Chapter 2

Cancer stem cells; important players in tumor therapy resistance

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Abstract

Resistance to tumor therapy is an unsolved problem in cancer treatment. A plethora of studies have attempted to explain this phenomenon and many mechanisms of resistance have been suggested over the last decades. The concept of cancer stem cells (CSCs), which describes tumors as hierarchically organized, has added a new level of complexity to therapy failure. CSCs are the root of cancers and resist chemo- and irradiation therapy explaining cancer recurrence even many years after the therapy ended. This review discusses briefly CSCs in cancers, gives an overview of the role of CSCs in therapy resistance, and discusses the potential means of targeting these therapy resistant tumor cells.
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Introduction

Well over 40 years ago president Nixon signed the national cancer act and officially started the war on cancer. 1 Despite this act and resulting significant improvements in therapy, the war has not ended and cancer is still one of the leading causes of death worldwide. To illustrate, in 2012 14.1 million adults in the world were diagnosed with cancer and in the same year an estimated 8.2 million people died from the disease 2 The main problem we face in cancer treatment is the presence or development of resistance to therapy for which a multitude of distinct reasons have been defined. For instance, acquisition of mutations in key signalling molecules, enhanced anti-apoptotic protein expression, presence of quiescent and/or resistant tumor cells or high expression of drug efflux pumps are all potential means that impair therapy efficacy. 3, 4 In the last decade a lot of attention has gone to the role of a specific subset of cancer cells called cancer stem cells (CSCs). In analogy with their normal counterparts, the stem cells, these cells display a high level of therapy resistance and can effectively repopulate the tumor.

CSCs are the tumorigenic core of tumors

CSCs are defined based on their tumor forming capacity in xenograft studies. 5 These cells normally represent a minority of the tumor cells and can be identified by a long list of markers, although most of these are not restricted solely to the CSC population. 6 CSCs can be selected \textit{in vitro} using spheroid growth in suspension and defined media compositions. Upon injection in mice CSCs, but not their more differentiated counterparts, can very efficiently form tumors that resemble the original tumor from which they were derived, including all differentiated cells. Moreover, re-isolation of the CSCs from xenografts allows for serial transplantation to secondary and tertiary mice, which is the gold-standard assay to prove that tumor cells are indeed CSCs. 5 CSCs were first defined in acute myeloid leukemia (AML) in 1994. 7 CD34+/CD38- expression has long been used to mark progenitor and pluripotent stem cells in the bone marrow. Intriguingly, a similar subpopulation was detected in AML and xenotransplantation of specifically this CD34+/CD38- leukemia cells resulted in leukemia in mice that reproduced many features of human AML. 7 A decade later CSCs were detected in solid tumors. Breast, glioblastoma, prostate and colorectal tumors are only some of the tumor types where CSCs were identified. 6, 8-12 The variety of tumors in which CSCs were identified suggests that it is a common feature in most cancers, although some observations indicate that it may not occur in all tumor types or alternatively at all stages of disease. 13-16
As mentioned above, CSCs are highly tumorigenic and therefore are also referred to as tumor-initiating cells. The name “CSC” does not refer to the fact that CSCs can be derived from normal stem cells, but rather points to the idea that CSCs display properties normally attributed to stem cells. Firstly, stem cells have the capacity to self-renew, i.e. to form a new stem cell upon division, and secondly stem cells can differentiate into the more specialized cell types that make up a tissue. Self-renewal and differentiation of stem cells is regulated by morphogenic pathways and interestingly these signaling pathways are also highly active in many CSCs, suggesting that equal regulatory principles exist in CSCs. One of the morphogenic pathways that is active in stem cells is the Wnt signaling. This pathway determines self-renewal and cell fate of hematopoietic stem cell (HSCs). High activity of this pathway is also observed in stem cells of other tissues like breast and colon. Next to Wnt signaling, Notch signaling is shown to be essential for stem cell maintenance. Notch signaling is highly active in HSC when compared to more differentiated cells and inhibition of Notch signaling promotes differentiation of HSC. Likewise, Hedgehog (HH) signaling regulates proliferation and self-renewal of stem cells and activation of HH signaling is able to expand HSC and brain stem cells \textit{in vitro} and \textit{in vivo}. In contrast to Wnt, Notch, and HH signaling BMP signaling is inhibiting stem cell expansion. Activation of BMP signaling results in suppression of Wnt signaling and this controls stem cells numbers. Combined these morphogenic pathways regulate stem cell fate and differentiation cues. Intriguingly, this regulatory network appears to extend to CSCs. In a high throughput screening in breast CSCs salinomycin was identified as a compound that eliminates CSCs. This antibiotic was shown to inhibit Wnt signaling and as a result is capable to differentiate breast CSCs. High Wnt pathway activity is also shown to be important for cell fate of CSCs from many tumors like CLL, breast, CRC, squamous cell carcinoma, and lung cancer. In these tumors inhibition of Wnt pathway activity, with e.g. salinomycin is detrimental for CSCs. Beside Wnt signaling, Notch signaling can also regulate CSC self-renewal. Inhibition of Notch signals can be achieved by neutralizing antibodies against DLL4, or treatment with a g-secretase inhibitor (DBZ). GBM, CRC and breast cancer stem cells need high Notch activity and inhibition of Notch results in loss of CSCs. Furthermore, Hedgehog signalling is highly active in CSCs, which is shown to be required for self-renewal of CSCs in various cancers like breast, lung, and CRC. Morphogenic pathways and inhibitors are depicted in figure 1.

In addition to the analogous usage of morphogenic pathways, stem cells and CSCs also appear to share high activity of DNA repair pathways. For example, HSC can
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Figure 1: Targeting morphogenic pathways in CSCs
Morphogenic pathways Notch (blue), Wnt (green) and Hedgehog (red) signaling pathways are highly active and important for self-renewal of stem cell and CSCs. Blue: Notch signaling is activated via direct cell-cell contact. A cell expressing Notch ligand (e.g. DLL4) contacts with another cell that expresses Notch receptor. When bound by a Notch ligand, the intracellular domain of the Notch receptor (IC-Notch) is cleaved by γ-secretase (γsec) and is targeted to the nucleus to activate transcription of downstream target gene that enhance self-renewal of CSCs. This pathway can be inhibited with a DLL4 antibody that neutralizes Notch ligand DLL4. Also γ-secretase inhibitors like DBZ are efficient in blocking Notch signaling. Green: Wnt ligands binds to the Frizzled-LRP receptor and inhibit a cytoplasmic destruction complex (APC-GSK3β-Axin) of β-catenin, which then enters the nucleus to activate transcription of Wnt target genes that are known to be important for CSCs maintenance. Wnt signaling can be inhibited with salinomycin. Red: The Hedgehog pathway is activated by Hedgehog ligands binding to the Patched receptor, which releases its inhibition of the Smoothened (Smo) transmembrane receptor. Smothened can then in turn activates Gli transcription factors, the final effectors of the Hedgehog pathway. The natural occurring compound cyclopamine is used to inhibit Smo receptor and thereby block Hedgehog signaling.
repair UV induced single-strand breaks faster when compared with more differenti-
ated cells \(^{40}\) and this was reported in CSCs as well. \(^{41}\) It is important to mention that
more and more evidence indicates that CSCs are not a fixed cell population, but rather
represents a state of tumor cells that appears inducible. Giving the right signals from
the micro-environment or introduction of new mutations can result in de-differentia-
tion of more differentiated tumor cells into CSCs. \(^{31, 42-44}\)
Not only signaling pathways, but also cell surface molecules are similarly expressed on
stem cells and CSCs. The pentaspan membrane glycoprotein CD133, also known as
Prominin-1, is expressed in normal stem cells, e.g. hematopoietic, neural, and intestinal
stem cells, \(^{45-47}\) but CD133 was also used to identify CSCs from different tumor types.\(^{8, 9, 41, 48}\) Moreover, in the last decade the G-protein coupled receptor Lgr5 received a lot
of attention because high Lgr5 expression was reported to mark stem cells in various
organs. This target gene of the Wnt signaling pathway is exclusively expressed by stem
cells of various organs \(^{49-52}\) and we and others have shown that Lgr5 also marks CSCs
in various tumors. \(^{53, 54}\) Most of the currently used markers have no known role in CSC
biology. In contrast, Aldehyde dehydrogenase isoform 1 (ALDH1) oxidizes aldehydes
to carboxylic acids and thus for instance catalyzes the conversion of retinol (vitamin
A) to retinoid acid. Inhibition of ALDH1 reduces retinoic acid levels and thereby
promotes HSC and breast CSCs self-renewal. \(^{55, 56}\) ALDH1 is highly expressed in many
stem cells and CSCs, and ALDH1 expression and its activity were used to isolate stem
cells and CSCs. \(^{57, 58}\) Taken together, it appears that stem cells and CSCs are wired in
the same way and share expression of several cell surface markers. Unfortunately, this
similarity extends to a more detrimental property, namely therapy resistance.

**The extreme survivors; CSCs and their therapy resistance**
The concept that CSCs selectively resist therapy stems from a multitude of observa-
tions in cell culture, animal models and cancer patients. In cell culture direct analysis
of apoptosis revealed that differentiated colon cancer cells are induced to die upon
chemotherapy treatment, while colon CSCs from the same cultures survive the toxic
insults. \(^{60}\) This differential sensitivity was not due to proliferation differences between
CSCs and more differentiated cells as it was also observed when using treatments that
are not dependent on cycling cells. \(^{60}\) Moreover, these surviving CSCs can re-estab-
lish the culture, confirming the idea that they are responsible for therapy failure. \(^{60}\)
Chemotherapy resistant CD133\(^+\) CSCs were described in liver and lung cancer as
well, \(^{61, 62}\) Similar observations were derived in pancreatic cancer where CSCs were
isolated from patient specimens and subsequently treated with gemcitabine. Also in
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this tumor type in vitro cell death was more pronounced in the differentiated CD133- cells as compared to the CD133+ cells. Finally, GBM CSCs and breast CSCs isolated from patient specimens, displayed selective resistance to various chemotherapies. Next to the in vitro evidence, escape from therapy was also evident from xenograft studies. Chemotherapy treatment of xenotransplanted CRCs resulted in an increase in CD133+ in the tumor. This indicates that CD133+ CSCs are more resistant to oxaliplatin in vivo when compared to differentiated CD133- cells. In vivo resistance of CRC CSCs is not restricted to oxaliplatin as mice bearing human CRC tumors treated with irinotecan show an increase in cells that express ESA+ CD44+ CD166+. Intriguingly, therapy resistance appears to be a general feature of these cells and is not restricted to chemotherapy, but observed with radiotherapy as well. Irradiated glioblastoma, either implanted subcutaneously or intracranially showed an increase of CD133+ cells when compared to non-irradiated tumors. This distinction was suggested to be clinically relevant as the authors extended these findings to ex vivo irradiation of surgically removed glioblastomas. Also for irradiation examples encompass other tumor types. MMTV-Wnt1 mice bearing breast tumors showed an increase in the CSC (Thy1+ CD24+ Lin-) fraction after irradiation and in the same study CSCs from primary human head and neck cancers proved radioresistant. Combined these data indicate that cell line or primary tumor-derived cells with CSC markers display decreased sensitivity to chemo and radiotherapy. It is important to realize though that one potential caveat with this conclusion is the fact that CSC markers are heavily debated, suggesting that the increases observed in marker expression may not represent enhanced stemness (for a review see Medema 2013). Moreover, xenotransplantation models may not adequately represent the normal situation in patients and select for distinct traits and/or markers. Nevertheless, a first direct hint that this CSC resistance concept could explain minimal residual disease and therapy failure in patients came from a study of the group of Luis Parada who used a genetically modified mouse model to study endogenously growing tumors in which CSCs could be traced using a Nestin reporter construct. Nestin+ tumor cells, which represent a quiescent CSC population, could fully repopulate the tumor after temozolomide chemotherapy, while selective deletion of these cells prevented tumor outgrowth. These data indicate that CSCs resist therapy and might be a poten-
tial cause of tumor relapse. In line with this observation, an increasing list of observations in patients support the crucial role of CSCs in tumor relapse after therapy. In patients with GBM, CRC, or breast cancer, increased CSC fractions using marker expression were measured after chemotherapy treatment. 71-74 A more direct evidence for increases in true functional CSCs came from a study in breast cancer. In contrast to other reports, here authors studied patient samples and performed functional assays. Increase in mammosphere formation capacity was seen after chemotherapy treatment, 72, 73 proving that stemness rather showed a relative increase than decrease upon therapeutic intervention. The growing body of evidence that points to a role for CSCs in resistance warrants a more detailed survey to increase our understanding of the mechanisms that determine resistance in order to target these survivors of therapy.

Mechanisms behind therapy resistance
Normal stem cells contain multiple mechanisms to control cell death, which aids to protect these crucial cells from cytotoxic insults. Elevated apoptosis resistance, drug-efflux pumps, enhanced DNA repair efficiency, detoxification enzyme expression and quiescence are all identified as pro-survival mechanisms. Intriguingly, all these mechanisms appear to be hijacked by CSCs. For instance, mitochondrial apoptosis is associated with loss of mitochondrial membrane integrity, which is maintained by a strict balance of anti-apoptotic BCL2 proteins (e.g. BCL2, BCLXL, and MCL1), pro-apoptotic BCL2 family members (BAX and BAK) and BH3 proteins (e.g. BIM, BAD, and NOXA). A cytotoxic insult-induced imbalance in the ratio of these molecules results in permeabilization of the mitochondrial outer membrane and subsequent activation of a caspase cascade. 75 In stem cells, but also in CSCs, an elevated anti-apoptotic protein expression increases the threshold for apoptosis induction and thereby directly protects the cells against apoptosis. For instance, in breast and AML CSCs, BCL2 and BCLXL are highly expressed. 76, 77 Similarly, in primary GBM cultures CD133+ CSCs had elevated expression of BCL2 and BCLXL compared to their more differentiated CD133- progeny. 78 In agreement with a role for apoptosis regulation in CSCs, direct proteomic analysis of CRC CSCs and differentiated cells revealed “apoptosis” as one of the main molecular pathways affected, involving differential expression of key anti-apoptotic proteins, including BIRC6. 79 Combined this suggests that CSCs have an elevated anti-apoptotic threshold. Recent data confirm this idea using so-called BH3 profiling, an assay to directly measure the apoptosis priming state of cells. 80 This revealed that CRC CSCs were less-primed as compared to differentiated cells, which at least in part explains their resistance to conventional chemotherapy. 80 In agreement, sublethal doses of BH3
mimetics can change this threshold and strongly sensitize CSCs to chemotherapy. Besides an elevated apoptotic threshold, CSCs display high expression of drug efflux pumps, like ATP-binding cassette (ABC) transporter family proteins. The proteins are important for efflux of chemotherapy across the plasma membrane. Various ABC transporter proteins are highly expressed in HSC and in AML CSCs (CD34+/CD38-) compared to the non stem (CD34+/CD38+) cells. Also in GBM and melanoma high expression of drug efflux pumps in CSCs are reported. In the latter, expression of ABC transporter ABCB5 in fact serves as a marker for CSCs. Surprisingly, in CRC a different scenario is reported. Here not CSCs, but rather the differentiated cells express high levels of the drug efflux pump ABCB1. The authors suggested that differentiated cells protect CSCs from chemotherapy treatment by forming a protective rim around the CSCs.

The above points to the fact that CSCs employ means to avoid the impact of therapy, which we can potentially circumvent using combination therapy. However a potentially more challenging problem is the recent observation that CSCs may exist that display quiescent properties. Selectivity of chemotherapy for cancer cells relies on the fact that chemotherapy mainly kills cells that are highly proliferative. As rapid uncontrolled proliferation is a standard feature of many tumor cells, chemotherapy is thought to target tumor cells selectively over non-proliferating normal cells, consistent with the observed toxicity in organs with rapidly dividing cells, such as bone marrow, digestive tract, and hair follicles. In contrast, slow proliferating or quiescent normal cells are largely protected from chemotherapy treatment. Importantly, this resistance also extends to quiescent tumor cells. In ovarian cancer CD24+ CSCs are less proliferative and more resistant to chemotherapy when compared to CD24- cells. Recent data point to the existence of CSCs that are quiescent. These can be identified using the dye PKH26, which dilutes out when cells proliferate and therefore only low or non-proliferative cells will retain the label. In primary melanoma cultures label retaining cells were detected with a very low doubling time of around 4 weeks in vitro. Although these cells are slow dividing they have increased sphere forming capacity in vitro suggesting that these label retaining cells are enriched in CSCs. Such quiescent cells are also identified in pancreatic adenocarcinoma and shown to be enriched for CSC markers like CD133, CD24+/CD44+ and ALDH. In agreement with this notion, these label retaining cells are more tumorigenic, indicating that cancer is not a disease of homogeneously rapidly proliferating cells, but also contains quiescent cells that can escape classical chemotherapy and subsequently induce regrowth of the tumor. Quiescent cells not only display decreased sensitivity to chemotherapy, but also
contain enhanced potential and/or time to repair the damage that is inflicted to them. As many chemotherapeutic agents as well as radiotherapy work by inducing DNA damage, cells that effectively repair DNA damage can potentially survive chemotherapy. Various reports have shown that CSCs, for instance from GBM, possess high DNA repair activity, which makes them resistant to radiation and chemotherapy. Similarly, in breast CSCs there is increased expression of DNA repair genes, indicating that high DNA repair pathway activity may aid in making CSCs resistant to tumor therapy. In conclusion, there are many ways for CSCs to resist tumor therapy. Figure 2 illustrates the reasons for therapy resistance in CSCs.

Killing CSCs, magic bullets or combination cocktails?
Although considered bad news, the efficient DNA repair of CSCs may also point to a dependency for these mechanisms and as such offer a means to target these cells. For example, CSCs in GBM have elevated activity of Chk1 and ATM and survive irradiation, but inhibition of the cell cycle checkpoint kinases Chk1 and Chk2 is sufficient to sensitize CSCs towards irradiation. Recently, it has been reported that a combined Chk1 and PDK1 inhibition is required to kill CSCs in GBM. Similarly, non-small cell lung cancer CSCs can be sensitized to chemotherapy by combining treatment with Chk1 and Chk2 inhibitors SB218078 or AZD7762. Mechanistically, inhibition of Chk1 results in active Cdc2-cyclin B complex that is followed by mitotic catastrophe. Although effective, these compounds are also relatively toxic and combination of the Chk inhibitor AZD7762 with gemcitabine showed cardiac toxicity. To overcome this toxicity an inhibitor of a downstream target of Chk1, Wee1, was developed. In the presence of DNA damage Wee1 arrests cells in G2 phase and allows cells to repair DNA before entering into mitosis. Interestingly, Wee1 is reported to be overexpressed in GBM CSCs. In the same report, the authors show that inhibition of Wee1 with PD0166285 sensitizes GBM CSCs towards irradiation. Besides targeting the core of the repair machinery, a lot of effort is put into targeting the execution machinery in cancer cells. Previously, we have used an inducible caspase-9 to target colon CSCs. Upon activation of caspase-9, colon CSCs were killed efficiently in vitro and in vivo suggesting that activation of caspases are sufficient to efficiently kill CSCs. As described, anti-apoptotic proteins are highly expressed in various cancers and especially in CSCs. Targeting these anti-apoptotic proteins using small molecules that have been developed therefore forms an attractive mechanism. For instance, ABT-737, a small molecule inhibitor that targets BCL2, BCLXL, and BCLW tips the apoptotic balance to a more pro-apoptotic state and reverts the resistance of colon CSCs. Unfortunately, Navitoclax (ABT-263), an orally bioavailable variant of ABT-737 with
Figure 2: Mechanisms of therapy resistance in CSCs
a) Four mechanisms that are used by CSCs to resist chemotherapy. Efficient DNA repair (orange), quiescence (red), increase ABC transporter expression (green), and decreased mitochondrial priming (blue).
b) Potential means of targeting therapy resistant CSCs.
the same specificity, seems to exert high toxicity for platelets, which depend on BCLXL for survival. Although more selective inhibitors, such as ABT-199 targeting only BCL2, have been developed to overcome this problem, our recent data indicates that also colon CSCs are dependent on BCLXL for survival. In agreement inhibition of BCLXL with ABT-737 or a BCLXL specific inhibitor (WEHI-539) is sufficient to kill colon CSCs. Similarly, lung CSCs are shown to be dependent on BCLXL and also in these cells inhibition eliminates lung CSCs in vitro and in vivo. Although this still raises the problem of toxicity, it is possible to use sublethal amounts of BCLXL inhibition, which is sufficient to strongly sensitize CSCs towards chemotherapy. It is currently not completely clear why colon CSCs acquire this dependency on BCLXL. One possible explanation is the observation by Todaro and colleagues showing an autocrine loop of IL4/IL4R in colon CSCs, which appears to maintain BCLXL levels and protect CSCs from chemotherapy. One thing that allows for the identification of CSCs is the presence of several cell surface markers. Various groups and companies therefore developed immunotoxins that directly target such CSC markers. For instance, antibodies against for instance the stem cell marker CD133 conjugated to paclitaxel or cytolethal distending toxin (Cdt) target CD133 expressing cells and show in vitro and in vivo elimination of tumors. Similarly, targeting of CD133+ cells can be achieved by generation of CD133 specific Measles viruses. These oncolytic viruses infect CD133 expressing cells and destroy them by lysis. Moreover, selective killing of CD133+ GBM cells was shown with CD133 antibodies coupled to single walled carbon nanotubes (SWNTs). These anti-CD133-SWNTs induce thermal destruction of cancer cells when it is combined with nearIR laser light. However, CD133 expression is not specific for CSCs but also expressed on normal stem cells, which should be protected from such therapies at all times. To minimize toxicity and deliver drug to cancer selectively photochemical internalization (PCI) was developed. This technique makes it possible to release drug in the tumor area specifically. Next to CD133, there is an increasing effort to target other cell surface molecules including the stem cell marker Lgr5. Nevertheless, as is true for CD133, toxicity with such an approach can be expected. Surprisingly, antibodies without toxins targeting other cell surface molecules are shown to be efficient in killing CSCs as well. Antibodies against CD47 give promising effects in various cancers. CD47 is a receptor that is involved in inhibition of so called “eat-me” signals and is highly expressed on CSCs compared to more differentiated cells. Blocking of this CD47 receptor with an antibody enables the phagocytosis of AML CSCs and thereby blocks tumor growth. In addition, CD47 inhibition also
blocks tumor growth in solid cancers, like breast cancer, CRC, ovarian cancer and GBM, which is also suggested to depend on facilitating phagocytosis of CSCs. 105

Next to phagocytosis induction, several antibodies have shown to delete essential signals from CSCs. For instance, direct targeting of breast CSCs can be achieved by using an antibody against CXCR1. The IL8 receptor CXCR1 is expressed almost exclusively on CSCs and Repertaxin, an inhibitor of CXCR1/2, or anti-CXCR1 treatment induces cell death in CXCR1+ breast CSCs, which appears to be mediated by AKT signaling inhibition. 64 Intriguingly, PI3K/AKT signaling addiction in colon CSCs was also reported in colon CSCs, where a CD44v6-positive subset was identified that is exclusively metastatic. These cells express high levels of PI3K, which, if inhibited, alter the viability of the cells and impede the capacity to migrate, 104 suggesting that PI3K signaling is crucial for CSCs.

As described, CSCs require signaling through morphogenic pathways for their maintenance, suggesting that these may be attractive targets for therapy as well. In agreement, a screen for CSC sensitizing compounds identified salinomycin, which inhibits Wnt signaling and eliminates breast and CLL CSCs. 28, 32 As CSCs in CML, AML and skin tumors are dependent on the Wnt pathway, inhibition can be clinically relevant. 29, 105-107 Furthermore, inhibition of Notch signalling pathway using a neutralizing antibody against DLL4 results in less tumor engraftment in secondary tumors suggesting in vivo differentiation of CSCs. Importantly, DLL4 antibody was also able to sensitize the tumor to irinotecan in vivo. 55 Inhibition of Notch signaling was also sufficient to deplete GBM CSCs and sensitize ovarian CSCs to chemotherapy. 35, 108 Lastly, HH signaling can be inhibited by using cyclopamine and this Smoothened antagonist sensitizes AML CSCs to Ara-c treatment. 109 Similarly, in GBM and in pancreatic cancer decreases in CSCs are observed after treatment with Smoothened inhibitors cyclopamine or CUR199691. 110, 111 These data point to a crucial role for HH signaling in cancer stemness and this is confirmed by knockdown of Smoothened, which results in loss of CML CSCs. 112 Antibodies can also be used to target CSC niche. Blood vessels maintain GBM CSCs in a stem like state. Targeting microenvironment with Bevacizumab, an antibody against VEGF is able to differentiate GBM CSCs. 113 In 2006 Jin et al showed that using an antibody against CD44 decreased homing of AML cells and thereby promoting the AML CSCs to differentiate to a more mature cancer cell progeny. This antibody inhibited AML growth in mice. 114 Forcing CSCs to non tumorigenic differentiated cells is clinically very relevant and is
reported to happen with bone morphogenetic protein 4 (BMP4) as well. In CRC BMP4 expression is exclusively expressed by differentiated cancer cells and shown to induce differentiation of CSCs and sensitization to oxaliplatin in vivo. In addition, BMP4 also forces GBM CSCs to differentiate and thereby inhibits their tumorigenicity. Not only inhibition of morphogenic pathways, but activation of signaling pathways can change CSCs cell fate as well. Activation of the unfolded protein response (UPR) induces differentiation of stem cells in the mouse intestine. In line with this, salubrinal induces UPR in colon CSCs and forces them to differentiate. In addition to inducing differentiation, UPR sensitizes cells to chemotherapy in vitro and in vivo (M.C.B. Wielenga and S. Colak unpublished observations). Although targeting CSCs by forcing them to differentiate or by the induction of apoptosis seem to be an attractive therapeutic options, the suggested flexibility of the system is a clear caveat. Even when CSCs are eliminated within a tumor, differentiated cells can de-differentiated and take the place of the CSCs that were deleted. Targeting the cues that induce de-differentiation or simply attacking both CSCs and more-differentiated cells needs to be achieved to eradicate a tumor. Altogether, direct CSCs targeting or CSC differentiation therapy are promising means to improve tumor therapy. Further studies are needed to investigate the most promising combination treatments that does not give severe toxicities.

**Summary**

Identification of CSCs in many tumors allowed for a better understanding as to why even many years after therapy tumors can relapse. There is increasing evidence that targeting these CSCs is important to improve therapies. Here we reviewed mechanisms that make CSCs resistant to therapy. A better understanding of these mechanisms and the way CSCs retain their tumorigenic stem cell capacities is crucial. The exiting new insight will undoubtedly provide new therapeutic tools in the years to come.

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