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# Breast Cancer Stem Cells

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## 1. Introduction

Breast cancer is the most common non-cutaneous type of cancer in women and the most common cause of cancer-related mortality among women worldwide, with more than 1,000,000 new cases and more than 410,000 deaths each year (Parkin et al., 2005; Anderson et al., 2006; Parkin & Fernandez, 2006). Even when breast cancer mortality is decreasing in developed countries due to primary prevention, screening, and improved therapies, there were still 130,000 deaths in europe (Boyle & Ferlay, 2005) and 40,000 deaths in US (Ries et al., 2008) during 2004. Moreover, in less developed countries breast cancer patients show poorer treatment outcomes and increased mortality rates as result of diagnosis at a more advanced stage (Boyle, 2005).

Therapy for breast cancer includes cytotoxic, hormonal, and immunotherapeutic agents. In general, these agents induce response rates ranging from 60% to 80% for primary breast cancers and about 50% of metastases (Guarneri & Conte, 2004; Gonzalez-Angulo et al., 2007). However, despite the frecuency of primary responses, the median duration of response to chemotherapy is 8 to 14 months (Pusztai & Hortobagyi, 1998). Consequently, 20% to 70% of patients show recurrent disease within 5 years (Pusztai & Hortobagyi, 1998; Pisani et al., 2002; Colleoni et al., 2004). The use of local radiotherapy in addition to chemotherapy reduces mortality by 17 to 30% and is particularly beneficial for patients with extensive nodal metastasis, which tend to contain a higher absolute number of chemotherapy resistant cells (Ragaz, 2009).

These data indicate that even though current treatments are active at the beginning of therapy, progression still occurs in the majority of patients. Furthermore, when recurrence appears, resistance to therapy is common increasing the risk of death (Gonzalez-Angulo et al., 2007). The failure of current treatments necessitates new approaches. Such approaches must consider the potential role of cancer stem cells (CSCs) in the initiation, maintenance, and clinical outcome of breast cancers.

## 2. Breast cancer stem cells

The cells within a tumor display functional heterogeneity, with different morphology, differentiation grade, proliferation rate, and invasiveness (Heppner & Miller, 1983). Recent

studies suggest that the ability of a tumor to proliferate and propagate relies on a small population of stem-like cells, called cancer stem cells (CSCs). CSCs share fundamental characteristics with normal adult stem cells: they divide asymmetrically producing one stem cell and one progenitor cell. In normal stem cells, this allows the continuation of the stem cell compartment and starts the production of cells that undergoes multilineage differentiation. Similarly, CSCs have the ability to perpetually self-renew and to produce tumors comprised of cells with different phenotypes. Since their discovery in leukaemia (Bonnet & Dick, 1997), the existence of a subpopulation of CSCs has been corroborated in several solid tumours, including breast, brain, colon, pancreas, prostate, lung, and head and neck tumors (Glinsky, 2007; Li et al., 2007; Prince et al., 2007; Eramo et al., 2008).

### 2.1 Identification and isolation of breast CSCs

The discovery of CSCs in human breast tumors was reported in 2003 by Al-Hajj and collaborators. They discovered a cellular population characterized by cell-surface $CD44^+/CD24^{-/low}/ESA^+$ markers, and $lineage^-$ (lack of expression of CD2, CD3, CD10, CD 16, CD18, CD31, CD64, and CD140b). As few as 200 of these cells were able to form tumors when injected into NOD/SCID mice while tens of thousands of other cells could not (Al-Hajj et al., 2003). The tumors that were generated recapitulated the phenotypic heterogeneity of the initial tumor, containing a minority of $CD44^+/CD24^{-/low}/lineage^-$ cells that can be serially passaged to form new tumors (Al-Hajj et al., 2003). The $CD44^+/CD24^-$ phenotype has been used extensively to identify and isolate cancer cells with increased tumorigenicity (Fig. 1).

Breast CSCs have also been isolated from patient samples after *in vitro* propagation (Ponti et al., 2005) and from breast cancer cell lines (Fillmore & Kuperwasser, 2008). The breast CSCs convey an ability to form mammospheres in culture. Mammosphere culture is a system that allows the propagation of mammary epithelial cells in an undifferentiated state, based on their ability to proliferate in suspension as non-adherent spheres (Dontu et al., 2003; Dontu et al., 2004). Accordingly, the capacity to form mammospheres is increased in early progenitor/stem cells. These cells have the ability to differentiate along all three mammary epithelial lineages and to generate complex functional structures in reconstituted 3D culture systems (Dontu et al., 2003; Dontu et al., 2004). The mammospheres from breast cancer cells are enriched in cells with the $CD44^+/CD24^{-/low}$ phenotype, and these cells retain tumor-initiating capability when injected into NOD/SCID mice (Fig. 1). However, only a fraction of $CD44^+/CD24^{-/low}$ cells is able to form secondary mammospheres (Ponti et al., 2005). Consistent with these findings, cancer cell lines that are enriched (90%) in $CD44^+/CD24^{-/low}$ cells are not more tumorigenic than cell lines that contain only 5% of cells with the same phenotype (Fillmore & Kuperwasser, 2008), indicating that only a subgroup within the $CD44^+/CD24^{-/low}$ cells are self-renewing.

As only a subpopulation of $CD44^+/CD24^-$ cells form tumors, additional markers have been investigated. Aldehyde dehydrogenase (ALDH) family of cytosolic isoenzymes are responsible for oxidizing intracellular aldehydes, leading to the oxidation of retinol to retinoic acid, an event that ocurrs in early stem cell differentiation. ALDH1 is the predominant ALDH isoform in mammalian cells. Increased ALDH activity has been described in human hematopoietic stem cells as well as in cancer stem cells of multiple tissues (Hess et al., 2004; Corti et al., 2006). Aldefluor staining for the identification of breast CSCs uses an uncharged ALDH substrate, BAAA (BODIPY-aminoacetaldehyde). BAAA is

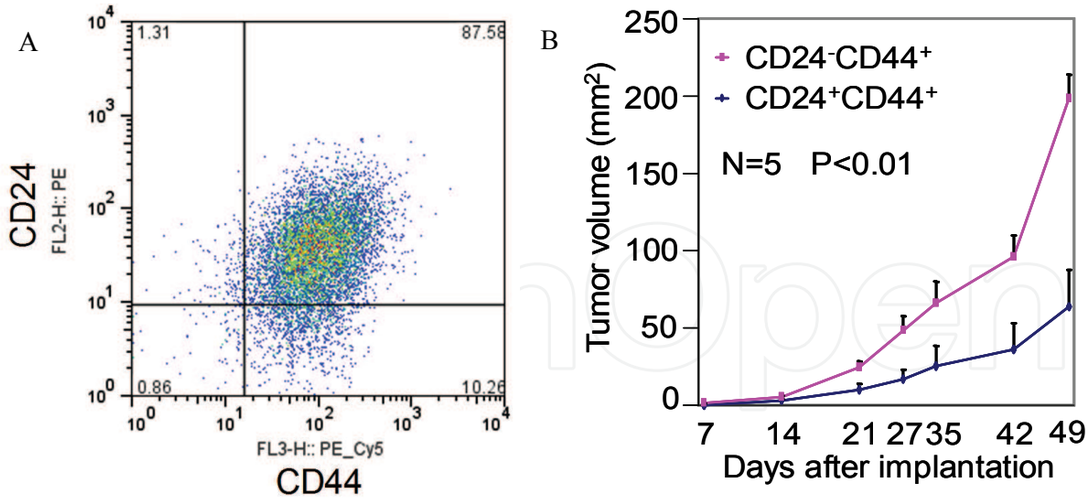



Fig. 1. Increased tumorigenicity of CD44+/CD24- Met-1 mouse breast cancer cells (from Wu et al., 2010 J Biol Chem, in press). A) Met-1 cells were separated by immunosorting based on the phenotypes CD44+/CD24- or CD44+/CD24+. B) Tumor regeneration after after subcutaneous implantation of 1000 sorted cells per mouse.

taken up by living cells through passive diffusion and converted by intracellular ALDH into a negatively charged reaction product BAA- (BODIPY-aminoacetate). BAA- is retained inside cells expressing high levels of ALDH, causing them to become brightly fluorescent (Christ et al., 2007). Thus, the ALDH-expressing cells can be detected in the green fluorescence channel (520-540 nm) of a standard flow cytometer (Fig. 2). Breast tumor cells positive for ALDH activity are able to generate tumors in NOD/SCID mice with phenotypic characteristics resembling the parental tumor, suggesting that the ALDH+ pool contain the CSC population (Ginestier et al., 2007). The cell selection using the CD44+/CD24-/ALDH+ phenotype increases the tumorigenicity of breast cancer cells in comparision with CD44+/CD24- or ALDH+ cells (Ginestier et al., 2007).

New strategies to improve the identification and isolation efficiency of breast CSCs have been recently reported (Cicalese et al., 2009; Pece et al., 2010; Sajithlal et al., 2010). The fluorescent dye PKH26 has been used to identify the fraction of stem cells in normal human mammary cells. Briefly, the method consists of labelling the cell membrane with PKH26 and then culturing the mammary cells in suspension to form mammospheres. After 7-10 days only the slow cycling cells retain the dye and can be sorted based on their PKH26 fluorescence intensity. The PKH26hi cells (0.2-0.4% of the total cell population) are able to form secondary mammospheres, divide asymetrically, express markers of pluripotentiality, and can reconstitute a normal mammary epithelium when transplanted into NOD/SCID mice, indicating that this population is highly enriched in stem cells (Pece et al., 2010). The same strategy has been successfully used to enrich breast CSCs in ErbB2 transgenic mice (Cicalese et al., 2009) and from human cancer cell lines (Fig. 3). Furthermore, analysis of the expression profile of PKH26hi cells allowed the identification of the CD49f+/DLL1hi/DNERhi phenotype as prospective markers of human breast CSCs (Pece et al., 2010). CD49f+/DLL1hi/DNERhi cells are present in human breast tumor samples, corresponding to 1.5-6% of the cancer cells. The CD49f+/DLL1hi/DNERhi fraction is enriched in CSCs, since the injection of only 500 of those cells was sufficient to produce tumors in NOD/SCID mice (Pece et al., 2010).

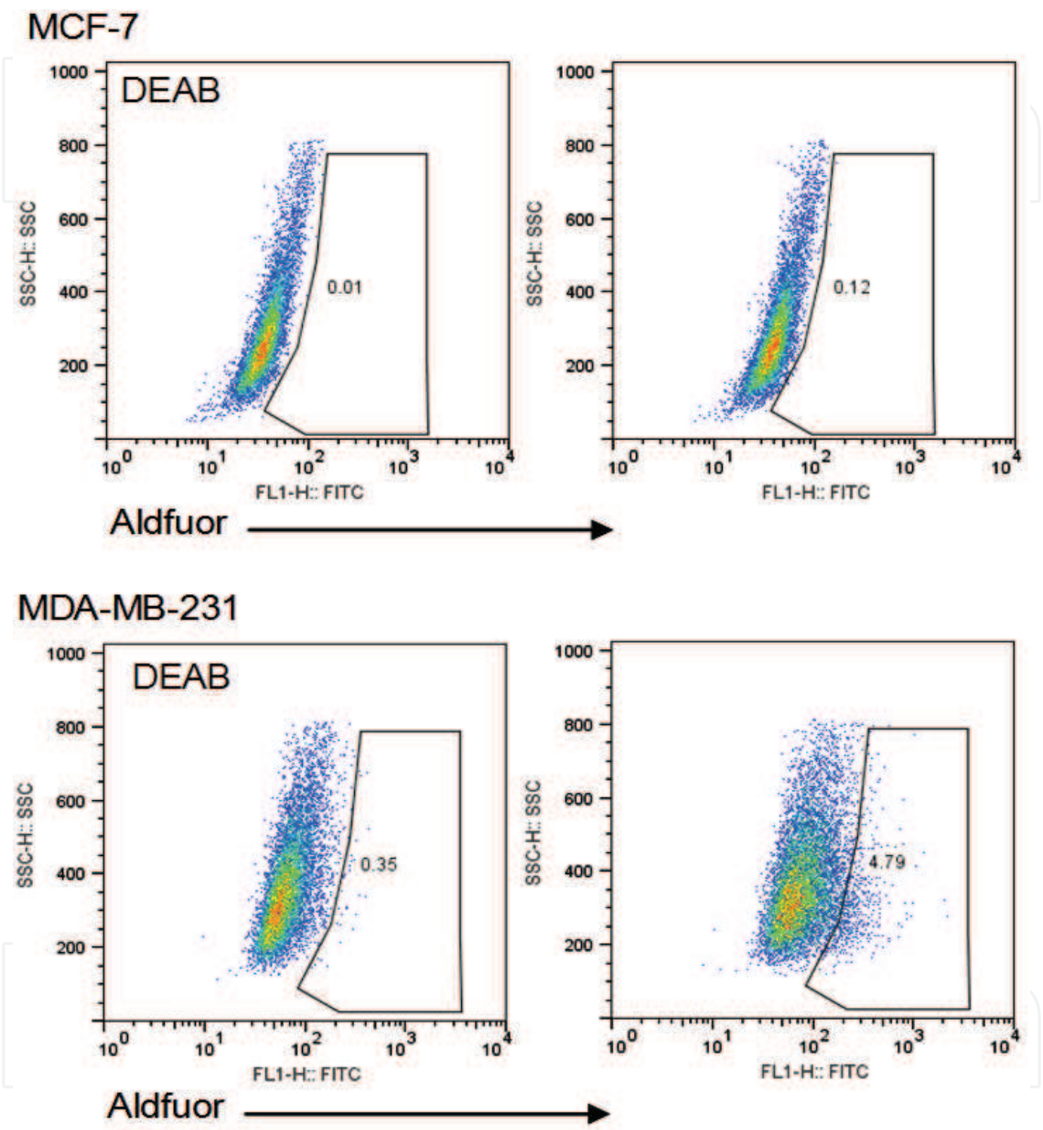
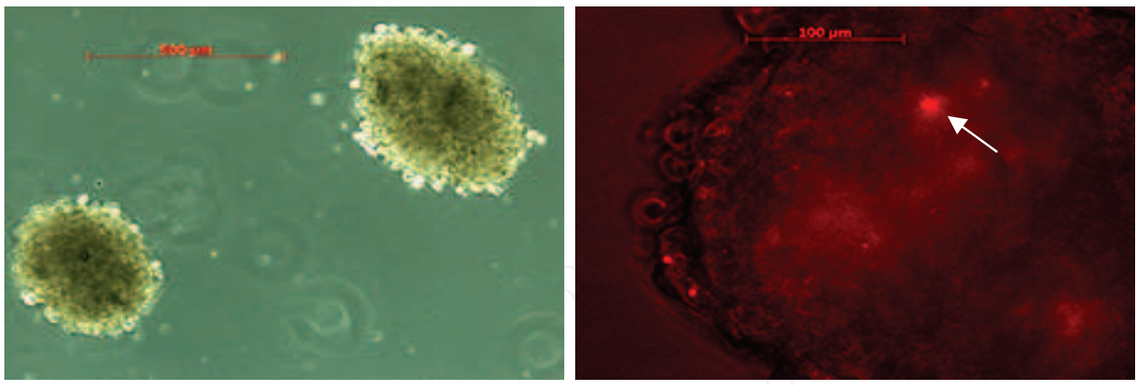



Fig. 2. Aldefluor assay of MCF-7 and MDA-MB-231 breast cancer cells (Jiao et al., unpublished). This assay allows the identification and separation of CSCs based on the activity of ALDH. DEAB is an inhibitor of ALDH used to increase specificity of the assay. Note that the ALDH$^+$ fraction in MCF-7 cells is almost undetectable.




(A) (B)

Fig. 3. PKH26 retention during mammosphere culture (Velasco-Velazquez et al., unpublished). A) Hs578T human breast cancer cells cultured in non-adherent conditions for 7 days (bright field). B) Fluorescence microscopy shows that only a few cells retain a high level of PKH26 (arrow). Those cells have properties of CSCs.

A different approach was recently reported by Sajithlal and collaborators (Sajithlal et al., 2010). They tagged the CSC population from human cancer cell lines with green fluorescent protein (GFP) under the control of the Oct3/4 promoter. In MCF-7 cells only 1% of the population expressed GFP, and the large majority of those cells were $CD44^+/CD24^-$. $GFP^+$ cells were sorted and maintained in culture. Unexpectedly, the $CD44^+/CD24^-/GFP^+$ phenotype remained stable for more than one year, suggesting that the incorporation of the promoter blocks CSC differentiation. As predicted, the GFP+ cells were 100-300 times more tumorigenic that the rest of tumor cells and displayed an increased resistance to cytotoxic drugs. Similar results were found when other breast cancer cell lines were stably transfected with the Oct3/4 promoter (Sajithlal et al., 2010). These cell lines may become valuable models in the study of CSC biology.

Other stem cell markers have been used to identify breast CSCs in murine models, including CD133 and the β1 integrin subunit (CD29). In tumor cell lines generated form Brca1 deficient mice, Wright and collaborators found two different populations of potential CSCs: one with the previously reported $CD44^+/CD24^-$ phenotype and the other being $CD133^+$ (Wright et al., 2008). Both subpopulations were able to repopulate cell fractions found in the parental cell lines, formed *in vitro* mammospheres, generated tumors in NOD/SCID mice, and expressed Oct4, a marker of pluripotency. In a similar manner, subpopulations of $CD24^{hi}CD29^{low}$ cells isolated from tumor cell lines exhibit the capacity of self-renewal, differentiation and tumorigenicity (Vassilopoulos et al., 2008). One possibility is that these cells with different immunophenotypes represent different origins of breast cancer stem cells. The $CD44^+/CD24^-$ population most likely represent basal breast cancer stem cells and cells with the $CD24^{hi}CD29^{low}$ signature most likely originate from the mammary luminal progenitor cells. These data, together with the fact that CD133 and CD29 have been used in the identification of normal and cancer stem cells from different tissues, indicate that CD133 and CD29 could be used as a marker of mouse breast CSCs. The diversity of mouse breast cancer stem cells may provide a tool to elucidate the hierarchy of breast cancer stem cells.

## 3. Therapeutic resistance in breast CSCs

Whether breast CSCs arise from normal stem cells or from progenitor cells that have gained the ability for self-renewal remains unclear. However, both of these hypotheses consider

that the different phenotypic characteristics of normal and cancerous stem cells are caused by genetic alterations that promote changes in the signalling pathways controlling the cell cycle, differentiation, and survival. These alterations promote changes in key CSC functions that are directly related to the clinical outcome of the tumor. In the case of breast cancer, a growing body of evidence indicates that CSCs are more resistant to chemo- and radiotherapy than the non-stem tumor cells. Accordingly with the cancer stem cell hypothesis, the surviving CSCs will be capable to repopulate treated-tumors and produce relapse. Moreover, since mutations can be passed on to all the stem cell's progeny, it is likely that the new tumor will display increased resistance to therapeutic regimens, allowing evolution towards malignancy over time. Elucidation of the molecular mechanisms by which CSCs survive therapy may identify new targets for breast cancer therapeutic intervention.

### 3.1 Chemoresistance and mechanisms involved

The role of chemotherapy in the selection and expansion of breast CSCs has been studied using different strategies. The proportion of *in vitro* self-renewing cancer cells from patients who received neoadjuvant chemotherapy has been compared with that of cells isolated from chemotherapy-naive patients. Mammosphere formation was 14-fold higher in tumor cells from the patients that had received chemotherapy (Yu et al., 2007). Enrichment of CSCs by chemotherapy was confirmed by studying paired specimens from patients obtained by biopsy prior to chemotherapy and at surgery following neoadjuvant chemotherapy. Mammosphere formation and the proportion of $CD44^{+}/CD24^{-/low}$ cells were increased approximately 10-fold after chemotherapy (Yu et al., 2007).

Additional evidence from mouse models supports that exposure to chemotherapeutic agents elicits a selective pressure and prevents differentiation of CSCs, increasing the proportion of CSC in the tumors. Yu and collaborators studied the properties of tumors generated by SKBR3 breast cancer cells after consecutive passage in mice receiving epirubicin. Those tumors were highly enriched in $CD44^{+}/CD24^{-}/lineage^{-}$ cells, and were able to form 20-fold more mammospheres than cells isolated from tumors generated with the parental cell line (Yu et al., 2007). The expansion of the CSC population after drug treatment contributes to drug resistance. Mammary tumors from Brca1/p53-mutated mice are sensitive to cisplatin, but a few months after treatment, tumors relapse at the same site. The proportion of $CD29^{hi}/CD24^{med}$ cells (tumorigenic cells) in tumors that arise after cisplatin treatment was 4-fold greater than in untreated primary tumors (Shafee et al., 2008). Interestingly, when $CD29^{hi}/CD24^{med}$ cells from relapse tumors were injected into $Rag1^{-/-}$ mice, they formed tumors that were only partially sensitive to cisplatin. A second round of selection and transplantation further increased the $CD29^{hi}/CD24^{med}$ fraction and generated tumors that were completely refractory to cisplatin (Shafee et al., 2008), indicating the appearance of cisplatin-resistant progenitor cells.

#### 3.1.1 Multidrug resistance transporters

The chemoresistance in breast CSCs is caused partially by the expression of ABC (ATP-Binding Cassette) transporters. A subpopulation of breast cancer cells with the capability to extrude the dye Hoechst 33342 (a measurement of ABC transporters activity) is enriched in CSCs (Patrawala et al., 2005; Christgen et al., 2007; Woodward et al., 2007). This subpopulation, called "side population" (SP), isolated from Cal-51 cells exhibited a 30-fold increased in ABCG2 mRNA expression in comparison to unsorted cells (Christgen et al.,

2007). After isolation and expansion, cells from the Cal-51 SP gave rise to a heterogeneous mix of SP and non SP cells in a proportion similar to the original cell line, in which the non SP cells lacked expression of ABCG2. Similarly, ABCG2 expression declined with *in vitro* differentiation of SKBR3 cells isolated from mouse xenotransplants (Yu et al., 2007). Thus, the expression of ABCG2 and the ability to efflux drugs is lost during differentiation of CSCs to cancer cells. These data partially explain why primary chemotherapy produces responses in the large majority of tumors but is ineffective in eradicating the cells that express ABC transporters and CSC properties.

### 3.1.2 Stem cell signalling pathways

Alterations in signalling pathways controlling self-renewal and cell fate, such as HER-2, Notch, Wnt, and Hedgehog, also contribute to drug resistance in breast CSCs (see (Charafe-Jauffret et al., 2008; Kakarala & Wicha, 2008) for recent reviews). For example, HER-2 may play a role in regulating breast CSC population. HER2 overexpression in breast cancer cell lines increased the CSC population as demonstrated by increased ALDH activity, mammosphere formation, tumorigenesis, and expression of stem cell related genes (Korkaya et al., 2008). ALDH1 has been reported as a major mediator of resistance to cyclophosphamide in CSCs (Dylla et al., 2008), suggesting that HER-2-medited signaling may favor resistance. Correspondingly, HER-2 inhibition with trastuzumab reduced by 50% the recurrence rate after conventional adjuvant chemotherapy (Slamon & Pegram, 2001).

HER-2-mediated CSC expansion may involve the activation of the Notch pathway, which regulates self-renewal of normal mammary stem cells (Dontu et al., 2004). Notch is aberrantly activated in human breast carcinomas (Pece et al., 2004; Stylianou et al., 2006) correlating with cyclin D1 overexpression. Notch directly induces cyclin D1 expression and Notch correlates with cyclin D1 expression during development (Stahl et al., 2006). HER-2 induced Notch-1 activation in breast cancer cells by increasing the expression of cyclin D1. In turn, cyclin D1 inhibited the expression of the Notch-1 negative regulator Numb (Lindsay et al., 2008). In ER-negative breast cancer cells, Notch-1 activation directly promoted the transcription of the antiapoptotic gene Survivin (Lee et al., 2008). In turn, increased survivin levels may deregulate multiple mitotic checkpoints, contributing to genetic instability (Lens et al., 2006) and inhibiting radiation- and drug-induced apoptosis (O'Connor et al., 2002; Ghosh et al., 2006). Additional evidence of the role of a Notch/survivin axis in breast CSCs survival and resistance include that: i) Notch-1 protects $CD44^{+}/CD24^{-/low}$ breast cancer-initiating cells from radiation (Phillips et al., 2006); ii) a neutralizing antibody against Notch-4 reduced mammosphere viability in primary cultures of ductal carcinoma *in situ* of the breast (Farnie et al., 2007); iii) the antiapoptotic protein survivin is overexpressed in breast CSC cultures (Ponti et al., 2005); and iv) chemoresitance displayed in CSCs isolated from MCF-7 cells is associated with increased expression of Notch-1 (Sajithlal et al., 2010). These data suggest that survivin and cyclin D1 may operate as a Notch-regulated cytoprotective factors that promote persistence of breast CSCs.

## 4. Role of CSCs in breast tumor metastasis

Metastasis is a highly complex process that comprises several sequential steps, that include escape from the primary tumor (intravasation), survival within the circulation, extravasation into a secondary site, and sustained growth in a distinct microenvironment (Woodhouse et al., 1997; Chambers et al., 2002; Pantel & Brakenhoff, 2004). Several lines of evidence indicate

that metastasis is a highly inefficient process. Depending on the experimental model, 0.02-0.1% of the cancer cells that reach the circulation can develop macrometastases (Weiss, 1990; MacDonald et al., 2002; Allan et al., 2006). Recently, CSCs capable of seeding distant metastasis have been identified (Li et al., 2007) supporting the model in which CSCs initiate and sustain secondary tumor growth. Accordingly, several authors have proposed a model in which CSCs appear as the active source of metastatic spread (Wicha, 2006; Li et al., 2007; Goss et al., 2008; Visvader & Lindeman, 2008).

In agreement with that model, a subpopulation of circulating tumor cells that express stem cell markers has been identified in metastatic breast cancer patients and a high percentage of $CD44^+/CD24^-$ tumor cells have been found in metastases. (Balic et al., 2006; Aktas et al., 2009; Theodoropoulos et al., 2010). Additionally, a gene signature of invasiveness (IGS), generated by comparing the gene-expression profile of $CD44^+/CD24^-$ tumorigenic breast cancer cells with that of normal breast epithelium, is strongly associated with metastasis-free survival (Liu et al., 2007). Finally, expression of the stem cell marker ALDH in samples of inflammatory breast cancer (IBC) correlates with the development of distant metastasis and decreased survival (Charafe-Jauffret et al., 2010).

The ability of breast CSCs to invade and proliferate at the metastatic sites has been studied both *in vitro* and *in vivo*. CSCs isolated from cancer cell lines exhibited increased invasiveness and elevated expression of genes involved in invasion (IL-1α, IL-6, IL-8, CXCR4, MMP-1, and UPA) (Sheridan et al., 2006). Accordingly, $ALDH^+$ cells isolated from breast cancer cell lines were more migratory and invasive than the $ALDH^-$ cells (Charafe-Jauffret et al., 2009; Croker et al., 2009). Intracardiac injection of $ALDH^+$ cells isolated from human breast cancer cell lines to NOD/SCID mice generated metastases at distinct organs; in contrast, $ALDH^-$ cells produced only occasional metastases limited to lymph nodes (Charafe-Jauffret et al., 2009; Charafe-Jauffret et al., 2010).

Molecular genetic analysis has identified key regulators of the breast cancer stem cell phenotype using knockout and transgenic mice including c-Jun (Jiao et al., 2010) , $p21^{CIP}$ (Liu et al., 2009), NFκB (Liu et al., 2010 Cancer Res, in press) and the retinal determination gene network (RDGN) (Micalizzi et al., 2009); Wu et al., 2010 J Biol Chem, in press).

Our group has shown that molecular signals that promote "stemness" in cancer cells also promote the acquisition of metastatic ability. Using bitransgenic mice encoding a floxed c-Jun allele and mammary targeted ErbB2 we have reported that the proto-oncogene c-Jun

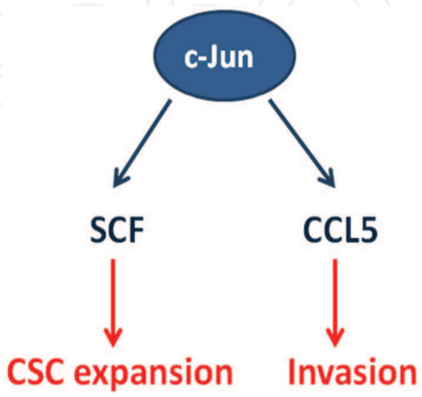



Fig. 4. Schematic representation of c-Jun-mediated cellular migration and CSC expansion via induction of SCF and CCL5 (RANTES) production (adapted from Jiao et al. 2010).

controls the transcriptional expression of SCF (Stem Cell Factor) and CCL5 (RANTES). Reduction in SCF causes a decrease in the proportion of cells expressing breast CSC markers and in CSC self-renewal, while c-Jun-mediated expression of CCL5 plays a key role in the autocrine control of the migration and invasion of breast cancer cells (Jiao et al., 2010). These studies demonstrated that a single cellular proto-oncogene is necessary to both, activate signaling pathways that promote features of CSC and maintain the invasive phenotype of mammary tumors (Fig. 4).

## 5. Targeting CSCs

The key roles of CSCs in breast cancer biology suggest that new therapies must target these cells. The main objective of those therapies would be the eradication of the CSC compartment with no harm to other cell types. Eradication of breast CSCs may include different strategies as summarized in Table 1.

Different approaches have been used to overcome ABC transporter-mediated chemoresistance. The anthracycline modified drug annamycin, which is not extruded by ABC transporters, was toxic to the resistant cell line MCF-7/VP (Perez-Soler et al., 1997). The plant alkaloid berberine decreased the expression of the ABCG2 transporter and reduced the "side population" of the MCF-7 cell line (Kim et al., 2008), suggesting that downregulation of ABC transporters may be useful for targeting breast CSCs. However, the ability to target drug transport in CSCs may be difficult since these cells express multiple ABC transporters (de Grouw et al., 2006). The use of inhibitors of ABC transporters simultaneously with anticancer drugs is an efficient approach to overcome resistance *in vitro* and in animal models (Ozben, 2006). However, clinical trials with this kind of inhibitors have shown that they produce serious side effects (Ozben, 2006). High-throughput screening identified the ionophore salinomycin as toxic to breast CSCs (Gupta et al., 2009). Salinomycin induced capase-independent apoptosis in human cancer cells of different origins that display multiple mechanisms of drug resistance, at concentrations that did not affected normal cell viability (Fuchs et al., 2009). Subsequent studies showed that salinomycin induces a conformational change of the ABC transporter MDR1/ABCB1 that reduces its activity (Riccioni et al., 2010). Therefore, salinomycin is particularly effective at inducing apoptosis in leukemia cells that display ABC transporter-mediated drug-resistance (Fuchs et al., 2010).

Targeting CSCs through their specific markers was partially succesful in acute myeloid leukemia (AML) (Sperr et al., 2005; Tsimberidou et al., 2006). Cytotoxic antibodies directed against CD33 (a common marker in leukemic stem cells) induced remission in some patients. However, the antibody produced cytopenia due to its effects on normal hematopoietic stem cells (Sperr et al., 2005; Tsimberidou et al., 2006). Similarly, a monoclonal antibody against CD44 induced terminal differentiation and apoptosis of AML cells in engrafted mice (Jin et al., 2006). Anti-CD44 antibodies conjugated with cytotoxic drugs or radiolabels have shown to reduce disease progression in breast cancer patients and animal models (reviewed by (Platt & Szoka, 2008)).

Other potential targets in breast CSC therapy include molecules that participate in self-renewal and cell fate. Inhibition of Hedgehog signaling in xenografts established from pancreatic cancer cell lines reduced the number of ALDH-overexpressing cells (Feldmann et al., 2008). The promoters of the MDR, hTERT, and Cox-2 genes are active in breast CSCs. Oncolytic adenoviruses driven by these promoters were effective in killing $CD44^{+}/CD24^{-/low}$ cells *in vitro*, and reducing tumor growth *in vivo* (Bauerschmitz et al., 2008).

Interruption of signals generated in the CSC microenvironment using antibodies or soluble ligands against adhesion receptors may be useful in CSC targeting. α6-integrin inactivation with antibodies or siRNA abrogated mammosphere-forming ability and tumorigenicity of breast cancer cells (Cariati et al., 2008). The IL-8 receptor CXCR1 inhibitor repertaxin reduced the breast CSC population, producing apotosis in the tumor population, and reduced metastasis (Ginestier et al., 2010).

| Target in breast CSCs | Strategy | Example |
|---|---|---|
| **ABC transporters** | Cytotoxic drugs that cannot be extruded by ABC transporters | Annamycin |
| | Reduce expression | Berberine siRNAs |
| | ABC transporters inhibitors | Salinomycin |
| **Membrane markers** | Antibodies conjugated with drugs or radioligands | Anti-CD44 |
| **Intracellular signalling molecules** | Small molecule inhibitors | --- |
| | Reduce expression | siRNAs |
| | Oncolytic virus activated by specific promoters | MDR promoter |
| **Signals from the microenvironment** | Small molecule receptor antagonists | Repertaxin |
| | Blocking antibodies | Anti-α6 integrin |
| | Blocking soluble ligands | Soluble HA |
| **Others** | Metabolic alteration? | Metformin |

Table 1. Strategies for the eradication of CSCs.

Metformin is an anti-diabetic drug that has found to reduce breast cancer incidence and improve survival of breast cancer patients with type 2 diabetics (Vazquez-Martin et al., 2010a). Recent studies showed that the drug metformin selectively reduces the breast CSC population. In human breast cancer cell lines, metformin reduced the $CD44^+/CD24^-$ population and their ability to form mammospheres (Hirsch et al., 2009). In a xenograft mice model, concurrent treatment with metformin and doxorubicin reduced tumor mass much more effectively than either drug alone (Hirsch et al., 2009). Metformin also targeted traztasumab-resistant CSCs that overexpress HER-2 (Vazquez-Martin et al., 2010b). The mechanism involved in the metformin effects on CSCs is unclear, but seem to be associated with its activator effect on AMP-activated kinase (AMPK) (Vazquez-Martin et al., 2010a). AMPK phosphorylates and inhibits Acetyl CoA carboxylase (ACACA), the limiting enzyme of the fatty acid synthesis. Thus, metformin may be affecting cancer cell metabolism and functioning of lipid raft platforms (Vazquez-Martin et al., 2010a).

## 6. Conclusions

CSCs have a central role in breast cancer progression since they are involved in tumorigenesis, therapy response, and metastasis formation. Diverse methodologies based on their phenotype or specific cellular functions have been described to isolate mouse and human breast CSCs. Combinations of these methodologies improve the efficiency of purification.

Development of new therapies for targeting and eradication of breast CSCs must consider both, the differences between CSCs cells and the rest of the tumor cells and the pathways shared between CSCs and normal stem cells. Elucidation of the specific mechanisms by which CSCs survive chemotherapy, regulate self-renewal, and interact with their primary and metastatic niches will be useful for the design of new therapeutic alternatives. Such approaches may become the basis for the generation of effective and clinically applicable therapies that prevent disease relapse, metastasis and enhance patient survival.

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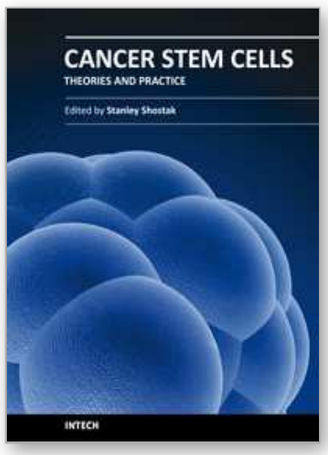


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Cancer Stem Cells Theories and Practice does not 'boldly go where no one has gone before!' Rather, Cancer Stem Cells Theories and Practice boldly goes where the cutting edge of research theory meets the concrete challenges of clinical practice. Cancer Stem Cells Theories and Practice is firmly grounded in the latest results on cancer stem cells (CSCs) from world-class cancer research laboratories, but its twenty-two chapters also tease apart cancer's vulnerabilities and identify opportunities for early detection, targeted therapy, and reducing remission and resistance.

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