs could be characterized by more specific phenotypic surface markers such as CD34+/CD38− in leukemia cells, CD44+/CD24− in solid tumors, or CD133+ in other tumors (Dragu et al. 2015). CD44 binds to a glycosaminoglycan (hyaluronic acid) which is present in extracellular matrix that helps in the CSC attachment contributing to proliferation and migration of the stem cells. It has been reported that the higher expression of CD44 is strongly associated with therapeutic drug resistance (Goodison et al. 1999). Similarly, CD133 is found to be highly expressed on CSCs of several cancers of different tissue origin that increased drug resistance. Thus, targeting of CD44 and CD133 molecules can be used to deliver chemotherapeutic drugs to eliminate CSCs.

CSCs can be isolated mainly by long-term cell culture using FACS (fluorescence-activated cell sorting) and MACS (magnetic-activated cell sorting) techniques. FACS can be used for CSC isolation and enrichment based on the expression of some specific cell surface markers such as CD24, CD34, CD44, and CD133. MACS is considered a standard method for CSC isolation based on specific stem cell markers as this technology aids in isolation of high-quality cells. At first, the cell surface markers are labeled with monoclonal antibody (mAb) or magnetic microbeads; then isolation is carried out. In the next step, marked cells are separated by positive selection removing the unmarked ones. Thus, isolation of target cells from a cell suspension can be efficiently done by one of the best and most direct methods called positive selection method. As mentioned above, CSCs express a set of cell surface markers that can be used for detection and separation by positive selection method.

One more functional method for CSC isolation and characterization includes colony-forming unit (CFU) assay. This quantitative and high-throughput method is believed to be analogous for in vivo transplantation. CFU assay is used to analyze the pattern of CSC proliferation and differentiation by their ability to form colonies in a semisolid medium. These colonies are formed by a particular number of input cells, which give the basic information about the proliferation and differentiation potential of CSCs. In brief, CSCs are non-adhesively cultured in serum-free medium supplemented with growth factors which can develop into tumorspheres. Usually cancer cells undergo anoikis (a suspension-induced apoptosis) in these conditions, whereas CSCs can survive and form tumorspheres on the colony basis. These tumorspheres exhibit higher CSC portions than the original tumor cells.

Similarly, CSCs can also be isolated based on the overexpression of drug efflux transporters specifically BCRP or ABCG2. For example, cell populations which efflux Hoechst 33342 dye maintain CSC properties in a variety of cancers, and presently this method is the most widely used for the isolation of CSCs.

Overall, currently a good set of tools is available to study the CSCs. A variety of in vitro assays must be used in combination, and due to their cost-effectiveness and high efficiency, these methods are very useful in the beginning of the study, and the results have to be carefully validated in vivo.

4.2.3 Dysregulated Pathways in Cancer Stem Cell's Survival

In quiescent stage, self-renewal and maintenance are key features of normal stem cells and CSCs. The cellular physiology of CSCs are regulated by different cellular signaling pathways in their tumor microenvironment. The regulation of such signaling pathways like Notch, Wnt, and Hedgehog plays an important role in regulating self-renewal, proliferation, and differentiation property of stem cells (Matsui 2016). The dysregulation of such pathways has been proved to attribute to the CSC growth, metastasis, and emergence of drug-resistant condition during cancer treatment. The dysregulation happens due to mutation or abnormal activation of such pathway's genes. For example, Notch pathway is one of the conservative signaling pathway of

multicellular organisms that is linked to self-renewal and CSC survival. The dysregulation in Notch signaling has led to the development of various types of cancers such as breast cancer and glioblastoma. The Notch protein also contributes to drug resistance, because decrease in Notch expression leads to enhanced drug sensitivity of cancer cells, inhibiting tumor regrowth, and reduces migration and invasion of cancer cells (Wang et al. 2012a).

Normally, the activation of Notch signaling initiates with the binding of a transmembrane ligand to the Notch transmembrane receptor (NOTCH1/NOTCH2/NOTCH3/NOTCH4) on a neighboring cell. This leads to proteolytic cleavage of the Notch receptor, thereby releasing constitutively active intracellular domain of NOTCH (NICD) which further translocates into the nucleus, where NCID binds to transcription factors CSL (CBF1/RBPJ/suppressor of hairless/Lag-1) and coactivator to activate the transcription of Notch-responsive genes. The development of Notch pathway targeting therapeutics is a primary focus for inhibition of CSC growth. Monoclonal antibodies like demcizumab (OMP-21 M18), OMP-52 M51, and OMP-59R5 are designed to target Notch pathway and currently being used in clinical trials for inhibiting CSC growth (Smith et al. 2014). Furthermore, the direct link of Notch pathway was established with the metastasis potential of CSCs. Gemcitabine-resistant pancreatic cancer cells overexpress Notch-2 and its ligand Jagged-1 that helps in maintaining EMT and acquisition of the CSC phenotype. EMT is a crucial process in which CSCs move from tumor lesions into the blood, whereas the opposite process of transition of mesenchymal epithelium was believed as the main mechanism of invasion of CSCs into the healthy organs. It has been investigated that disruption in Notch signaling by using siRNA leads to reversal in the EMT phenotype partially. Therefore, Notch signaling activation and progression of EMT can be directly related to the resistance to gemcitabine in pancreatic cancer. Thus, the inhibition of Notch pathway could be a potent approach for overcoming drug resistance and metastasis in clinic.

Wnt signaling is also one of the important pathways that plays a vital role in embryogenesis and development of cancer. The Wnt proteins act as growth factors that help in the normal stem cell maintenance and proliferation. Mutations in Wnt/β-catenin pathway lead to the development of various types of cancers including leukemia, colon, epidermal, breast, and cutaneous carcinoma (Polakis 2012). Moreover, Wnt/β-catenin signaling pathway also plays a vital role in ABCB1/MDR-1 transcription factor-driven colorectal carcinogenesis (Correa et al. 2012). The dysregulation of such signaling pathway involves in the chemoresistance of pancreatic cancer (Cui et al. 2012). Studies demonstrated that silencing of Wnt activity using siRNA against β-catenin was able to efficiently inhibit the proliferation and drug resistance in lung cancer cells (Cai et al. 2017). Similarly, inhibition of Wnt activity leads to the reversal of 5-fluorouracil resistance in colon CSCs (Deng et al. 2010). Recently, studies have demonstrated that the Wnt pathway is associated in the maintenance of stemness such as the self-renewal capacity, and heterogeneity of breast CSCs is promoted by proliferating cell nuclear antigen-associated factor (PAF) (Wang et al. 2016). Further, the latent competent cancer cells have been isolated from lung and breast carcinoma cell lines and demonstrated

that Sox2 or Sox9 expression induces DKK1 (a natural Wnt inhibitor) allowing the cells to enter a quiescent state. This results in lower expression of natural killer (NK) cell ligands and weak innate immunity, thereby conferring CSCs with the quiescent state for a long time (Malladi et al. 2016).

Similarly, activation of the Hedgehog (Hh) signaling pathway leads to the development of various types of cancer, such as basal-cell carcinoma, breast, brain, and pancreatic tumors. Hh signaling maintains the self-renewal property in glioblastoma, breast, and myeloma stem cells. Several studies have reported the role of the Hh pathway in the development of metastasis particularly through the EMT initiation via activation of some protein expressions such as MMP-9 (matrix metalloproteinase 9) and E-cadherin. In inactive state during the absence of Hh, smoothened (Smo) gets inhibited by Ptch1, a transmembrane receptor. During an active state, Hh is secreted by the adjacent cells; thereby, the Smo will be activated by the Ptch receptor. After Smo activation, Gli1/2 is to be translocated into the nucleus, which then leads to activation of h-associated genes. It has been reported that high expression of Hh signaling molecules such as Smo and Gli1 was attributed to the tamoxifen resistance in breast cancer (MCF-7 and T47D) cells (Villegas et al. 2016). Moreover, breast, colon, and pancreatic CSCs have shown sensitivity to the Hh pathway inhibitors. Numerous compounds targeting Hh pathway have demonstrated potential efficacy in preclinical studies and are now in phase I and II clinical trials (Takebe et al. 2015).

4.2.4 Molecular and Cellular Therapeutic Targets (Biomarkers) in Drug-Resistant CSCs

Current therapeutic strategies against cancer such as chemo- and radiotherapy have multiple limitations that frequently result into treatment failure and relapse in cancer patients. These therapies are not specific to target CSCs leading to toxicity in healthy tissues; thereby, the risk of disease relapse or recurrence increases in patients. Thus, CSC elimination is very crucial for preventing tumor relapse. Recently, multiple novel strategies have been investigated with the specific aim of killing CSCs and altering their niche. These specific targets include both slight differences in surface marker expression and altered signaling pathways. Several studies have concentrated on dysregulation of signaling pathways in CSCs to develop a new and advanced approach for cancer treatment. This way of finding might be promising because most of the cancers are associated with dysregulation of the same signaling cascades. In this context, CSCs can be diagnosed by expression of surface markers but also by the signals sent by them to the tumor microenvironment (Dragu et al. 2015). Investigators often use the surface markers as important targets for therapy. They choose the ligands or antibodies for surface markers and used them as an adjunct to chemotherapy, radiotherapy, and surgery. Most importantly, monoclonal antibody development is highlighted in targeting CSCs. At the moment, some therapeutic strategies are successfully used in clinic, while others are still under preclinical evaluation.

Some of the important CSC-based markers along with strategies for targeting them are as follows:

CD133 (Prominin-1) It is a cell surface glycoprotein, widely expressed on many types of CSCs in solid tumors including glioma, lung, and breast cancer. Patients with large CD133 subpopulation have shown poor clinical outcomes. For this reason, strategies for anti-CD133 therapy represent a promising approach for cancer treatment. Paclitaxel-loaded polymeric nanoparticles functionalized with CD133 antibody were investigated to efficiently reduce the number of cell and colony formation in colorectal adenocarcinoma Caco-2 cells. Moreover, these drug-loaded nanoparticles have shown better efficacy as compared to free paclitaxel in xenograft model (Swaminathan et al. 2013). Similarly, anti-CD133 scFv and pseudomona exotoxin 38 (PE-38)-based fusion construct (immunotoxin) exhibited tumor regression property after several intraperitoneal injections of anticancer drug for about 4–6 weeks in ovarian cancer xenograft model. This resulted in cancer-free survivors for a long period of time (Skubitz et al. 2013). These studies prove that anti-CD133 therapy is associated with drug delivery and drug antibody constructs that might increase the efficacy of CD133+ CSCs by abolishing them. Similarly, CD133 + -based cell therapy showed antiproliferative activity resulting in reduced tumor-initiating ability in sarcoma CSCs (Stratford et al. 2013). Furthermore, this cell therapy also showed similar type of results in pancreatic and hepatic CSCs (Huang et al. 2013). Recently, anti-CD133-conjugated carbon nanotubes in combination with irradiation of near-infrared laser light could selectively kill the CD133+ glioblastoma cells (Wang et al. 2011).

CD44 It is a transmembrane protein and found to be overexpressed on different cancer cells such as breast, prostate, gastric, pancreas, ovary, colorectal, bladder, hepatocellular, head and neck, and leukemia CSCs. Thus, targeting CD44 by using monoclonal antibody proves as a promising strategy to kill CSCs. Further, the efficacy of combining antihuman CD44 monoclonal antibody with cyclophosphamide and doxorubicin is reported in preventing relapse of metastatic breast cancer (Marangoni et al. 2009). Anti-CD44 antibody has also shown much efficacy in killing leukemic stem cells in acute lymphoid leukemia (ALL) disease (Huang et al. 2017). Presently, numerous antibodies are approved by FDA and are being used for the treatment of different solid and hematological cancers in clinic such as anti-CD20 (rituximab), anti-EGFR (cetuximab), anti-ER2 (trastuzumab), anti-VEGF-A (bevacizumab), etc. (Kwiatkowska-Borowczyk et al. 2015).

Dysregulation of signaling pathways in CSCs is one of the mechanisms by which they are able to avoid or survive cancer therapeutics (Chen et al. 2013). Moreover, inactive proapoptotic and parallel active antiapoptotic pathways are sizzling points attracting researchers. Targeting Notch signaling by monoclonal antibodies has shown good results (Fischer et al. 2011). In addition, Notch1 inhibition also reduced the CD44+/CD24 CSC subpopulation, thereby inhibiting the

condition of brain metastasis during breast cancer treatment (McGowan et al. 2011). Elevated levels of β-catenin have been shown to contribute CSC tumorigenicity property in colon cancer (Vermeulen et al. 2010). Numerous pharmaceuticals and monoclonal antibodies against Wnt signaling are under preclinical investigation for clinical trials and have shown promising efficacy in cancer treatment (Chen et al. 2013). Recently, it has been investigated that Hedgehog inhibitors have exhibited potential effect in inhibiting systemic metastases in pancreatic orthotopic xenograft mice models. Moreover, a large decrease in ALDH-positive cells was observed indicating reduction in tumor-initiating population in pancreatic cancer (Feldmann et al. 2008). Many researchers have proved the efficacy of an SMO signaling inhibitor (cyclopamine) of Hedgehog cascade in inhibiting the growth, invasion, and metastasis of prostate, breast, and brain tumors in both in vitro and in vivo conditions. Synergistic effect of both cyclopamine and gemcitabine was also reported to inhibit the growth of ALDH high pancreatic CSCs (Feldmann et al. 2007). Furthermore, the synergistic effect of cyclopamine and temozolomide (TMZ) has shown to reduce the cell number of glioma CSCs in in vivo condition (Clement et al. 2007).

4.2.5 Current Therapies and Challenges in Cancer Stem Cell Therapy

As mentioned earlier, curative therapies should target both CSCs and non-CSCs. In recent studies, multiple novel therapeutic strategies have been designed for killing CSCs. However, some of these drugs are in preclinical development, and some are in clinical trials that can specifically eliminate or suppress CSCs. The surface marker differences and alterations in signaling cascades are alluring therapeutic targets for CSC therapy. Moreover, researchers have also investigated some more potential CSC therapeutic targets including ABC transporter-binding protein, microenvironment niche protein, etc. As discussed earlier, CSCs can be eradicated by using treatment targeting the signaling pathways such as Notch, Wnt, and Hedgehog. However, most of the signaling pathways are common in both normal and cancer stem cells that become critical to target the cancer stem cells specifically without harming the normal stem cells. Luckily, CSCs seemed to have their own particular enhanced signaling pathways (Vinogradov and Wei 2012). Furthermore, as mentioned above, the surface markers exploited for isolation of CSCs are also vital targets for CSC treatment. Antibodies that are used in immunotherapy for targeting surface markers overexpressed on CSCs are often used in combination with conventional therapies for better efficacy. CSCs are also known to express ABC transporter proteins at high levels. These proteins help in protecting CSCs from therapeutic agents. Thus, downregulation of these proteins may prove as a promising approach for overcoming the drug resistance to current conventional cancer therapies. However, studies conducted in chemoresistant patients have not yet

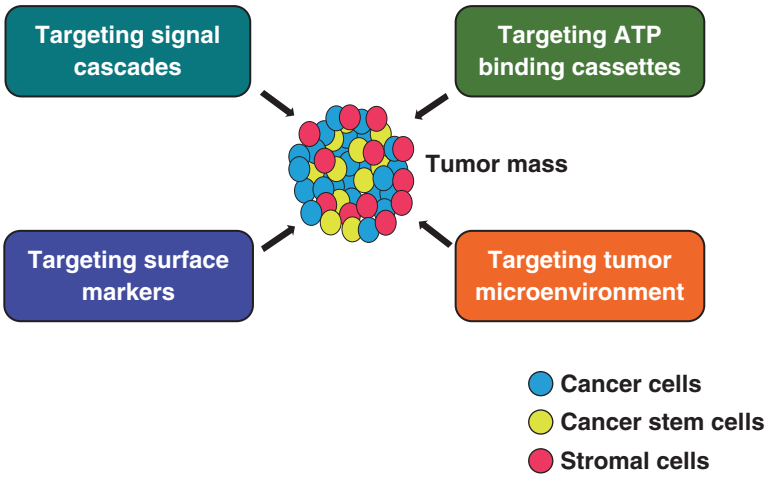

Fig. 4.2 Elimination of CSCs based on different targeting approaches such as tumor microenvironment, surface marker expression, dysregulated signal cascades, and ABC transporters to get rid of relapsed condition

shown the advantage of using these ABC transporter protein inhibitors for overcoming disseminated condition associated with CSCs. Therefore, there is a need to add some extra therapeutic agents in order to eradicate CSCs for better long-term therapy. Thus, inhibition of drug efflux activity in combination with conventional cancer therapies could be one of the promising strategies for CSC treatment in the near future. Last but not the least, CSC niche impairment might also be a potential approach for targeting CSCs. The tumor microenvironment helps to nurse and protect the CSCs from outside toxic agents. Several studies have investigated the role of stromal cells in bone marrow microenvironment and secondary lymphoid organs to favor the disease progression (Chen et al. 2013). Hence, the discussed major therapeutic approaches for CSC therapy are shown in Fig. 4.2.

In addition, tumor angiogenesis is also one of the important factors related to CSC survival and chemoresistance, which is initiated by vascular endothelial growth factor (VEGF). Earlier studies demonstrated that targeting VEGF with bevacizumab in mouse glioblastoma leads to normalization of tumor vasculature resulting to dramatic reduction in glioblastoma stem cell number (Burkhardt et al. 2012). Also, the combination of VEGFR2 antibody DC101 and cyclophosphamide has shown more efficiency against C6 glioma xenografts in vivo than the single therapeutic agent alone (Folkins et al. 2007).

The above discussed avenues represented some of the possible ways of therapeutically targeting CSCs. Over the past few years, many novel approaches have been designed for targeting CSCs, such as a nanoparticle functionalized with a targeting ligand specific to CSC containing an anticancer drug molecule to eliminate CSCs in combination with a chemosensitizer to overcome drug resistance (such as an ABC transporter inhibitor) and an imaging agent to facilitate tumor diagnostics. Such

combinational approaches might exert the anti-tumor effect more effectively with lesser side-effects. Moreover, this approach would facilitate exact identification of the primary tumor localization and its metastases. Although all the alternative therapies are very effective, some approaches are not specific and might affect the normal tissue also. Moreover, CSCs have many ways to evade treatment as these cells reside in low oxygen area (Hypoxia) far from vascularized region, thereby preventing the efficient delivery of the therapeutic agents. Future challenges should involve the development of newer strategies for targeting CSCs specifically in an efficient manner by avoiding toxicity on normal healthy tissue stem cells.

Furthermore, these new strategies should also aid in easy delivery and retention of the drugs in the CSCs. As a result, these new therapies should increase the efficiency of the current drugs against cancer, thereby preventing tumor relapse and enhancing patient survival.

4.3 Nanomedicine-Based Cancer Stem Cell Therapy

4.3.1 Importance and Urgent Utility of Nanomedicine in Cancer Stem Cell Therapy

In clinic, cancer patients are generally treated through surgery, chemotherapy, and radiation therapy by individual drug or their combinations. In chemotherapy, most of drug-sensitive cancer cells are killed during treatment cycle, but unfortunately, tumor relapse condition was observed later due to the presence of cancer stem cells (CSCs) or tumor-initiating cells (TICs) (Shen et al. 2016). Targeting of CSCs has become a biggest challenge in modern medical science. It has reduced the therapeutic potential of current anticancer drugs. Therefore, the elimination of CSCs is an important aspect to prevent cancer drug-resistant condition by targeting drug efflux transporters (Singh and Settleman 2010), reprogramming of metabolic processes (Zhao et al. 2013a), and activation of antiapoptotic signaling pathways (Zhao et al. 2009).

In past few years, the identification of anticancer modalities which can kill CSCs has increased in significant numbers. Recently, the anticancer properties of antibiotics, bioactive compounds, therapeutic peptides, nucleic acids, and small molecule inhibitors have been broadly reviewed with their limitations. However, the clinical application of these anticancerous agents is limited due to their peculiar characteristic features like nonspecificity, poor water solubility, short circulation time or rapid clearance from blood, instability, and nonspecific biodistributions, which lead to low therapeutic values. Targeted killing of CSCs alone will not fulfill the complete eradication of cancer disease as cancer cells have plasticity and heterogeneity, which can reverse their phenotype into CSCs. Hence, there is urgent need to pay more attention toward the development of novel therapeutic approaches, which can kill both multiple drug-resistant CSCs and bulk malignant tumor cells simultaneously (Barenholz 2012).

Since the last two decades, nanotechnology has made several significant contributions in the field of biomedical science. Notably, nanoparticle-based drug delivery system (liposomes, dendrimers, metal oxide nanoparticles, polymeric nanomicelles, and carbon nanotubes) is one of them, which has gained substantial attention on translational point of view. Moreover, nanomedicines are smaller in size (~200 nm) which can easily cross through blood capillaries and reach the target site. The drug loading capacity, biocompatibility, and pharmacokinetic properties of these nanodrug carriers can be optimized by different modifications of nanoparticle's surface. Several other characteristic features like size control, tunable surface properties, and surface-to-volume ratios, attractive surface functional groups for bioconjugation, lesser nonspecific biodistribution, and minimal side effects have shown nanomedicine as a promisable tool to overcome aforementioned limitations. Recently, researchers have made attempts and formulated many nanodrug formulations for CSC therapy (Zhao et al. 2013a). Some of them were clinically approved for cancer treatment including doxorubicin-loaded liposomal formulation (Doxil), albumin-bound paclitaxel (Abraxane), and PEG-L-asparaginase (Oncaspar) (Barenholz 2012). Furthermore, novel and bioengineered multifunctional nanoparticles are being developed for CSC treatment, which are discussed further.

4.3.2 Examples of Nanomedicine for Cancer Stem Cell Therapy

Different types of nanomaterial such as polymeric nanoparticles, metal-based nanoparticles, carbon nanotube, magnetic nanoparticles, and liposome have been used to formulate targeted nanodrug carriers for CSC targeting using chemo-drugs, antibiotics, nucleic acids, peptides, and proteins. These therapeutic modalities target downstream cellular signaling pathways, CSC survival-associated genes, cell surface markers, and metabolic pathways, as shown in Fig. 4.3.

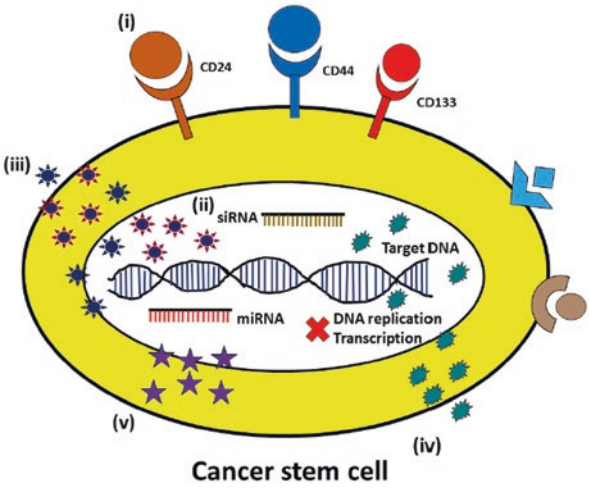

Fig. 4.3 CSC targeting by functionalized nanoparticles in combination with anticancer therapeutic agents: **(i)** targeting surface markers, **(ii)** targeting genes associated with CSCs, **(iii)** chemo- or radiotherapy drugs, **(iv)** small molecular inhibitors (Wnt, Notch, Hh), and **(v)** targeting metabolic targets (ROS)

4.3.2.1 Nucleic Acid-Loaded Nanomedicines against CSCs (miRNA, siRNA, Aptamer)

Nucleic acids have been used as therapeutic agents to treat several cancers of different origins with targeting CSCs also (Liu et al. 2011; Wu et al. 2011). Interestingly, wtp53 plasmid, miRNAs, siRNAs, and aptamers were used to modulate the gene expression by targeting cancer-specific oncogenic mRNAs for inhibiting cancer development, metastasis, and recurrence condition.

The mutations in tumor suppressor genes lead to the development of primary tumor. Restoration of such mutations in tumor suppressor genes can increase the survival rate of patients. For example, GBM is the most aggressive and lethal form of brain tumor in adults. GBM patients have poor prognosis and low survival rate due to the development of recurrence condition by CSCs and drug-resistant cancer cells. The frequent mutations in p53 gene are responsible for ~30% and ~65% risks of primary and secondary brain tumor (GBM), respectively. Temozolomide (TMZ) is an alkylating chemo-drug for the first line treatment of GBM with its methylation action at guanine bases to trigger unsuccessful mismatch repair leading to cell cycle arrest and apoptosis. O^6- methylguanine-DNA methyltransferase (MGMT) is a ubiquitous DNA repair enzyme that repairs DNA methylation and mismatch generated by TMZ. The research evidence suggests that wild-type p53 gene was found to be linked with MGMT expression. It negatively regulates the expression of MGMT in GBM-associated CSCs and drug-resistant cancer cells, which increases the therapeutic efficacy of TMZ. However, the effective delivery of wtp53 gene to target tumor across the blood-brain barrier is a notable challenge at present. Recently, researchers developed a novel nanocarrier based on cationic liposome (scL) [1,2-dioleoyl-3-trimethylammonium propane (DOTAP)/dioleoyl-phosphatidyl ethanolamine (DOPE)] for combinatorial delivery of wtp53 and TMZ specifically to GBM-associated CSCs and drug-resistant cancer cells using anti-transferrin receptor (TfR) single-chain antibody fragment (scFv). The scL-wtp53 does not only cause the cell cycle arrest and apoptosis in GBM but also increases sensitivity of GBM CSCs to TMZ in in vitro study. This approach was subjected in both preclinical and clinical study through systemic administration of scL nanodrug carriers (Kim et al. 2014).

Micro-RNA (miRNA) is a small noncoding RNA of 21–25 nucleotide length and is involved in the posttranscriptional regulation of genes. Earlier, several studies reported the alternations in miRNA expression leading to the development of cancer. Moreover, miRNA expression is usually found to be upregulated in cancer cells, called as oncogenic miRNA, used as biomarker for early diagnosis of cancer, while some miRNA expressions are found to be downregulated, called as tumor suppressor miRNA, used as novel therapeutic agents for removal of drug-resistant cancer and CSCs. Moreover, miRNA-based therapeutic approaches have several limitations in clinic such as higher instability, poor cellular uptake, lesser endosomal release, and risk of systemic toxicity. Therefore, the development of effective nanodrug carriers is essential for delivering therapeutic miRNAs to drug-resistant cancer and CSCs. For example, solid-lipid nanocarrier (DDAB) was used to deliver miRNA-34a in $CD44^+$-B16F10 CSCs. These vehicles protected miRNA-34a from

nuclease degradation in serum and enhanced their bioavailability at target site. As a result, miSLNs-34a inhibited the migratory, invasive, and metastasis properties of B16F10 cells by attenuating CD44 expression and induced apoptosis at both in vitro and in vivo platform. Such nanoparticulate delivery system augmented the therapeutic effect of miRNA-34a for lung CSC therapy (Shi et al. 2013). In another approach, spherical nucleic acid (SNA) was used to determine the anticancerous activity of miRNA-182 in orthotopic GBM xenograft mice model. This nucleic acid-based nano-formulation was prepared through immobilization of miRNA-182 on gold nanoparticles that helped in crossing the blood-brain barrier (BBB) and tumor vascular network. Also, the systemic delivery of 182-SNA initiated the neutralization of Bcl2L12, c-Met, and HIF-2α oncogene expression in GBM, due to which the drug sensitivity of patient-derived glioma-initiating cells (GICs) increased to TMZ and receptor tyrosine kinase inhibitors (RTK-Is) by curbing stem cell-associated mRNA signatures. Further, antitumor property of 182-SNA is tested with glioma cells, GICs, and glioma-bearing mice. In result, 182-SNAs reduced GBM burden and increased animal life expectancy without any side effects (Kouri et al. 2015). Furthermore, a combinational drug delivery approach was developed to deliver chemo-drugs with miRNAs during drug-resistant cancer treatment. For example, solid-lipid nanoparticles (SLNs) were used for codelivery of both miRNA-200c and paclitaxel (PTX) to breast CSCs (BCSCs), where miRNA-200c restored the sensitivity to PTX by downregulating the expression of class III β-tubulin (TUBB3). As a result, the cellular cytotoxicity of PTX-loaded SLNs was increased against BCSCs. It revealed that combinational delivery is a novel therapeutic approach for CSC treatment (Liu et al. 2016).

Likewise, there is another class of therapeutic nucleic acid called small interfering RNA (siRNA) which is responsible for posttranscriptional gene silencing (PTGS) by RNA interference (RNAi) pathway. It is a double-stranded nucleic acid of 20–25 nucleotides long. It has been widely used in the treatment of many diseases by knocking down disease-specific gene. Generally, the delivery of siRNA to target tissue and cells is achieved through virus-like particles, metallic nanoparticles, lipidic and polymeric nanoparticles, and so on. In recent years, the application of siRNA has been broadly extended to drug-resistant cancers. Generally, cancer cells need more fuel (glucose) than healthy cells as they divide because cancer cells are more dependent on glycolysis for energy production rather than oxidative phosphorylation (in presence of oxygen) for building their biological macromolecules. Usually, cancer cells express more glucose transporters (GLUT) for glucose influx than normal cells. Among these, GLUT3 is highly expressed in GBM and particularly in GBM CSCs because it has fivefold higher affinities with glucose than GLUT1 which is predominantly found in normal tissues for key biological functions; therefore, knockdown of GLUT3 was targeted for GBM treatment using siRNA (siGLUT3) encapsulated with PEG-PLA cationic-lipid nanoparticles (as shown in Fig. 4.4), due to which the glucose uptake decreased in glioma cells leading to downregulation of stemness and proliferation of U87MG and U251 cells inhibited in a glucose-limited environment. Further, this nanoformulation was systemically administered through intravenous injection (i.v.) into U87MG xenograft

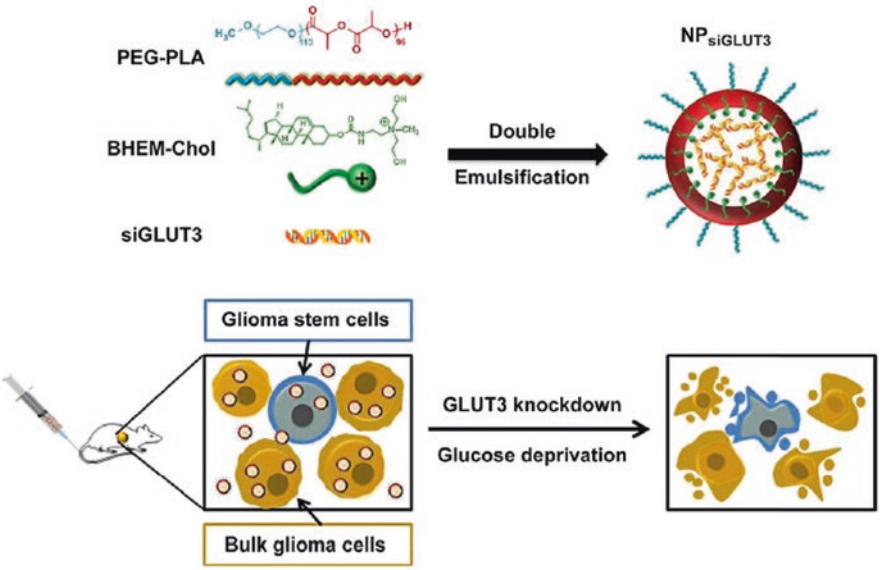

Fig. 4.4 Schematic illustration of the preparation of NPsiGLUT3 and the mechanism of NPsiGLUT3-mediated tumor growth inhibition in U87MG xenograft mice model. After intravenous injection, NPsiGLUT3-mediated GLUT3 knockdown caused glucose deprivation in glioma stem cells and bulk glioma cells, which inhibited the glucose metabolism and the growth of tumor. (Permission obtained from Elsevier press, Ref. no. 58)

murine model. As a result, NPsiGLUT3 significantly inhibited the growth of GBM in mice due to reduced expression of GLUT3 transporters and decreased the stemness of glioma cells. This study concluded that siRNA is a potential anticancer agent for cancer stem cell targeting (Xu et al. 2015b). Similarly, another study used siRNA-loaded nanocarriers to knockdown MDR1 gene-encoding drug efflux transporters. In this study, both MDR1 siRNA and paclitaxel (PTX) were encapsulated in cross-linked lipid nanocomplex composed of PEI-lipid in 1:16 ratio. Such nanocarrier system reduced the MDR1 expression in CD133+ human colon CSCs resulting in increased chemosensitivity to paclitaxel drug. Moreover, the synergistic action of siMDR1 and paclitaxel increased the therapeutic efficacy compared to drug alone (Liu et al. 2009). Recently, the siPlk1-encapsulated cationic lipid-polymeric nanoparticles were developed to target TGF-β signaling pathway for BCSC therapy. The nanoparticles carrying siPlk1 efficiently eliminated breast CSCs derived from MDA-MB-231 cells in vitro. However, LY364947 (an inhibitor of TGF-β type 1 receptor) increased the permeation of these polymeric nanoparticles in tumor tissue via vascular leakage leading to nanoparticle accumulation in drug-resistant cancer and CSCs. The synergistic action of LY364947 and siPlk1inhibited the tumor growth and reduced BCSC population in vivo (Zuo et al. 2016). In another study, siPLK1/SSB-encapsulated HA-PEI/PEG nanocarriers were developed to target CD44 receptors in resistant A549 lung cancer cells. The nanodrug carriers

showed dose-dependent therapeutic effect and targeted specific gene knockdown at both in vitro and in vivo platform. Further, these HA-based nanosystems can deliver any siRNA for systemic targeting of CD44 overexpressed on cancers (Ganesh et al. 2013). Overall, nanocarriers have gained considerable attention among the researchers for delivery of siRNAs, miRNAs, and other nucleic acid-based therapeutic agents for CSC treatment. However, a novel discovery of another class of nucleic acid molecules that has replaced the use of antibodies is called aptamers, used for targeted delivery of therapeutic agents in drug-resistant cancer treatment. As we are discussing about nucleic acid aptamers, it is a single-stranded oligonucleotide molecule that forms highly stable 3D structure to bind a wide range of small molecules or even cells with high affinity and specificity. Functionally, it mimics like antibody and modulates several protein functions. The exciting significant future of aptamers is due to its very small size, low systemic toxicity, and non-immunogenicity. These properties make aptamers a novel therapeutic carrier to deliver specific drugs and molecules into diseased cells. Presently, aptamers have been utilized in the designing and formulation of target-specific drug delivery systems in the field of nanobiotechnology. Unnatural aptamers are discovered through an in situ method called SELEX (systematic evolution of ligands by exponential enrichment) with high specificity and stability in a cost-effective manner (Sun et al. 2014b). Based on its application, aptamers can be easily modified with any functional group. Recently, a variety of aptamers are developed with high affinity to target CSC surface markers. Generally, these aptamers are functionalized on the surface of drug-loaded nanoparticles. For example, a CD30-specific RNA aptamer and PEG were functionalized on the surface of hollow gold nanospheres (HAuNS) via covalent S-Au bonds and doxorubicin which were loaded via charge force. The formulated Apt-HAuNS-Dox NPs were highly sensitive to acidic pH. As a result, 80% Dox release was shown in 2 h at pH 5.0. However, gold nanosphere without aptamer conjugation (HAuNs-Dox) released 55% drug at the same pH, which concluded that aptamer conjugation favored pH-induced drug release that selectively killed lymphoma cells and CSCs (Zhao et al. 2013b). Interestingly, PEGylated aptamer against EpCAM (a CSCs marker) showed higher rate of penetration into tumorsphere core after nanocarrier administration and remained in the core for at least 24 h, while EpCAM antibody displayed limited tumor penetration after 4 h incubation. In xenograft tumor, the PEGylated EpCAM aptamers were sustained for 26 h, which was 4.3-fold longer than EpCAM antibody. Also, the accumulation of PEGylated aptamers was 1.67-fold and 6.6-fold higher than antibody in xenograft tumor mice model at 3 h and 24 h after i.v. administration, respectively. Moreover, EpCAM aptamers were detected 200 μm far away from blood vessels in 3 h after i.v. administration than EpCAM antibodies, which were found to be distributed around the blood vessels in xenograft tumors. This study indicated that aptamers are better to antibodies in cancer theranostic application due to their uniform biodistribution, enhanced penetration, and higher retention in tumor sites (Xiang et al. 2015). Furthermore, 19-mer EpCAM RNA aptamer-conjugated PEG-PLGA-Dox nanopolymersomes were developed for targeting EpCAM-overexpressed CSCs isolated from adenocarcinoma cell lines. The in vitro results showed the efficient cell uptake and

internalization of nanopolymersomes that exhibited the higher cytotoxicity toward EpCAM+ MCF7 cells (Alibolandi et al. 2015). Also, curcumin-loaded lipid-polymer-lecithin hybrid nanoparticles were synthesized and functionalized with EpCAM RNA Apts for targeting colorectal adenocarcinoma cells. The PEG core of this hybrid nanoparticle was modified with lecithin which is a well-known dispersing agent to improve drug loading capacity and stability. The Apt-CUR-NPs showed enhanced binding affinity to HT-29 cells and increased cellular uptake, due to which cell cytotoxicity was improved and higher curcumin bioavailability was seen over a period of 24 h during in vivo studies after systemic administration of biconjugate nanoparticles (Li et al. 2014). Thereby, several aptamer-conjugated nanodrug formulations were prepared recently, in which most of them are under phase II and III trials. As we have discussed earlier about CD44 expression on CSC surface, a 2'-F-pyrimidine-containing RNA aptamer (Apt1) was designed and conjugated on the surface of PEGylated liposome for targeting CD44+ CSCs. The results showed that Apt1-loaded lipid NPs had higher sensitivity and selectivity compared to blank liposomes. It concluded that Apt1-Lip has promising potency as a specific drug delivery system for CD44+ CSCs (Alshaer et al. 2014). Meanwhile, spherical capsules with alginate-enclosing, chitosan-coated (AEC), iron-saturated bovine lactoferrin, and EpCAM RNA Apt-conjugated calcium phosphate nanoparticles were prepared to reduce the viability of Caco-2 cells. These nanocarriers reduced the expression of CSC markers like CD133+/survivin/CD44+ in xenograft colon cancer models. These nanoparticles induced apoptosis by targeting survivin in drug-resistant cancer and CSCs. During treatment, such nanoparticles maintained iron, zinc, and calcium levels (Kanwar et al. 2015). Next, the application of aptamers has been extended for effective delivery of therapeutic nucleic acids like tumor suppressor gene-carrying plasmid, siRNA, and miRNA. In this context, aptamer-siRNA chimeras were developed to knockdown Plk1 gene in EpCAM+ cancer cells in vitro and in biopsy tissues. The Plk1 EpCAM-AsiCs inhibited EpCAM+ basal and luminal TNBC growth in nude mice and also stopped mammosphere formation in vitro, which provided an efficient approach for treating epithelial cancers (Gilboa-Geffen et al. 2015). Besides drug delivery, the use of aptamers was also extended to photothermal therapy for cancer. Gold nanorods (AuNRs) are well-known nanomaterial that can absorb near-infrared light and generate heat in its surrounding environment for photothermal therapy of cancer. Based on this, two aptamers (Apt CSC1 and Apt CSC13) were functionalized on the surface of gold nanorods to target both prostate cancer and CSCs. A beam of near-IR light was passed on AuNR internalized CSCs and cancer cells by which the temperature increased from 25° to 55 °C in target cells. Such heating caused the destruction of cellular organelles and their membrane which induced apoptosis, while untargeted cells were rarely affected without significant adverse side effects (Wang et al. 2013). All studies discussed here have been summarized in Table 4.1.

As several preclinical and clinical studies are needed to definitively assess how CSCs respond to current therapies, on this basis, the development of effective therapeutic strategies against CSCs is needed to increase the efficacy of conventional cancer treatment. Such potential approaches generally include targeting

Table 4.1 Therapeutic nucleic acid functionalized nanoparticles for CSC therapy

Therapeutic nucleic acids	Chemotherapeutic drugs	Target CSCs and markers	Nanocarrier	Mode of function	References
scL-p53	Temozolomide (TMZ)	GBM CSCs	Cationic liposome 1,2-dioleoyl-3-trimethylammonium propane (DOTAP)/ dioleolylphosphatidyl ethanolamine (DOPE)	Upregulating p53 and modulating O^6-methylguanine-DNA methyltransferase enzyme	Kim et al. (2014)
miRNA-34a	–	Lung CSCs, CD44 + B16F10 cells (CSCs like cell population)	Solid-lipid nanoparticles (SLNP)/ dimethyldioctadecylammonium bromide (DDAB)	Inhibiting tumor development and tumorigenicity	Shi et al. (2013)
miRNA-200c	Paclitaxel (PTX)	Breast CSCs	Cationic lipid 1,2-dioleoyl-3- trimethylammonium-propane	Reducing the expression of class III β-tubulin (TUBB3) gene	Kouri et al. (2015)
miRNA-182	Tyrosine kinase inhibitors (RTK-is) and TMZ	Bcl2L12, c-Met, and HIF-2α in GBM	Spherical nucleic acid	Suppressing stem cell-associated mRNA signatures	Liu et al. (2016)
siGLUT3	–	GBM CSCs, GLUT-3	PEG-PLA cationic lipid nanoparticles	Knockdown of GLUT3 gene	Xu et al. (2015b)
siMDR1	Paclitaxel	CD133+ human colon CSCs	PEI-Lipid (1:16)	Knockdown of MDR1 gene	Liu et al. (2009)
siPlk1	TGF-β type 1 inhibitor	Breast CSCs derived from MDA-MB-231 cells	Lipid-polymeric nanoparticles	Knockdown of Plk-1 gene	Zuo et al. (2016)
CD30 RNA Apt	Doxorubicin	CD30 overexpressed cancer and CSCs cells	Hollow gold nanosphere	Targeting specific drug cytotoxicity	Zhao et al. (2013b)

2'-F-pyrimidine-containing RNA aptamer (Apt1)	–	CD44+ CSCs	PEGylated liposome	Targeting specific drug carrier	Alshaer et al. (2014)
EpCAM RNA apt	Curcumin	EpCAM+ colon CSCs	Lecithin modified PLGA–PEG NPs	Inducing apoptosis	Li et al. (2014)
EpCAM RNA apt	Iron-saturated bovine lactoferrin	CD133+/CD44+/ survivin colon CSCs	Alginate-enclosed, chitosan-coated, calcium-phosphate (Ca-P) NPs	Inducing apoptosis in colon CSCs	Kanwar et al. (2015)
CSC1 and CSC13 apt	–	Prostate CSCs	Gold nanorods (AuNRs)	Photothermal destruction	Wang et al. (2013)

Abbreviations: *CSCs* cancer stem cells, *CD* cluster of differentiation, *NPs* nanoparticles; *Apt* aptamer, *EpCAM* epithelial cell adhesion molecule, *TGF-β* transforming growth factor β, *GLUT-3* glucose transporters-3, *MDR-1* multidrug resistance-1, *Plk1* polo-like kinase-1, *GBM* glioblastoma multiforme, *HIF-2α* hypoxia inducible factor-2α, *c-met* hepatocyte growth factor receptor, and *Bcl2L12* Bcl-2 like protein-12

CSCs, inhibition of ATP-binding cassette (ABC) transporters, blocking of essential self-renewal and cell survival signaling pathways, or disruption of tumor microenvironment.

4.3.2.2 Chemotherapeutic Drug-Loaded Nanomedicines Against CSCs

The main application of nanomedicine is to deliver hydrophobic drugs that have several serious issues related to its solubility, stability, and toxicity. The use of nanomedicine generally increases the solubility and stability of hydrophobic drugs with reduced side effects. This approach can be useful for such drug candidates that have the aim to eliminate drug-resistant cancer and CSCs. For example, doxorubicin (Dox) is the most commonly used chemo-drug at present time in clinic. To enhance its therapeutic efficacy, a nanodrug formulation based on gold nanoparticles was prepared, called Dox-Hyd-PEG-AuNPs, for targeting BCSCs. This formulation inhibited the mammosphere formation capacity in vitro and further removed all tumor cell subpopulations enriched with CSCs in orthotopic xenograft breast cancer mice model (Sun et al. 2014c). Like Dox, salinomycin (SA) is also a widely used chemo-drug having very low therapeutic efficacy in clinic. Therefore, several nanodrug carriers were used to enhance its clinical use. In an earlier study, SA drug was conjugated to hyaluronic acid (HA)-based nanogels, i.e., made up of cholesterol. These drug-loaded nanogels targeted and killed CD44+ BCSCs in both drug-resistant tumor cell subpopulations and multicellular tumor spheroids (Wei et al. 2013). In addition, paclitaxel (PTX) is the most common drug in the treatment of several solid cancers. The clinical efficacy of this drug was enhanced through the preparation of several nanodrug formulations. For example, PTX-loaded poly(D,L-lactide-coglycolide) nanoparticles were synthesized. Further, these drug-loaded nanoparticles were functionalized with anti-CD133 antibody for targeted drug delivery that resulted into higher cytotoxicity on HepG2 and Huh7 cells in both in vitro and in vivo studies. Also, these actively targeted nanoparticles eliminated CD133+ liver CSCs, which made it a promising candidate for testing in different phase trials (Jin et al. 2014).

The most important advantage of nanocarriers is to deliver multiple drugs at a time. For instance, a combined therapeutic approach was developed using nanoparticle-encapsulated Dox (pluronic F127-chitosan-Dox NPs) and cryoablation technology, which killed almost CD44+ and CD133+ CSCs in a 3D mammosphere model (Rao et al. 2014). In another study, SA drug was used in combination with other therapeutic modalities to eliminate other CSCs. By this approach, a combinational therapy using SA drug with polyelectrolyte-conjugated AuNPs (Au/SA@PDC) was developed and showed a synergistic BCSC inhibition in drug-resistant MCF-7 cells (Xu et al. 2014). Additionally, two different nanodrug formulations like octreotide-conjugated PTX-loaded PEG-b-PCL (Oct-M-PTX) and salinomycin-loaded PEG-b-PCL (M-SA) polymeric micelles were prepared. It was shown that M- SA micelles eliminated the large proportion of CD44+/CD24- BCSCs in a potent manner compared to SA alone. Also, Oct-M-PTX micelles inhibited a large population of MCF-7 cells as compared to M-PTX micelles, while

the anticancerous effect of Oct-M-PTX and M-Sal micelle combination was very strong in both in vitro and in vivo system. Hence, this combinational therapy improved the treatment of breast cancer with elimination of BCSCs (Zhang et al. 2012a). Further, both PTX and SA drugs were also conjugated to anti-CD44 functionalized SWCNTs via hydrazine linker. Such pH-responsive nanodrug carriers released both drugs at acidic tumor microenvironment after targeting CD44+ BCSCs and increased the combined therapeutic effect of both drugs in xenograft mice models (Al Faraj et al. 2016). The nanodrug carriers were also used in the codelivery of MDR inhibitors with cytotoxic drugs. These inhibitors increase the antitumor effect of existing drugs after sensitizing CSCs. For example, a combination of ABCG2 mAb- and PTX-conjugated Fe3O4 nanoparticles was tested on multiple myeloma (MM) $CD138^{-}CD34^{-}$CSCs in a xenograft MM NOD/SCID mouse model. The combination induced a strong therapeutic response compared to current conventional regimens for MM patients which is due to instantaneous inhibition of ABC transporters by antibodies and delivery of PTX to CSCs via magnetic nanoparticles (Yang et al. 2015). Nanomedicines were also used to carry such therapeutic modalities that affect CSC's development and maintenance pathways. These agents were reached into different phases of clinical trials but could not be used for a longer time due to their high toxicity, lesser solubility, and nontargeted drug delivery. For example, cyclopamine is a natural Hedgehog (Hh) pathway inhibitor. When this drug was conjugated with HPMA (N-(2-hydroxypropyl)-methacrylamide) copolymer, its systemic toxicity was decreased and aqueous solubility increased. Such polymer-drug conjugate was used as a CSC-selective macromolecular therapy, which removed a large subpopulation of CD133+ CSCs from human prostate cancer epithelial cells (RC-92a/hTERT cells) (Zhou et al. 2012). In another study, HPI-1 is used as a potent antagonist of Hh transcription factor (Gli1) that blocks the downstream signaling events of Smo. A nanoformulation based on HPI-1-encapsulated PLG PEG nanoparticles (NanoHHI) was prepared. The NanoHHI increased the systemic bioavailability of HPI-1 inhibitor and improved its solubility. Such nanoformulations inhibited the growth of Ptch(−/+), Trp53 (−/−) medulloblastoma in xenograft mice models. Especially, the combined therapeutic effect of NanoHHI with gemcitabine significantly inhibited the growth of orthotopic Pa03C pancreatic cancer xenografts (Chenna et al. 2012). Also, NanoHHI decreased the population of CD133+ CSCs in hepatocellular carcinoma (HCC) and potentially inhibited the tumor growth in orthotopic HCC xenografts (Xu et al. 2011). Conclusively, the Gli1 inhibition through NanoHHI displayed antitumor effect in both pancreatic cancer and HCC models. All studies discussed here are summarized in Table 4.2.

4.3.2.3 Targeted Therapy- and Immunotherapy-Based Nanomedicines against CSCs

In few years, the field of drug delivery has become more advanced with the development of actively targeted nanodrug carriers, i.e., basically based on ligand-receptor interactions. The main advantage of such delivery is to increase the therapeutic efficacy of chemo-drugs, small molecular inhibitors and nucleic acid-based therapeutic

Table 4.2 Chemotherapeutic drug loaded different nanodrug carriers for CSC therapy

Nanocarrier	Chemotherapeutic drugs	Target CSCs and markers	Composition	Mode of function	References
Gold nanoparticles (AuNPs)	Doxorubicin	Breast CSCs	PEG- Hyd-Dox, AuNPs	Reducing the no. of tumor cell subpopulations enriched with CSCs	Sun et al. (2014c)
Hyaluronic acid (HA)-nanogel	Salinomycin	CD44+ breast CSCs	HA, cholesterol	Targeting CD44+ CSCs and inhibiting tumor growth	Wei et al. (2013)
Anti-CD133 antibody conjugated poly (D,L-lactide-coglycolide) NPs	Paclitaxel	CD133+ liver CSCs	Anti-CD133 antibody, PLGA NPs	Suppressing CD133+ CSCs population	Jin et al. (2014)
Pluronic F127-chitosan NPs	Doxorubicin	CD44+ CD133+ CSCs	Pluronic F127, chitosan	Combined killing of CD44+ and CD133+ CSCs via Dox and cryoablation technology	Rao et al. (2014)
PDC-AuNPs	Salinomycin	Breast CSCs	PDC, AuNPs	Synergistic inhibition of BCSCs via hyperthermia and SA treatment	Xu et al. (2014)
Octreotide-PEG-b-PCL polymeric micelles	Paclitaxel Salinomycin	Breast CSCs	PEG-b-PCL, octreotide	Eliminating BCSCs to improve breast cancer treatment	Zhang et al. (2012a)
Anti-CD44 antibody functionalized SWCNTs	Paclitaxel Salinomycin	CD44+ breast CSCs	Anti-CD44 antibody, SWCNTs, hydrazine linker	Combined therapeutic effect against BCSCs in xenografts	Al Faraj et al. (2016)
Iron oxide NPs	Paclitaxel	MM $CD138^{-}CD34^{-}$CSCs	ABCG2 mAb, Fe3O4 NPs	Inhibiting ABC transporters by antibody and targeting of CSCs by PTX-loaded magnetic NPs	Yang et al. (2015)
HPMA	Cyclopamine	CD133+ CSCs	HPMA polymer	Removing a large population of CD133+ CSCs from human prostate cancer epithelial cells	Zhou et al. (2012)

PLGA-PEG NPs	HPI-1 gemcitabine	Medulloblastoma, pancreatic cancer	PLGA-PEG copolymer	Inhibiting medulloblastoma growth and Pa03C pancreatic cancer in xenografts	Chenna et al. (2012)
PLGA-PEG NPs	HPI-1	CD133+ CSCs hepatocellular carcinoma (HCC)	PLGA-PEG copolymer	Inhibiting tumor growth in HCC xenografts	Xu et al. (2011)

Abbreviations: *CSCs* cancer stem cells, *CD* cluster of differentiation, *NPs* nanoparticles, *PDC* poly (dimethyldiallylammonium chloride), *PEG* poly ethylene glycol, *PCL* poly-caprolactone, *SWCNT* single-walled carbon nanotube, *HPMA* N-(2-hydroxypropyl) methacrylamide, *HPI* Hedgehog pathway inhibitor, *mAb* monoclonal antibody, and *ABCG* ATP-binding cassette subfamily G

agents without any risky side effects, achieved through the practice of therapeutic antibodies functionalized on nanoparticle surface. Moreover, antibodies have also eliminated CSCs and drug-resistant cancer cells from tumor cell population like other therapeutic modalities. As a result, the patient's survival rate increases during clinical trials (Vinogradov and Wei 2012). For example, an anti-CD133 antibody-decorated SN-38-loaded PEG-PCL nanoparticle-based nanoformulation was prepared to target CD133+ HCT116 cells and showed higher toxicity in colorectal cancer cells compared to nontargeted particles (PEG-PCL-SN38). Further, CD133Ab-PEG-PCL-SN38 NPs significantly reduced the tumor growth in orthotopic HCT116 xenograft mice model compared to CPT-116 and PEG-PCL-SN38 NPs. However, tumor relapse condition was observed in all treatment groups during the off-therapy stage, but no relapse condition was observed in CD133Ab-PEG-PCL-SN38 NP treatment group. Also, the mouse body weight decreased during the treatment stage that indicated better inhibition of tumor growth and then remained constant in the rest of the experimental period as shown in Fig. 4.5. In end, it was concluded that several other targeted nanoparticles can be designed using this cutting-edge study for delaying tumor relapse condition (Ning et al. 2016). Mostly, mAb-functionalized nanodrug carriers are under different clinical phase trials, while there is a concern related to the use of mAb and its origin. In preclinical studies, mAbs generally target human antigens and cannot cross-react with murine antigens, due to which the systemic toxicity and other side effects of mAbs could not be observed. Therefore, there is a need to characterize mAbs in a very efficient manner on different preclinical and clinical platforms. Further, anti-CD44 mAb was developed to target CD44+ CSC population. Based on this, an anti-CD44 mAb-conjugated liposomal nanoparticles loaded with doxorubicin and triple fusion gene were prepared for hepatocellular carcinoma (HCC) treatment. These nanoparticles killed CD44+ CSCs via chemotherapy and gene therapy to reduce the side effects of conventional chemotherapy (Wang et al. 2012b). In addition to therapeutic antibodies, aptamers have also shown their better potential for the development of targeted CSC therapeutics and molecular imaging agents as described earlier in Sect. 4.3.2.1. In another study, two different nanodrug formulations like PLGA-PEG-PTX-CD44 and PLGA-PEG-PTX-CTX were developed to target CD44+ breast and EGFR+ colon cancer cells. These targeted nanoconjugates displayed significantly more therapeutic efficacy in tdTomato+ MCF-7, MDA-MB-231, HCT116, and HCT8 cells. Using this fluorescent CSC model, it was concluded that active targeting sensitized CSCs to PTX treatment (Gener et al. 2015). Furthermore, EGFR and EGFRvIII receptors are also highly expressed on glioblastoma multiforme (GBM) neurospheres and GBM stem-like cells (GSCs). To target this receptor, a nanoformulation of cetuximab-conjugated iron oxide nanoparticles (CTX-IONPs) was formulated that showed a significant antitumor effect with increased apoptosis in EGFRvIII+ GSCs and EGFR+ GBM neurospheres. Further, the survival rate of GBM xenografts was increased with substantial tumor regression after treatment (Kaluzova et al. 2015). Recently, an NTP-conjugated, paclitaxel-loaded biodegradable polyglutamic acid polymer-based nanoformulation was prepared to target NCAM-overexpressed CSCs in Wilms tumor. The results showed the proliferation

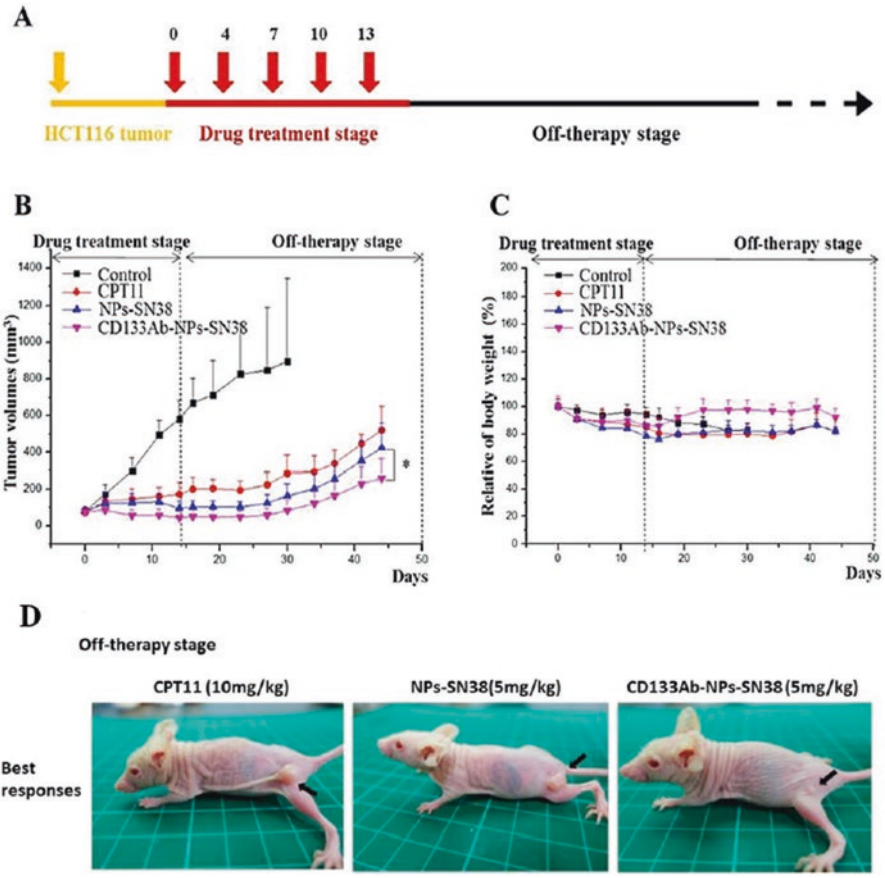

Fig. 4.5 In vivo anticancer efficacy of CD133Ab-NPs-SN-38 in HCT116 tumor xenograft model (**a**) Scheme of the in vivo treatment schedule. (**b**) Tumor volume and (**c**) the changes in body weight of HCT116 tumor-bearing mice treated with control (n = 6), CPT-11 (10 mg/kg, n = 6), NPs-SN-38 (5 mg/kg of SN-38, n = 6), and CD133Ab-NPs-SN-38 (5 mg/kg of SN-38, n = 6). (**d**) Pictures of tumor size in the mice with the best responses in treated groups. * = $P < 0.05$. (Reprinted with permission from Ning, S. T., Lee, S. Y., Wei, M. F., Peng, C. L., Lin, S. Y. F., Tsai, M. H. et al. (2016). Targeting Colorectal Cancer Stem-Like Cells with Anti-CD133 Antibody-Conjugated SN-38 Nanoparticles. *ACS Applied Materials & Interfaces*, *8*(28), 17,793–17,804. Copyright 2018. American Chemical Society, Ref. no. 82)

and migration inhibition in xenograft-derived WT cells. Also, NTP-PGA-PTX conjugate reduced the tumor size in a patient-derived WT xenografts with dramatic reduction in NCAM-overexpressed CSCs. Further, this nanoconjugate was used in targeting drug-resistant cell population (Markovsky et al. 2017). In fact, these antigens are mainly responsible for tumor development and progression; therefore, the search for novel antigens and the development of new therapeutic antibodies are still going on. The nanodrug carriers have been also utilized in the codelivery of

multiple drugs and imaging agents to the drug-resistant cancer for cancer theranostic applications. For instance, bifunctional nanoparticles were designed for tumor imaging and targeted drug delivery in the treatment of hormone refractory prostate cancer. The single-chain prostate-specific antigen (PSA) antibody-conjugated PLGA-SPIO/docetaxel nanoformulation was prepared. These nanoparticles increased antitumor efficacy and improved MRI imaging in vitro via targeted delivery of Dtxl and SPIONS to PC3M cells. In addition, such dual activity comprising of nanoparticles provided a negative MRI contrast enhancement and tumor growth inhibition in PC3M xenograft mice models (Gao et al. 2012). As CSCs overexpress significant biomarkers on their surface for their isolation and targeting, no targeted therapy has the capability to eliminate all CSCs from tumor cell subpopulation. It may be due to the localization of CSCs in tumor necrotic area, where nanodrug carriers are difficult to reach. Thereby, targeted nanodrug carriers can be more suitable in such cancer treatment, where CSCs are freely available like leukemia stem cells (LSCs) in hematological malignancies. All studies pertaining to targeted nanodrug carriers are summarized in Table 4.3.

In recent years, nanoparticle-based delivery has gained a significant attention as potential carriers for cancer vaccine delivery. These nanocarriers have delivered vaccine antigens, adjuvants, and immunomodulatory agents to the specific target sites. In fact, most of the vaccines require additional adjuvants to induce cellular immunity, but nanoparticle-based vaccine delivery reduced the additional use of adjuvants (Shima et al. 2013). As nanoparticles can enter easily inside the cell, it interacts with Toll-like receptors (TLRs) to further improve its efficacy as vaccine adjuvant (Nguyen et al. 2012). The nanocarriers have also increased the antigen-specific cytotoxic T-cell (CD8+) responses that are critical regulators of anticancer immunity. If nanoparticles are manipulated by their surface charge, particle size, particle core hydrophobicity, and surface-bound ligands, then it can easily enter into antigen-presenting cells (APCs) and modulate humoral immune responses to tumor antigens leading to improved anticancer immunity (Cruz et al. 2012). In addition, nanoparticles have also destroyed or decreased the no. of tumor-associated macrophages (TAM) which are responsible for CSC growth in its niche. For example, a siCCR2-encapsulated lipid nanoparticle containing C12–200 lipid, PEG-DMG, cholesterol, and disteroylphosphatidyl choline was formulated. The monocyte-targeted siRNA nanomaterials silenced CCR2 at mRNA, protein, and functional levels in monocyte subsets, preventing their accumulation at inflammation site in both lymphoma-modeled mice and colorectal xenografts. It was already known that inflammatory monocytes differentiate into tumor-associated macrophages under host response. The results showed that siCCR2 decreased the no. of TAMs followed by CSC growth inhibition leading to the reduction in tumor size (Leuschner et al. 2011). Furthermore, several immunotherapeutic molecules were delivered to CSCs through aptamers. For instance, bispecific oligonucleotide aptamer conjugates were used to deliver 4-1BB costimulatory molecules to prostate cancer cells and enhanced T-cell-based antitumor immunity. Nevertheless, the effects of such costimulatory aptamers on CSCs are less understood (Pastor et al. 2011). Also, CART cells were developed for CSC therapy. Up to date, only three animal studies are available on

Table 4.3 Different types of targeted nanodrug carriers for CSC therapy

Nanocarrier	Chemotherapeutic drugs	Ligand/receptor	CSC source and their marker	Mode of function	References
CD133Ab-PEG-PCL-SN38 NPs	SN-38	Anti-CD133 antibody/CD133	Colorectal cancer, HCT-116 cells/CD133+	Inhibit tumor growth in CD133+ HCT116 xenografts	Ning et al. (2016)
Anti-CD44 Ab-liposomal NPs	Doxorubicin	Anti-CD44 antibody/CD44	CD44+ HCC CSCs (HepG2 cells)	Eliminate CCD44+ CSCs and induce apoptosis via chemo and gene therapy	Wang et al. (2012b)
PLGA-PEG-PTX-CD44 PLGA-PEG-PTX-CTX	Paclitaxel	Anti-CD44 antibody/CD44 Cetuximab/EGFR	CD44+ breast CSCs EGFR+ olon CSCs	Inhibit MCF-7, MDA-MB-231, HCT116 and HCT8 tumor cells growth	Gener et al. (2015)
CTX-IONPs	–	Cetuximab/EGFR and EGFRvIII	CD133- EGFR+ GBM neurospheres and EGFRvIII+ GSCs	Inhibit tumor growth and increase survival rate of GBM xenografts	Kaluzova et al. (2015)
NTP-PGA-PTX	Paclitaxel	NCAM targeted peptide(NTP)/NCAM	NCAM + WT CSCs	Reduce the tumor size in a patient derived WT xenografts	Markovsky et al. (2017)
scAb-PLGA-SPIO/Dtxl	Docetaxel	Single-chain PSCA antibody/PSCA	PSCA+ PC3M CSCs	Inhibit tumor growth in PC3M xenograft	Gao et al. (2012)

Abbreviations: *CSCs* Cancer Stem Cells, *CD* Cluster of Differentiation, *NPs* Nanoparticles, *PEG* Poly Ethylene Glycol, *PCL* Poly-Caprolactone, *Ab* Antibody, *PTX* Paclitaxel, *CTX* Cetuximab, *SPIONP* Super Paramagnetic Iron Oxide Nanoparticles, *PLGA* Poly (Lactic-Co-Glycolic) Acid, *PGA* Polyglutamic Acid, *DTX* Docetaxel, *scAb* Single-Chain PSCA Antibody, *EGFR* Epidermal Growth Factor Receptor, *PSCA* Prostate Stem Cell Antigen, *NCAM* Neural Cell Adhesion Molecule, *HCT* Human Colorectal Carcinoma, *HCC* Hepatocellular Carcinoma, *WT* Wilms Tumor, *GBM* Glioblastoma Multiforme, *GSC* Glioblastoma Stem Cells, and *PC* Prostate Cancer

CSC-targeted CART cells in which the first study used an anti-CD133 CART cell that killed patient-derived glioblastoma stem cells both in vitro and in vivo platform (Zhu et al. 2015). In the second study, EpCAM-targeted CART cells showed an antitumor efficacy against prostate cancer in both in vitro and animal models (Deng et al. 2015). Further, in the third study, similar EpCAM CART cells were used to treat peritoneal carcinomatosis in xenograft mice model (Ang et al. 2017). Significantly, a CSC-based dendritic cell vaccine was developed to induce anti-CSC immunity in an effective manner. The DC vaccine displayed a strong antitumor effect in neurospheres compared to glioma xenografts (Ning et al. 2012; Toda 2013). In another study, a CSC-based vaccine prevented the liver metastasis from colon cancer and reduced the tumor size with low incidence in a rat colon carcinoma syngeneic model (Duarte et al. 2013). In few years, cancer immunotherapy has gained a large attention to both the clinicians and scientists because of its use; the survival rate of patients has increased with elimination of relapsed state. Thereby, in future, the combination of cancer immunotherapy with nanotechnology may open novel avenues with several breakthroughs for patient's treatment.

4.3.2.4 Metabolic Target-Based Nanomedicines Against CSCs

The targeting of metabolism in CSCs and drug-resistant cancer cells has always been a challenging task. When CSCs are treated through radiation, then it induces DNA damage through ROS (reactive oxygen species) generation derived from water molecules. Such damages can be seen in long- and short-term consequences. The short-term DNA damage disturbs the DNA metabolism such as DNA replication and RNA transcription. If DNA repair does not work, then it leads to the genomic instability and subsequently tumor development in a long-term manner. In CSC population of different tumors like lung, breast, glioblastoma, and prostate, the DNA repair mechanism is highly active due to the activation of ATR-Chk1 and ATM-Chk2 pathways (Krause et al. 2016). In cellular physiology, ROS is mainly produced during oxygen metabolism leading to the control of different cellular processes like proliferation, differentiation, and survival (Schieber and Chandel 2014). If ROS level is higher inside the cells, then it leads to irreversible oxidative stress and cell death. Thereby, ROS level is maintained through several scavenging molecules like catalase, peroxidase, glutathione, dismutase, and superoxide (Trachootham et al. 2009). In CSCs, the ROS level is generally found to be lower that contributes to the high resistance to genotoxic stress. Furthermore, it was already known that CSC populations reside in hypoxic region of tumors where oxygen level is very low. If tumor oxygenation is carried out, then CSCs can be more susceptible to current treatments (Kobayashi and Suda 2012). In several studies, nanomedicines have been utilized for increased ROS generation that induces necrosis and apoptosis in cancer cells with various morphological and physiological changes, for example, hyperthermia, a noninvasive treatment procedure that usually kills drug-resistant cancer and CSCs via heat shock and tumor reoxygenation. In addition, SPION nanoparticles were developed to generate heat in a localized tumor cell population area under an alternating magnetic field. Such NPs induced magnetic hyperthermia in MDA-MB-231 and A549 cells. Further, several

CSC-associated assays were showing the removal of ALDH+ CSCs in SPION-treated tumor cell subpopulations. During treatment, CSC killing was achieved through higher ROS generation and acute necrosis. In end, these results concluded that magnetic hyperthermia has the ability to eliminate the tumor relapse state compared to conventional cancer treatments (Sadhukha et al. 2013). Likewise, iron oxide magnetic nanoparticles were functionalized with epidermal growth factor for targeting EGFR receptor overexpressed in breast cancer cells. Such actively targeted nanoparticles were found to enter into the lysosomes of MDA-MB-231 cells. Under the effect of alternating magnetic field, these nanoparticles disrupted the lysosomal membrane and killed EGFR+ breast cancer cells with increased ROS production. Hence, the lysosome-mediated cell death pathway is an alternative independent mechanism to kill drug-resistant cancer cells, when apoptosis pathways become resistant (Domenech et al. 2013). In recent studies, breast CSCs were found to be resistant for traditional hyperthermia. Later, this resistance was observed due to higher expression of HSP90 in breast CSCs. Next, the PEG-coated MWCNTs (multiwalled carbon nanotubes) were designed to kill breast CSCs via thermal treatment that was activated through NIR (near infrared) irradiation. In contrast to magnetic hyperthermia, MWCNT-mediated photothermal therapy increased the survival time of mice with complete tumor regression (Burke et al. 2012). Overall, these electromagnetic field-responsive nanoparticles are in their initial stages of development, but such thermal effect will also lead to the development of other novel anti-CSC therapeutics in the future. In another study, the mitochondria-targeted PEGylated liposomes were formulated and encapsulated with daunorubicin and quinacrine drugs. To achieve mitochondrial targeting, the dequalinium regulator was attached on the surface of the liposomes. The results showed the accumulation of such targeted liposomes into the mitochondria which induced the proapoptotic Bax protein activation, reduced the mitochondrial membrane potential, opened the mitochondrial permeability transition pores, released the Cytochrome-C (Cy7C) from mitochondria to cytosol, and activated downstream caspase signaling. Finally, such nanoformulations induced apoptosis in MCF-7 CSCs and reduced the growth of relapsed tumors at large extent arising from MCF-7 CSCs in female NOD/SCID mice after the combined i.v. injection of daunorubicin and quinacrine liposomes as shown in Fig. 4.6 a, b (Zhang et al. 2012b).

4.4 Future Directions in Nanomedicine-Mediated Cancer Stem Cell Therapy

The current state of drug delivery technology clearly suggests the development of novel nanodrug carriers to enhance the therapeutic efficacy of existing anti-CSC therapies. Among all types of drug delivery systems, polymer-based drug delivery vehicles have gained a major attention related to its widespread use. These polymeric nanodrug carriers are designed with few considerations like controlled drug release profile, batch-to-batch reproducibility, and narrow size distribution. Moreover, such nanodrug carriers can also provide synthetic versatility according to

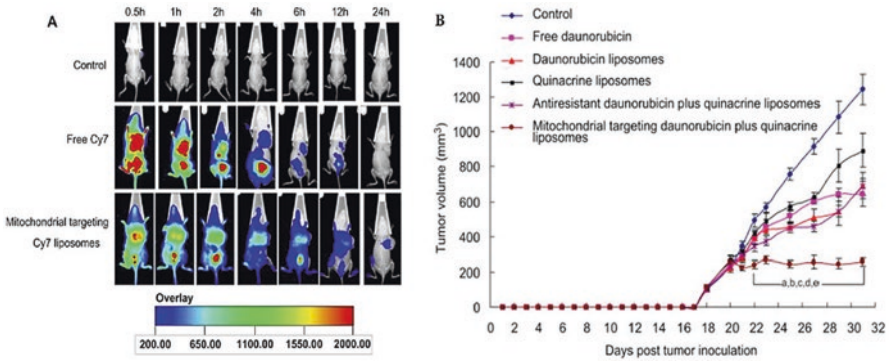

Fig. 4.6 (**a**) In vivo real-time imaging of the relapsed tumors arising from MCF-7 CSCs in female NOD/SCID mice after intravenous injecting PBS (pH 7.4), free Cy7, or mitochondrial targeting Cy7 liposomes. (**b**) Efficacy of mitochondrial targeting daunorubicin plus quinacrine liposomes in treating the relapsed tumors arising from MCF-7 cancer stem cells in female NOD/SCID mice. (Permission obtained from Elsevier press, Ref. no. 107)

the type of application. For example, the surface of polymeric nanodrug carriers can be modified with cancer targeting ligands for site-specific delivery to tumor site. Still, the application of drug delivery technology in CSC-targeted therapies is in an early stage with several unsolved issues. However, mostly nanodrug carriers are under different clinical phase trials and very few of them have been clinically approved. While there is need of more basic and applied research to advance the field of drug delivery for increasing the therapeutic effect of anti-CSC therapy in clinic, we here discuss several less explored issues with more attention to take advantage of such innovative and more effective strategies.

4.4.1 Synthesis of Highly Efficient Targeted Nanoparticles for CSC Therapy

The concept of designing targeted nanoparticles holds their great promises in CSC therapy as these nanoparticles can increase the drug concentration in CSCs for their elimination inside tumor mass. Therefore, the synthesis of such targeted nanoparticles will enhance the therapeutic efficacy of anti-CSC drugs in clinic and reduce the treatment course with patient's better outcome, but still, more attention should be given to the design and optimization of effective nanodrug carriers. If targeting moiety will be modified to achieve higher therapeutic efficacy, then several issues like additional complexity, regulatory barriers, and cost will come into consideration because these problems cannot be ignored. In terms of practice also, many questions were raised related to nanoparticle targeting and drug accumulation in the selected tumor and CSC subpopulation. In this scenario, a fundamental paradox infers that addition of targeting moiety onto the surface of nanoparticles generally compromises with the stealth feature of nanoparticles and

can increase their clearance by reticuloendothelial system from host body. For instance, actively and passively targeted doxorubicin-loaded liposomal nanocarriers were used to elucidate the effect of targeting moiety on blood circulation time and tumor-bearing animal survival. Further, the results showed that no difference was observed related to animal survival between both types of targeted nanocarriers. Also, intratumoral doxorubicin concentrations were equal in both treatments (McNeeley et al. 2007). Although high avidity of nanoparticles is another enigma that has been always seen as an advantage, in case of targeted nanoparticles, such effects reduced their infiltration inside the tumor core (Lee et al. 2010). It was also seen that some CSC populations are found in necrotic region of tumors, i.e., very challenging to reach (Keith and Simon 2007). Overall, targeted nanocarriers can be suitable for treating such types of cancer, where CSCs are easily accessible like leukemia diseases.

As most of the CSC markers are used for the development of targeted nanocarriers, they are also expressed on normal stem cells. In result, it can lead to the unwanted toxicities (Xia 2014). Thereby, the search of highly CSC-specific ligands has always been a very challenging task. We also suggest that targeting of CSCs should be more inclusive and circumvent all the discussed downsides to achieve the ultimate objective of enhanced cancer therapeutic efficacy.

4.4.2 Synthesis of Nanoparticles with Deep Penetration Potentials for Effective CSC Therapy

Several evidence suggested that tumors are heterogeneous in nature which contain two different types of cell populations in their microenvironment, i.e., CSCs and non-CSCs. The CSC population are generally found in hypoxic region (low oxygen level responsible for stemness), while non-CSC population can be seen in vascularized region. For example, CD133+ ALDH+ breast CSCs were located in the central region of tumor tissues (Liu et al. 2014). The non-CSCs and CSCs near to vascularized region were killed easily using therapeutic agents, but CSCs enriched in hypoxic region of tumor core could not be easily targeted due to poor penetration of nanodrug carriers or therapeutic drug molecules. Therefore, to achieve such penetration, several nanodrug carriers could be modified on several aspects including particle size, PEG coating, surface charge, and conjugation of tissue-penetrating peptides. By these modifications, the penetration and retention behavior of nanodrug carriers could be enhanced. Based on this, several intelligent and stimuli-sensitive nanoparticles are designed further. These smart nanoparticles contributed the controlled drug release profile and efficient delivery of therapeutic agents in tumor core. For instance, a pH-sensitive, doxorubicin-encapsulated DLC-PEG liposomal dendrimers were prepared for long circulation and better tumor accumulation. However, the drug-loaded dendrimers were released that further penetrated deeply inside the tumor, where doxorubicin was accumulated and killed the MCF-7 cells (Sun et al. 2014d).

4.4.3 Synthesis of Nanoparticles for Better Cellular Internalization for Effective CSC Therapy

In order to eliminate CSCs, the rationally designed nanodrug carriers have the capacity to deliver any types of therapeutic agents to the CSC-enriched target site. Further, these carriers are characterized with advantages like better internalization rate, higher retention time in the blood, and higher accumulation at tumor sites. While these drug delivery systems were not enough to overcome all limitations, therefore intelligent and stimuli-responsive drug delivery systems such as pH, temperature, and tumor microenvironment responsive were designed. Usually, nanoparticles were PEGylated or modified with other hydrophilic polymers to improve their stability and bioavailability, lessen immunogenicity, and prolong circulation time in the blood. Despite such enormous activity, PEGylation decreased the cellular uptake of nanoparticles resulting in the blockage of intracellular trafficking pathways for diminishing the anticancer therapeutic efficacy (Knop et al. 2010; Mishra et al. 2004). Therefore, to overcome this situation, the PEG molecules were conjugated on nanoparticle surface via stimuli-responsive linker. When these nanoparticles entered the cells, then PEG molecules cleaved under specific stimuli. For example, a liposomal formulation having pH-sensitive PEG-coating and cell-penetrating peptide was prepared, in which PEG molecules were conjugated to phosphatidylethanolamine (PE) lipid molecules via hydrazone (HZ) bonds. When such carriers entered into acidified tumor microenvironment, PEG molecules were removed due to cleavage of HZ bonds and showed site-specific delivery to cells due to TATp moieties (Kale and Torchilin 2010). Further, stimuli-sensitive PEG shield is used to coat several types of nanoparticles to enhance their intracellular delivery. However, this is not well studied that such stimuli-sensitive nanodrug carriers may facilitate their interaction with CSCs. Therefore, it can be a worthy area for its exploration.

4.4.4 Development of Nanoparticle-Mediated Genome Engineering for CSC Targeting

As we discussed in Sect. 4.3.1, there are two major therapeutic nucleic acids used in CSC therapy, miRNA and siRNA. Both nucleic acids suppress those genes responsible for CSC survival via RNA interference at mRNA, protein, and functional level. The main drawback of such mechanism is to repress gene expression incompletely. In result, it may lead to the progression of several diseases, where complete ablation of gene functions is required for therapy. Also, RNA interference exhibits off-target effects, which pose a safety issue and reduce the efficacy of gene therapy (Mittal 2004; Jackson and Linsley 2010). However, the recent advances in gene-editing technology like CRISPR-CAS9 system could harness their potential in manipulation or removal of diseased genes with on-target effects. Also, the CRISPR-CAS9 technology has been used with its huge potential to study genomic rearrangements, analyze gene functions, and inactivate deleterious mutations, insertion of therapeutic transgenes, and introduction of protective genetic mutations for treating

hereditary disorders (Cong et al. 2013; Ran et al. 2013). In the context of anti-CSC therapy, such RNA-guided CRISPR-CAS9 technology completely inhibited the expression of ABC transporters leading to drug accumulation inside CSCs and their killing (Qi et al. 2013). Also, CRISPR-CAS9 technology introduced the BMP4 gene inside the CD133+ hepatocellular carcinoma CSCs (Zhang et al. 2012c). As a result, CSCs underwent differentiation and lost their self-renewal capacity. The main drawback of gene-editing technology is its delivery as nucleic acids cannot reach easily to tumor core enriched with CSCs. Therefore, there is need of nanodrug delivery vehicles to harbor such gene-editing technology like what cationic lipid nanoparticles have shown (Zuris et al. 2014). It also appeared that CRISPR-CAS9 technology has been used at different preclinical and clinical platforms before entering into therapeutic pipeline. Still, there is concern going on related to its safety, efficacy and specificity. As gene-editing technology provides several possibilities to treat various diseases, therefore the advances in drug delivery technology will further increase its performance against several diseases.

4.5 Conclusions

The efficacy of chemotherapy or other therapeutic modalities is found to be reduced in the relapsed cancer patients. Therefore, the search and development of novel anticancer drugs to circumvent drug resistance and more effective treatment is of utmost importance. In this context, recent developments through nanotechnological advancements toward targeting CSCs along with conventional treatment could be the best strategy to overcome the resistance of anticancer drugs. However, the complexity and very limited understanding of tumor organization hamper the progress of nanotechnological approach in this direction. Therefore, the role of multidisciplinary fields is required to develop multidrug delivery systems that would be essential to improve the clinical translation of anticancer drugs in the near future.

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