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Breast Cancer

The Role of Cancer Stem Cells in Breast Cancer Initiation and Progression: Potential Cancer Stem Cell-Directed Therapies

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ABSTRACT

Recent studies have identified a small population of highly tumorigenic cells with stem cell properties in human breast and other solid tumors that are considered to be the source of tumor initiation and maintenance; these cells are referred to as cancer stem cells (CSCs). Preclinical data suggest that current breast cancer treatment strategies lead to CSC enrichment, contributing to chemotherapy and radiotherapy resistance, although a strong correlation with clinical parameters and prognosis is yet to be established. Importantly, overcoming treatment failure by effective targeting of CSCs may be an appealing approach, potentially leading to improved clinical outcomes for patients with breast cancer. Several preclinical studies provide promising results that support this hypothesis. The purpose of this review is to summarize the role of CSCs in breast cancer recurrence and resistance and to discuss current attempts of CSC targeting. *The Oncologist* 2012;17:1394–1401

INTRODUCTION

During the past several years, experimental data support that tumors, including breast cancer, are composed of heterogeneous cell populations with different biological properties [1–5]. It has been suggested that the tumorigenic process is preserved by a small subpopulation of cells referred to as cancer stem cells (CSCs), accounting for only 1%–5% of all tumor cells [5]. CSCs are defined by their ability to initiate tumors in immunocompromised mice and to differentiate into neoplastic cells forming the tumor bulk, due to their capacity of self-renewal and triggering differentiation in their progeny [6, 7].

CSCs display several features that can be of great importance in the understanding of carcinogenesis. They exhibit high invasive capacity, clonal evolution, and dormancy; promote blood vessel formation; and trigger cell motility [8, 9]. In addition to driving tumorigenesis, there is increasing evidence that they contribute to tumor progression [10] and metastasis [11, 12].

The role of CSCs in breast cancer is recently becoming elucidated. Highly tumorigenic cells with properties consistent with those of CSCs have been isolated from breast cancer by virtue of expression of cell surface and other markers. More specifically, breast CSCs (B-CSCs) bear the phenotype $CD44^+/CD24^{low}$ [5] and overexpress aldehyde dehydrogenase (ALDH) 1, a detoxifying enzyme that regulates the oxidation of intracellular aldehydes and plays a role in stem cell differentiation [13]. More recently described identifying markers include reduced 26S proteasome activity and α6- and β1-integrins [14, 15]. Furthermore, B-CSCs can be isolated by formation of spherical clusters (mammospheres) in suspension cultures as a result of their self-renewal capacity [16]. Finally, they can be identified by the so-called side population cells, which pump out the fluorescent dye H33342 via ABCG2, a transmembrane transporter that is being overexpressed in B-CSCs [17].

In this review, we summarize the existing preclinical evidence that indicate a role of CSCs in response to chemotherapy and radiation in breast cancer. We also discuss the potential impact of CSCs in clinical outcomes of patients with Breast

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Cancer. Most importantly, we refer to the exciting development of future CSC-directed therapies.

The CSC Hypothesis

Tumor heterogeneity has been long recognized, and the concept that tumors might originate from a rare population of cells with stem cell properties was suggested 150 years ago [18]. However, the CSC hypothesis has only recently been appreciated and supported due to advances in molecular biology, which allowed the development of new techniques and animal models of carcinogenesis that recapitulate human disease. The CSC hypothesis is now gaining ground against the classical model of oncogenesis that emphasizes random mutations as the primary source of tumor transformation [19].

It is known that tissues normally originate from organ-specific stem cells that undergo self-renewal and differentiation into the cell types that comprise each organ [20]. According to the CSC hypothesis, tumors arise from either tissue stem cells or their immediate progeny, which acquire infinite capacity to self-renew. When a CSC undergoes an asymmetrical division, it generates one daughter cell that is an exact copy of the original CSC and is able to initiate tumors, and another daughter cell that has limited self-renewing potential but high proliferation rate. Consequently, tumors contain a cellular subcomponent that retains key stem cell properties and a large amount of rapidly dividing cells that form the bulk of the tumor [21, 22]. Interestingly, in breast cancer, it has been recently shown that several early oncogenic events can play a role in the procedure. More specifically, HER2/neu amplification, which is found in 15%–20% of human breast cancers, results in more frequent and symmetric self-renewing divisions of CSCs, contributing to increasing numbers of CSCs in tumoral tissues; it has been also suggested that its continuous expression is required to sustain tumorigenesis [23, 24]. Similarly, loss of the phosphatase and tensin homolog (*PTEN*) gene, a defect found in approximately 40% of breast cancer cases, has been reported to increase the number of CSCs [25].

In breast cancer, the CSC hypothesis might have implications in prevention, detection, and treatment [26]. Furthermore, the heterogeneity of breast cancer is attributed by some investigators to be a function of CSCs, which constitute its originating cells [27]. It is also suggested that the CSC hypothesis can be incorporated in the molecular staging of breast cancer, in a sense that CSCs can generate cells with a certain type of limited and aberrant differentiation, which can translate into breast molecular subtypes [28, 29]. The claudin-low molecular subtype of breast cancer, which includes triple-negative invasive carcinomas, is suggested to be the most stem-like tumor, because CSC-like features, such as $CD44^+/CD24^{low}$ phenotype and ALDH1 expression, are highly found within it [30].

On the other hand, there is a growing body of evidence that a strong association exists between B-CSCs and epithelial-mesenchymal transition (EMT) [31, 32]. EMT is widely documented to play a key role in converting both normal and neoplastic epithelial cells into derivatives with a more mesenchymal phenotype. In the context of neoplasia, passage through an EMT results in the acquisition of cell-biological traits associated with high-grade malignancy, including motility, invasiveness, and an increased resistance to apoptosis—features associated with metastasis [33, 34]. Thus, in addition to conferring malignant cell-biological traits, it is suggested that forced passage of both normal and neoplastic mammary epithelial cells through an EMT confers on the resulting cells many of the properties of B-CSCs [31].

The presence of CSCs may contribute to the development of therapeutic resistance and relapse in breast cancer. Current therapeutic agents are directed against rapidly proliferating cells rather than cells that divide infrequently, such as CSCs, thus failing to address the tumor initiating and renewing compartment [8]. Consequently, it could be argued that if CSCs have different sensitivity to therapy than the majority of cancer cells, treatment will not succeed in complete cancer eradication because the shrinkage of the tumor reflects the effect on the differentiated non-CSC cell component. On the other hand, isolated targeting of CSCs may not be sufficient, especially in advanced cancer. Apparently, the simultaneous elimination of both the CSC population and non-CSC neoplastic cells might be the most effective treatment strategy [27].

Preclinical Evidence for the Role of CSCs in Breast Cancer

Response to Chemotherapy

Successful isolation of breast CSCs was followed by an effort to investigate their potential effect on response to chemotherapy agents commonly used in patients with breast cancer. Several preclinical studies indicate that CSCs are relatively resistant to antineoplastic agents. Most of these studies were generally performed in vitro in isolated breast cancer cells or single cell suspensions established from breast cancer tumor biopsies, or in vivo in mammary tumor models.

Several studies conducted in breast cancer mammary models have shown survival or significant enrichment of $CD44^+/CD24^{low}$ cells after administration of chemotherapy. One study demonstrated that 1 week after administration of paclitaxel/epirubicin in TM40D murine breast cancer cells, the vast majority of surviving cells expressed the CSC phenotype $CD44^+/CD24^{low}$ [35]. Because the combination of paclitaxel/epirubicin is widely used in first-line treatment for breast cancer, survival of CSCs, which can then go on to generate more tumor cells, might be implicated in relapse after treatment with these agents. Similar findings were demonstrated in studies performed in breast cancer tumor biopsies [36, 37]. A molecular signature for both $CD44^+/CD24^{low}$ cells and mammosphere cultures enriched in self-renewing cells has also been identified. An increase in cells bearing this gene signature was observed after treatment with docetaxel, consistent with survival of CSCs [37].

The impact of CSCs on response to chemotherapy has also been investigated in the context of HER2-positive breast cancer. Survival of Sca1-positive cells from tumor spheres derived from HER2-positive mammary carcinomas has been noted after treatment with doxorubicin [38]. In the clinical setting, the HER2-positive status is associated with better response to anthracycline therapy [39]; however, CSC survival might be im-

plicated in breast cancer relapse after initial treatment. In a more recent study, HER2 expressing MC7 mammary tumor cells were treated with trastuzumab and natural killer cells that are responsible for the so-called antibody-dependent cell-mediated cytotoxicity (ADCC), which is thought to contribute to the therapeutic effects of trastuzumab [40]. Interestingly, treatment resulted in selective survival of cells that had the characteristics of B-CSCs. When re-expanded, these cells could initiate tumor cell cultures that exhibited the same HER2 expression and ADCC sensitivity with the primary cell cultures but they were more tumorigenic due to a higher proportion of CSCs. Taken together, these data suggest that CSCs might be the source of clinical relapse and progression in HER2-positive breast cancer because they could regenerate the tumor after initial therapy-induced regression. Furthermore, it has been suggested that readministration of trastuzumab could be beneficial for relapsed tumors, since the regenerated cell cultures displayed identical HER2 expression and ADCC susceptibility. This corresponds with clinical reality because retreatment with trastuzumab can be considered in relapse, alone or in combination with other agents [41]. Finally, lapatinib has been shown to decrease the percentage of $CD44^+/CD24^{low}$ cells when given as neoadjuvant therapy for patients with HER2-positive breast cancer, although statistically not significant [42]. It could be therefore suggested that lapatinib could be used to target CSCs in combination with chemotherapy agents. This might explain antitumor activity of lapatinib when administered in combination with capecitabine in metastatic breast cancer [43].

It is worth mentioning that the role of CSCs in chemoresistance has also been studied in BRCA1-positive breast cancer tumors. In spontaneous BRCA1-positive mammary tumor models, treatment with cisplatin resulted in tumor shrinkage but subsequent regrowth [44]. Interestingly, secondary tumor transplants generated from CSC cells were found not only to be platinum-refractory but also to have an increased proportion of CSCs compared to primary transplants that were partially platinum responsive. This suggests a model of chemoresistance, where the platinum resistant CSCs expand and increase their proliferation rates. Although cisplatin is not commonly used in breast cancer treatment, this study indicates that clonal evolution of CSCs might contribute to treatment resistance in BRCA1-positive tumors.

Recent data indicate that CSCs act as a subpopulation of drug resistant cells that survive chemotherapy and repopulate the tumor. Several explanations might account for CSC chemoresistance. Firstly, stem cells are not actively dividing cells; they are slowly proliferating in the G0 phase of the cell cycle and therefore resistant to cell-cycle active chemotherapy agents [8]. Additionally, resistance to apoptosis due to increased expression of antiapoptotic proteins such as bcl-2 might be a contributing factor [45]. Furthermore, CSCs express high levels of multifunctional efflux transporters from the ATP-binding cassette (ABC) gene family that have been known to play an important role in multidrug resistance of tumor cells. More specifically, they express the transporter-encoding genes *ABCG2* and *ABCB1* that constitute the principal multidrug resistance genes [46]. Finally, the enzyme ALDH, which is a molecular marker of CSCs, is able to metabolize chemotherapeutic agents, such as cyclophosphamide, which is widely used in front-line treatment for breast cancer [47].

It is worth mentioning that among all studies investigating the role of CSCs in breast cancer chemotherapy resistance, there is one study demonstrating contradictory results. Surprisingly, a statistically significant drop in $CD44^+/CD24^{low}$ cells has been shown in breast cancer tumor biopsies after neoadjuvant treatment with the regimen epirubicin/cyclophosphamide [48]. This finding questions the proposed role of $CD44^+/CD24^{low}$ cells as the cause of chemoresistance. Interestingly, in another recent study performed in breast cancer tumor biopsies, an increase of the population of ALDH1-positive cells but not $CD44^+/CD24^{low}$ cells has been observed after neoadjuvant treatment with paclitaxel and epirubicin/cyclophosphamide/fluorouracil [49]. Taken together, these results challenge the role of CSC molecular markers for the identification of CSCs in terms of chemoresistance and emphasize the need for further investigation.

Response to Endocrine Therapy

Increasing evidence supports the role of CSCs in resistance to endocrine therapy in breast cancer. Recently, a subpopulation of estrogen receptor (ER)−/progesterone receptor (PR)−/CD44+/CK5+ cells that share the properties of CSCs has been identified in ER+/PR+ breast cancer xenografts [50]. Interestingly, treatment with tamoxifen or fulvestrant led to selective enrichment of these cells, whereas the population of ER+/PR+ cells was decreased [51]. This subpopulation of ER−/PR−/CK5+ cells that are resistant to hormonal therapy by virtue of their ER negativity might play an important role in ER-positive breast cancer treatment failure. Similar findings have been reported in breast cancer tumors that are characterized by strong enhancement of the $CD44^+/CD24^{low}$ signature after treatment with letrozole [37].

Response to Radiotherapy

There are few studies assessing the role of CSCs in response to radiotherapy in breast cancer. Overall, these studies are performed in vitro in breast cancer cell lines and demonstrate that B-CSCs exhibit increased radiation resistance, showing enrichment and survival after irradiation [52–54]. Several mechanisms might be responsible for this phenomenon.

In two of these studies, a significantly low level of reactive oxygen species (ROS) was observed in mammospheres, as well as cells derived from human and murine breast cancer tumors [54, 55]. ROS generate several forms of harmful DNA effects, such as base damage, single-strand breaks, and double-strand breaks that can cause cell death [56]; thus, decreased levels of ROS might contribute to CSC survival after irradiation. In addition, CSCs were found to overexpress genes involved in ROS metabolism that act as antioxidant defense systems and lead to increase ability to scavenge radiation-induced free radicals [55]. Furthermore, another study suggests that increased survival of CSCs after irradiation is

attributed to their reduced tendency to undergo senescence due to low p21 expression and increased telomerase activity [53]. Importantly, an increase in DNA repair capacity might be also implicated in B-CSC radioresistance. It has been shown that CSCs could contribute to breast cancer radioresistance by preferential activation of the DNA damage checkpoint response, such as increased activation of Ataxia Telangiectasia Mutated (ATM) protein signaling. Interestingly, targeting ATM activation by an ATM inhibitor overcomes CSC radioresistance and provides a therapeutic model for eradication of radiation resistance in breast cancer [57].

Breast CSCs not only have been found to survive after irradiation, but also to retain their self-renewal ability over several generations, defined by increased sphere-forming capacity, after fractionated radiotherapy [52]. Therefore, breast tumors might contain a proportion of tumorigenic cells (CSCs) that provoke repopulation of tumor cells during gaps of radiotherapy and lead to radioresistance.

The Role of CSCs in Clinical Outcome of Patients with Breast Cancer

A number of studies suggest a potential role of CSCs in resistance to therapy in breast cancer, by virtue of CSC enrichment after chemotherapy, radiation, and hormonal therapy. However, the majority of these studies fail to show significant correlation of CSC enrichment with prognosis and clinical outcome of patients with breast cancer.

In contrast, one recent study demonstrated correlation of the percentage of CSCs with poor clinical response to chemotherapy and decreased overall survival in breast cancer [58]. More specifically, the proportion of CSCs was determined (by the ALDH1 enzymatic assay, $CD44^+/CD24^{low}$ phenotype and mammosphere formation assay) in human breast cancer biopsies that were obtained prior to the administration of neoadjuvant chemotherapy. Interestingly, the percentage of CSCs was higher in biopsies of patients who had stable or progressive disease compared with those who had complete or partial response to treatment. Moreover, ALDH1+ expression was lower in breast cancer biopsies of patients who had higher response rates. This study suggests that the percentage of CSCs correlates with chemotherapeutic resistance and suggests that their quantification could be a useful tool for the prediction of chemotherapy sensitivity.

A number of studies are also assessing the prognostic significance of CSC molecular markers in breast cancer. First, several studies assessed the role of ALDH1 expression in the clinical outcome of patients with breast cancer. Two studies have shown a significant correlation of ALDH1 expression with triple-negative breast cancer tumors and with the unfavorable clinical parameter of advanced nodal status [59, 60]. In one of the two studies, ALDH1 expression has also been associated with HER2-positive status [60]. In addition, ALDH1 expression has been shown to correlate with systemic metastasis and decreased survival of patients with inflammatory breast carcinoma [61]. These data suggest that expression of this CSC molecular marker might correlate not only with more aggressive disease but also with breast cancer subtypes of known adverse prognosis.

CD44 is a cell-adhesion molecule involved in the binding of cells to hyaluronic acid; it is shown to be overexpressed in both in situ and invasive breast carcinoma [62] and to be involved in migration and metastasis of cancer cells [63]. Similarly to ALDH1, $CD44^+/CD24^{low}$ phenotype has been found to be associated with the basal-like breast cancer tumor subgroup [64], but also with BRCA1 tumors [64, 65], suggesting that it might be indicative of aggressive molecular subtypes. Recently, it has been demonstrated that CD44 isoforms can be expressed differently in several breast cancer subgroups, suggesting that the CD44 molecule might be part of a tumor progression program that leads to development of distinct molecular subtypes [66]. Most importantly, because both CSC molecular markers, ALDH1 and $CD44^+/CD24^{low}$ phenotype, seem to correlate with molecular subtypes of breast cancer with adverse prognosis, the role of CSCs in biological behavior of aggressive tumors is an area of active investigation. However, the fact that the expression of two CSC markers does not always overlap in breast cancer tumors [13, 60–61] might indicate that these two distinct markers symbolize CSCs of different origins [66].

CSC-Targeted Therapies

Previous studies indicate that B-CSCs comprise a small population of cells within the tumor that are both resistant to drugs and provide the source of new tumor growth. Theoretically, if these cells were deleted, the remaining cells would be unable to promote new tumor growth [42, 67]. This concept has led to the formulation of various potential drug-candidates, which are mainly molecules targeting regulatory and self-renewal CSC pathways that according to CSC hypothesis are dysregulated in tumor formation [21]. The majority of the studies are still preclinical, performed in vitro in breast cancer cell lines or in vivo in mouse breast cancer mammary models.

The first approach is to target CSC surface markers. Example of such approach is to target CD44 with the specific antibody P245; this results in growth inhibition of human breast cancer xenografts [68]. Furthermore, P245 treatment of xenografts originating from human basal breast cancer during tumor remission decreases the frequency of tumor recurrence [68]. Similarly, targeting the CSC marker ALDH1 with specific CD8+ T cells eliminates the number of CSCs and inhibits growth and metastasis in xenograft-bearing immunodeficient mice [69]. Taken together, these data suggest that combining chemotherapeutic drugs with either specific antibodies or T-cell based immunotherapy that selectively target CSC surface markers could be of potential benefit.

Another signaling pathway that is critical for normal breast development and CSC self-renewal is the Notch pathway [70]. Notch receptors 1 and 4 bind to several ligands that trigger their cleavage by the enzyme γ-secretase, leading to activation of genes involved in cell proliferation [71]. On account of its functional implications but also due to aberrant expression of Notch intracellular domain in both ductal carcinoma in situ (DCIS) and invasive ductal carcinoma [72], Notch signaling

consists one of the most appealing potential therapeutic targets. Pretreatment of mammosphere cultures, derived from DCIS samples, with the Notch γ-secretase inhibitor (GSI) DAPT (N-[N-(3,5-difluorophenacetyl)-1-alanyl]-S-phenylglycine t-butyl ester) or a Notch-4 neutralizing antibody, has shown to decrease mammosphere efficiency [73]. This finding suggests that Notch inhibitors could be used as chemoprevention in DCIS in order to reduce its progression to invasive disease.

Furthermore, targeting Notch in breast cancer cell lines or breast cancer mammary models has led to elimination of CSCs and eradication of tumor formation [74–77]. Many methods of Notch inhibition have been tested, such as treatment with GSIs [75], genetic inhibition (production of shRNA knockout cell lines) [75, 77], and immunotherapy (cytotoxic lymphocytes against Notch proteins) [74]. GSIs are currently undergoing clinical trials for the treatment of advanced breast cancer. In a recent phase I clinical trial that included 24 patients with breast cancer, oral GSI MK-0752 was well tolerated at a weekly dosing, but no clinical benefit was observed in patients with breast cancer [78]. Interestingly, one study showed that specific inhibition of Notch-4 using shRNA had greater effect in reducing B-CSC activity than GSIs [75].

Furthermore, it has been shown that Notch signaling is activated in HER2-overexpressing cells [79]. Specific Notch1 inhibition reduces HER2 cell surface expression and results in lower sphere-forming efficiency in breast cancer xenografts [79]. In addition, treatment of HER2-positive breast cancer mammary models with GSIs has been demonstrated to eliminate CSCs [80]. These data suggest that inhibition of Notch pathway could be used as CSC-directed therapy to increase therapeutic efficiency of trastuzumab or lapatinib in HER2-positive tumors. Recently, a study has shown promising results, suggesting that combined use of GSIs MRK-003 and LY 411 575 and trastuzumab can reduce tumor recurrence in trastuzumab-sensitive breast cancer xenografts or partially reverse trastuzumab resistance in resistant breast cancer xenografts [81]. Contradictory results have been reported by others, where Notch pathway has been shown to play a role in HER2-negative breast cancer; specifically, antibody blocking of Notch-1 results in sensitization of breast cancer cells to radiation in HER2-negative tumors [82].

Additional pathways that are possibly involved in B-CSC regulation are the phosphatidyl-inositol 3-kinase (PI3K) and the Wnt pathways. Specifically, the PI3K/Akt/mammalian target of rapamycin (mTOR)/signal transduction and activator of transcription (STAT3), and *PTEN* signaling, form a complex signaling network that is considered to be dysregulated and also to serve as a modulator of drug resistance in breast cancer [83]. It has been demonstrated that combined inhibition with both PI3K inhibitor LY294002 and mTOR inhibitor rapamycin reduces side population fraction in breast cancer cell lines and tumor formation in mice [25]. Furthermore, treatment with Akt inhibitor perifosine leads to a decrease in number of CSCs and tumor growth in breast cancer xenografts [84] and sensitizes CSCs to radiation in p53 null mice [85].

Furthermore, there is growing evidence of Wnt dysregulation in human breast tumors [86–88]. Recently, inhibition of Wnt signaling by dietary polyphenols curcumin and piperine has been shown to decrease mammosphere formation and percentage of ALDH1-positive cells [89]. Most importantly, these drugs did not have impact on differentiated cells in this study, which may account for limited toxicity on normal tissues and may favor the administration of these drugs in combination with chemotherapeutic agents to enhance their efficacy. However, the effect and safeness of these agents in patients must be tested in clinical trials.

As previously mentioned, one of the main characteristic features of CSCs is resistance to apoptosis. It could be therefore hypothesized that antiapoptotic proteins may also play an important role in survival of CSCs. Recently, it has been shown that genetic suppression of antiapoptotic FLICE-Like Inhibitory Protein (c-FLIP) using murine specific siRNA (FLIPi) selectively and repeatedly targets B-CSCs independent of hormone receptor status and sensitizes them to chemotherapy agent tumor necrosis factor-related apoptosis inducing ligand (TRAIL) [90], an anticancer agent that has been shown to have limited therapeutic potential in breast cancer cell lines [91]. Importantly, lack of toxicity on normal cells was demonstrated, which might enable the use FLIPi/TRAIL without adverse effects. In another study, an active mutant of proapoptotic gene *BIK* named BikDD was shown to reduce B-CSCs in breast cancer cell lines, without demonstrating toxicity on normal cells [92]. In addition, BikDD was found to have synergistic effect with lapatinib in HER2-positive cells and with paclitaxel in HER2-negative cells [92]. These results suggest that BikDD molecule could enhance the therapeutic efficacy of both lapatinib and chemotherapy without major side effects.

Finally, several drugs used in other diseases have been tested in preclinical trials for their potential impact on B-CSCs. One of the most promising drugs is the common anti-diabetic drug metformin that has been shown to selectively decrease CSCs in breast cancer cell lines [93]. Strikingly, combined administration of both metformin and doxorubicin in cell cultures and xenografts results in eradication of both CSCs and non-CSC tumor cells, whereas treatment with doxorubicin alone fails to eliminate CSCs [93]. Furthermore, metformin has been also found to synergistically interact with trastuzumab to reduce mammosphere formation and mammosphere size in trastuzumab-resistant HER2-positive tumors [94]. It is therefore tempting to suggest that concurrent treatment with metformin and chemotherapeutic agents or anti-HER2 molecular therapies could add significant benefit to tumor debulking.

Among other drugs, antineoplastic agent cyclophosphamide has been shown to display anti-CSC activity in breast xenografts [95]. This sounds like a paradox because ALDH1 expression, a CSC feature, can detoxify cyclophosphamide active metabolites [96]. However, this discrepancy might be explained by the fact that most direct data demonstrating this are in the context of ALDH1 gene transfer, leading to overexpression [97]. In this study, endogenous levels of ALDH1 may not be sufficient to confer resistance in CSCs at the cellular level at the clinically relevant dose given; other mechanisms, possibly

related to increased DNA repair efficiency induced by cyclophosphamide, might be implicated in CSC sensitivity [95]. In addition, salinomycin, an antibacterial potassium ionophore, has been reported to reduce dramatically the percentage of CSCs in cell lines and to inhibit tumor growth in mice [98]. Compared to paclitaxel, which is commonly used in breast cancer, it provokes a drop in CSCs by 100-fold, implying that the combination of the two drugs could be a potential therapeutic challenge [98]. Similarly, selective eradication of B-CSCs has been demonstrated with dofequidar fumarate, an inhibitor of ABCG2 gene expressing ABC-T transporters [99].

Conclusion

The CSC concept has important implications not only for our understanding of carcinogenesis, but also for the development of cancer therapeutics. There is a growing body of preclinical evidence that cancer stem cells contribute to chemotherapy and radiation resistance in breast cancer. However, to date, no significant impact on clinical outcome has been identified. The development of more effective therapies to overcome treatment resistance may include the simultaneous targeting of CSCs. Current published data from preclinical studies are promising, but they have still not been translated to the clinic in their entirety because the clinical efficacy of drug-candidates for the targeting of CSCs remains to be demonstrated in clinical trials. The use of drugs that interfere with stem cell self-renewal represents the strategy of choice but also a great challenge because many pathways are shared by cancer stem cells and their normal counterparts. In addition, cancer stem-cell regulatory pathways are highly interconnected, which suggests that the use of combinations of targeting agents may be necessary to effectively eliminate this cell population. Furthermore, new strategies need to take into account the role of microenvironment that may alter the response to therapeutic targets.

Among drugs tested to date, metformin and salinomycin electively inhibit CSCs and have shown important anti-CSC activity. In particular, metformin is a well-studied drug, commonly used in the treatment of diabetes mellitus, that displays a safe toxicity profile and can be used in clinical trials. Importantly, some CSC-directed therapies do not affect normal differentiated cells [89–90, 92] and could be easily administrated in the clinical setting in combination with chemotherapeutic agents due to limited toxicity. Furthermore, several targeted therapies show synergistic interaction with chemotherapy agents, molecular targeted therapies, or radiation [79, 85, 90, 92, 94], raising the possibility of combined efficacy of these different treatment agents. In addition, HER2 has emerged as an important regulator of B-CSCs. Recent studies have suggested that the remarkable clinical efficacy of HER2-targeting agents may relate to the ability to target B-CSCs. Further research efforts are necessary to improve the understanding of the role of CSCs in breast cancer and expand the knowledge of possible CSC-directed therapies for the benefit of patients with breast cancer.

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