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Breast Cancer Stem Cells

Marco A. Velasco-Velázquez^{1,2},
Xuanmao Jiao¹ and Richard G. Pestell¹

¹*Kimmel Cancer Center, Thomas Jefferson University*

²*Facultad de Medicina, Universidad Nacional Autónoma de México*

¹*USA*

²*México*

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 frequency of primary responses, the median duration of response to chemotherapy is 8 to 14 months (Puztai & Hortobagyi, 1998). Consequently, 20% to 70% of patients show recurrent disease within 5 years (Puztai & 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, CD16, 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 occurs 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

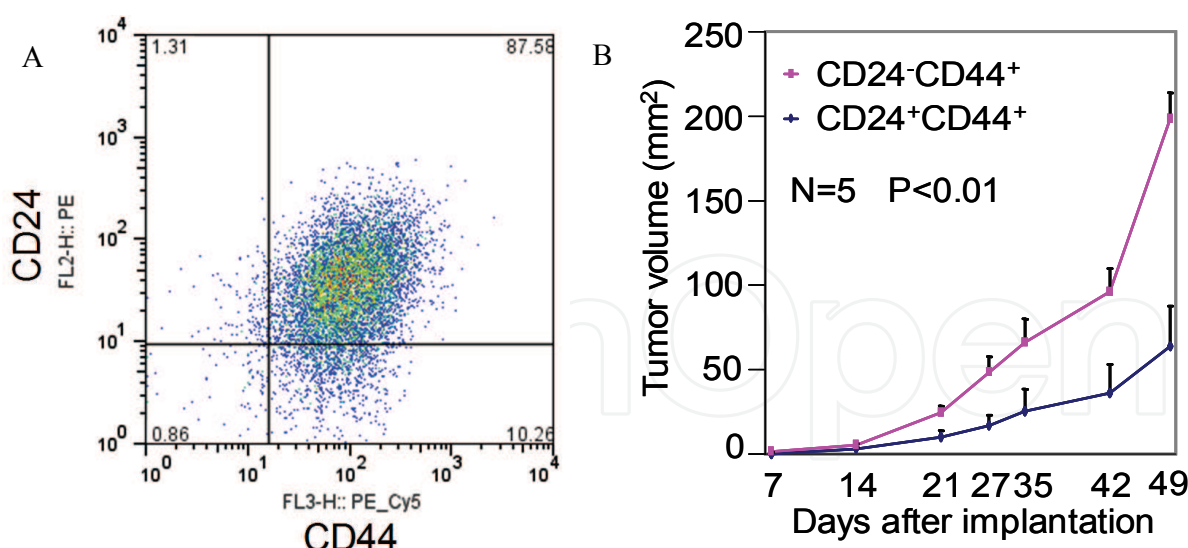


Fig. 1. Increased tumorigenicity of CD44⁺/CD24⁻ Met-1 mouse breast cancer cells (from Wu et al., 2010 J Biol Chem, in press). A) Met-1 cells were separated by immunosorting based on the phenotypes CD44⁺/CD24⁻ or CD44⁺/CD24⁺. B) Tumor regeneration after subcutaneous implantation of 1000 sorted cells per 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 comparison 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 PKH26^{hi} cells (0.2-0.4% of the total cell population) are able to form secondary mammospheres, divide asymmetrically, 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 PKH26^{hi} cells allowed the identification of the CD49f⁺/DLL1^{hi}/DNER^{hi} phenotype as prospective markers of human breast CSCs (Pece et al., 2010). CD49f⁺/DLL1^{hi}/DNER^{hi} cells are present in human breast tumor samples, corresponding to 1.5-6% of the cancer cells. The CD49f⁺/DLL1^{hi}/DNER^{hi} 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).

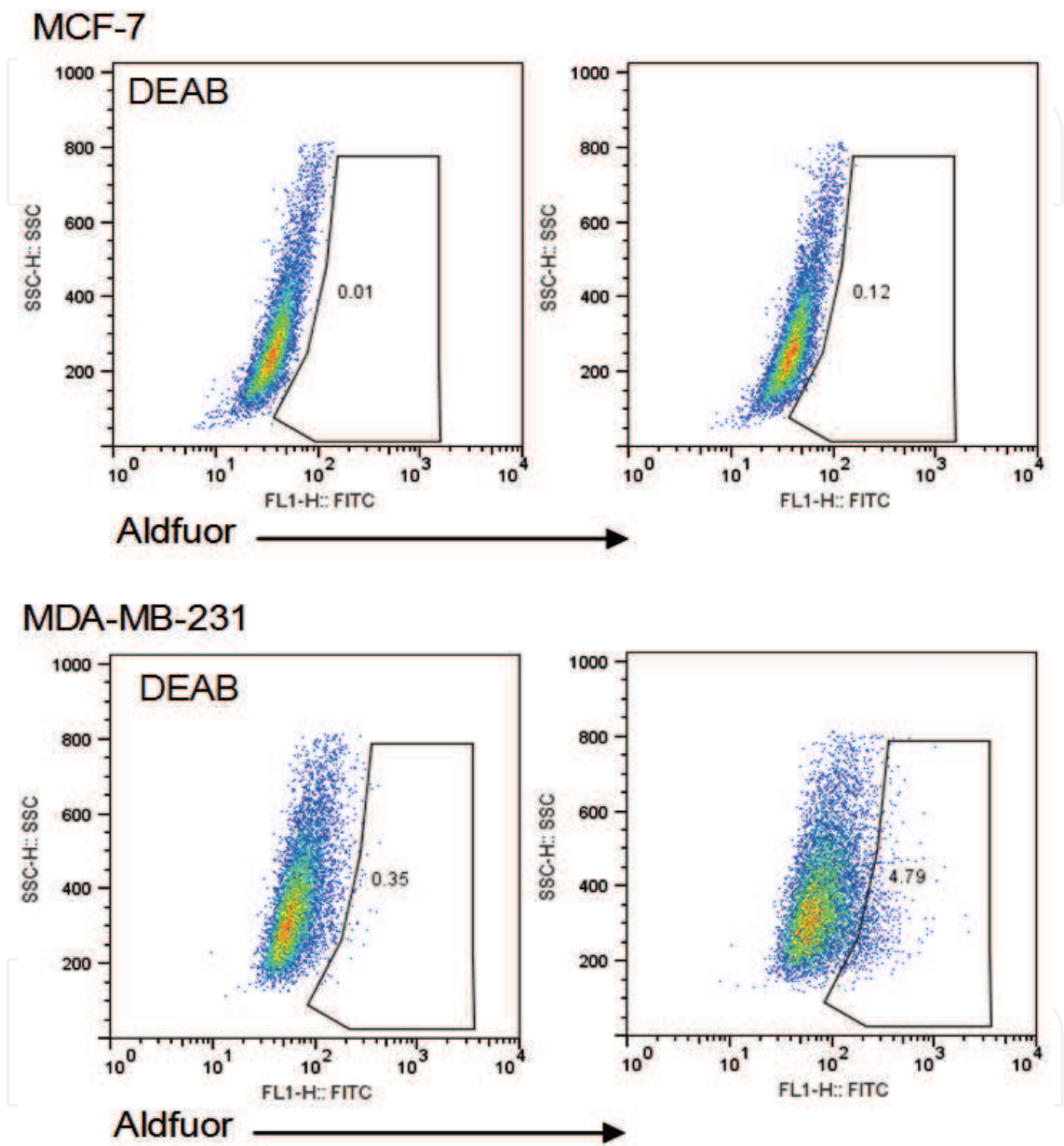


Fig. 2. Aldefluor assay of MCF-7 and MDA-MB-231 breast cancer cells (Jiao et al., unpublished). This assay allows the identification and separation of CSCs based on the activity of ALDH. DEAB is an inhibitor of ALDH used to increase specificity of the assay. Note that the ALDH⁺ fraction in MCF-7 cells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

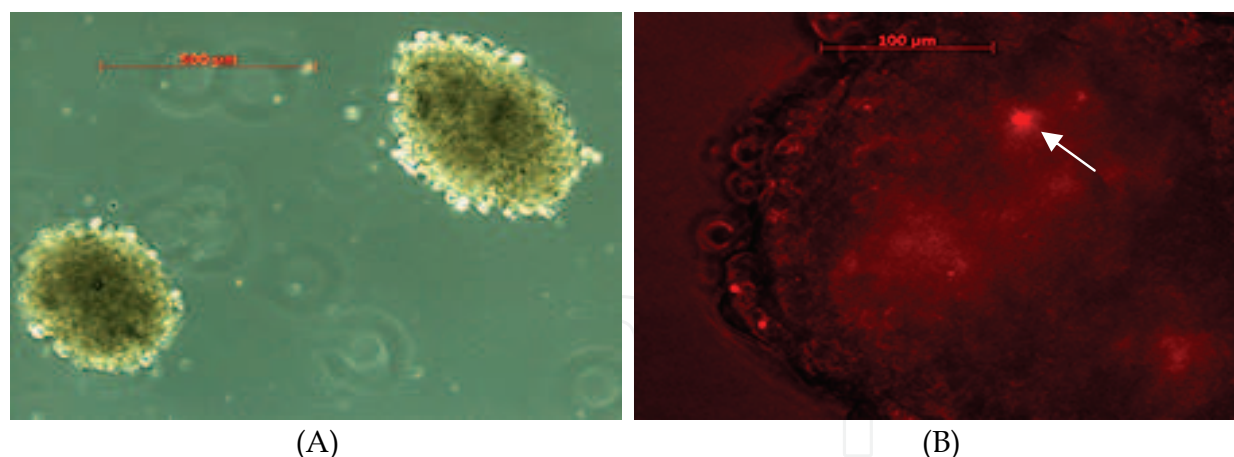


Fig. 3. PKH26 retention during mammosphere culture (Velasco-Velazquez et al., unpublished). A) Hs578T human breast cancer cells cultured in non-adherent conditions for 7 days (bright field). B) Fluorescence microscopy shows that only a few cells retain a high level of PKH26 (arrow). Those cells have 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 than 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 β 1 integrin subunit (CD29). In tumor cell lines generated from 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-naïve 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-mediated 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) chemoresistance 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-1 α , 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), NF κ B (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

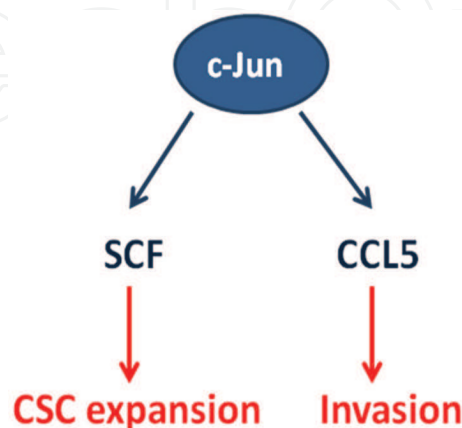


Fig. 4. Schematic representation of c-Jun-mediated cellular migration and CSC expansion via induction of SCF and CCL5 (RANTES) production (adapted from Jiao et al. 2010).

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 caspase-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 successful 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. $\alpha 6$ -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 apoptosis 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- $\alpha 6$ 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.

7. References

- Aktas, B., Tewes, M., Fehm, T., Hauch, S., Kimmig, R. & Kasimir-Bauer, S. (2009). Stem cell and epithelial-mesenchymal transition markers are frequently overexpressed in circulating tumor cells of metastatic breast cancer patients, *Breast Cancer Res* 11(4): R46.
- Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J. & Clarke, M.F. (2003). Prospective identification of tumorigenic breast cancer cells, *Proc Natl Acad Sci U S A* 100(7): 3983-8.
- Allan, A.L., Vantyghem, S.A., Tuck, A.B. & Chambers, A.F. (2006). Tumor dormancy and cancer stem cells: implications for the biology and treatment of breast cancer metastasis, *Breast Dis* 26: 87-98.
- Anderson, B.O., Yip, C.H., Ramsey, S.D., Bengoa, R., Braun, S., Fitch, M., Groot, M., Sancho-Garnier, H. & Tsu, V.D. (2006). Breast cancer in limited-resource countries: health care systems and public policy, *Breast J* 12 Suppl 1: S54-69.
- Balic, M., Lin, H., Young, L., Hawes, D., Giuliano, A., McNamara, G., Datar, R.H. & Cote, R.J. (2006). Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype, *Clin Cancer Res* 12(19): 5615-21.
- Bauerschmitz, G.J., Ranki, T., Kangasniemi, L., Ribacka, C., Eriksson, M., Porten, M., Herrmann, I., Ristimäki, A., Virkkunen, P., Tarkkanen, M., Hakkarainen, T., Kanerva, A., Rein, D., Pesonen, S. & Hemminki, A. (2008). Tissue-specific promoters active in CD44+CD24-/low breast cancer cells, *Cancer Res* 68(14): 5533-9.
- Bonnet, D. & Dick, J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell, *Nat Med* 3(7): 730-7.
- Boyle, P. (2005). Breast cancer control: signs of progress, but more work required, *Breast* 14(6): 429-38.
- Boyle, P. & Ferlay, J. (2005). Cancer incidence and mortality in Europe, 2004, *Ann Oncol* 16(3): 481-8.
- Cariati, M., Naderi, A., Brown, J.P., Smalley, M.J., Pinder, S.E., Caldas, C. & Purushotham, A.D. (2008). Alpha-6 integrin is necessary for the tumorigenicity of a stem cell-like subpopulation within the MCF7 breast cancer cell line, *Int J Cancer* 122(2): 298-304.
- Chambers, A.F., Groom, A.C. & MacDonald, I.C. (2002). Dissemination and growth of cancer cells in metastatic sites, *Nat Rev Cancer* 2(8): 563-72.
- Charafe-Jauffret, E., Ginestier, C., Iovino, F., Tarpin, C., Diebel, M., Esterni, B., Houvenaeghel, G., Extra, J.M., Bertucci, F., Jacquemier, J., Xerri, L., Dontu, G., Stassi, G., Xiao, Y., Barsky, S.H., Birnbaum, D., Viens, P. & Wicha, M.S. (2010). Aldehyde dehydrogenase 1-positive cancer stem cells mediate metastasis and poor clinical outcome in inflammatory breast cancer, *Clin Cancer Res* 16(1): 45-55.
- Charafe-Jauffret, E., Ginestier, C., Iovino, F., Wicinski, J., Cervera, N., Finetti, P., Hur, M.H., Diebel, M.E., Monville, F., Dutcher, J., Brown, M., Viens, P., Xerri, L., Bertucci, F., Stassi, G., Dontu, G., Birnbaum, D. & Wicha, M.S. (2009). Breast cancer cell lines

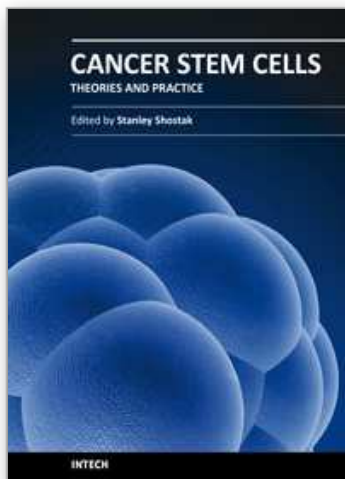
- contain functional cancer stem cells with metastatic capacity and a distinct molecular signature, *Cancer Res* 69(4): 1302-13.
- Charafe-Jauffret, E., Monville, F., Ginestier, C., Dontu, G., Birnbaum, D. & Wicha, M.S. (2008). Cancer stem cells in breast: current opinion and future challenges, *Pathobiology* 75(2): 75-84.
- Christ, O., Lucke, K., Imren, S., Leung, K., Hamilton, M., Eaves, A., Smith, C. & Eaves, C. (2007). Improved purification of hematopoietic stem cells based on their elevated aldehyde dehydrogenase activity, *Haematologica* 92(9): 1165-72.
- Christgen, M., Ballmaier, M., Bruchhardt, H., von Wasielewski, R., Kreipe, H. & Lehmann, U. (2007). Identification of a distinct side population of cancer cells in the Cal-51 human breast carcinoma cell line, *Mol Cell Biochem* 306(1-2): 201-12.
- Cicalese, A., Bonizzi, G., Pasi, C.E., Faretta, M., Ronzoni, S., Giulini, B., Brisken, C., Minucci, S., Di Fiore, P.P. & Pelicci, P.G. (2009). The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells, *Cell* 138(6): 1083-95.
- Colleoni, M., Viale, G., Zahrieh, D., Pruner, G., Gentilini, O., Veronesi, P., Gelber, R.D., Curigliano, G., Torrisi, R., Luini, A., Intra, M., Galimberti, V., Renne, G., Nole, F., Peruzzotti, G. & Goldhirsch, A. (2004). Chemotherapy is more effective in patients with breast cancer not expressing steroid hormone receptors: a study of preoperative treatment, *Clin Cancer Res* 10(19): 6622-8.
- Corti, S., Locatelli, F., Papadimitriou, D., Donadoni, C., Salani, S., Del Bo, R., Strazzer, S., Bresolin, N. & Comi, G.P. (2006). Identification of a primitive brain-derived neural stem cell population based on aldehyde dehydrogenase activity, *Stem Cells* 24(4): 975-85.
- Crocker, A.K., Goodale, D., Chu, J., Postenka, C., Hedley, B.D., Hess, D.A. & Allan, A.L. (2009). High aldehyde dehydrogenase and expression of cancer stem cell markers selects for breast cancer cells with enhanced malignant and metastatic ability, *J Cell Mol Med* 13(8B): 2236-52.
- de Grouw, E.P., Raaijmakers, M.H., Boezeman, J.B., van der Reijden, B.A., van de Locht, L.T., de Witte, T.J., Jansen, J.H. & Raymakers, R.A. (2006). Preferential expression of a high number of ATP binding cassette transporters in both normal and leukemic CD34+CD38- cells, *Leukemia* 20(4): 750-4.
- Dontu, G., Abdallah, W.M., Foley, J.M., Jackson, K.W., Clarke, M.F., Kawamura, M.J. & Wicha, M.S. (2003). In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells, *Genes Dev* 17(10): 1253-70.
- Dontu, G., Jackson, K.W., McNicholas, E., Kawamura, M.J., Abdallah, W.M. & Wicha, M.S. (2004). Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells, *Breast Cancer Res* 6(6): R605-15.
- Dylla, S.J., Beviglia, L., Park, I.K., Chartier, C., Raval, J., Ngan, L., Pickell, K., Aguilar, J., Lazetic, S., Smith-Berdan, S., Clarke, M.F., Hoey, T., Lewicki, J. & Gurney, A.L. (2008). Colorectal cancer stem cells are enriched in xenogeneic tumors following chemotherapy, *PLoS ONE* 3(6): e2428.
- Eramo, A., Lotti, F., Sette, G., Piloizzi, E., Biffoni, M., Di Virgilio, A., Conticello, C., Ruco, L., Peschle, C. & De Maria, R. (2008). Identification and expansion of the tumorigenic lung cancer stem cell population, *Cell Death Differ* 15(3): 504-14.
- Farnie, G., Clarke, R.B., Spence, K., Pinnock, N., Brennan, K., Anderson, N.G. & Bundred, N.J. (2007). Novel cell culture technique for primary ductal carcinoma in situ: role of Notch and epidermal growth factor receptor signaling pathways, *J Natl Cancer Inst* 99(8): 616-27.
- Feldmann, G., Fendrich, V., McGovern, K., Bedja, D., Bisht, S., Alvarez, H., Koorstra, J.B., Habbe, N., Karikari, C., Mullendore, M., Gabrielson, K.L., Sharma, R., Matsui, W. &

- Maitra, A. (2008). An orally bioavailable small-molecule inhibitor of Hedgehog signaling inhibits tumor initiation and metastasis in pancreatic cancer, *Mol Cancer Ther* 7(9): 2725-35.
- Fillmore, C.M. & Kuperwasser, C. (2008). Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy, *Breast Cancer Res* 10(2): R25.
- Fuchs, D., Daniel, V., Sadeghi, M., Opelz, G. & Naujokat, C. (2010). Salinomycin overcomes ABC transporter-mediated multidrug and apoptosis resistance in human leukemia stem cell-like KG-1a cells, *Biochem Biophys Res Commun* 394(4): 1098-104.
- Fuchs, D., Heinold, A., Opelz, G., Daniel, V. & Naujokat, C. (2009). Salinomycin induces apoptosis and overcomes apoptosis resistance in human cancer cells, *Biochem Biophys Res Commun* 390(3): 743-9.
- Ghosh, J.C., Dohi, T., Raskett, C.M., Kowalik, T.F. & Altieri, D.C. (2006). Activated checkpoint kinase 2 provides a survival signal for tumor cells, *Cancer Res* 66(24): 11576-9.
- Ginestier, C., Hur, M.H., Charafe-Jauffret, E., Monville, F., Dutcher, J., Brown, M., Jacquemier, J., Viens, P., Kleer, C.G., Liu, S., Schott, A., Hayes, D., Birnbaum, D., Wicha, M.S. & Dontu, G. (2007). ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome, *Cell Stem Cell* 1(5): 555-67.
- Ginestier, C., Liu, S., Diebel, M.E., Korkaya, H., Luo, M., Brown, M., Wicinski, J., Cabaud, O., Charafe-Jauffret, E., Birnbaum, D., Guan, J.L., Dontu, G. & Wicha, M.S. (2010). CXCR1 blockade selectively targets human breast cancer stem cells in vitro and in xenografts, *J Clin Invest* 120(2): 485-97.
- Glinsky, G.V. (2007). Stem cell origin of death-from-cancer phenotypes of human prostate and breast cancers, *Stem Cell Rev* 3(1): 79-93.
- Gonzalez-Angulo, A.M., Morales-Vasquez, F. & Hortobagyi, G.N. (2007). Overview of resistance to systemic therapy in patients with breast cancer, *Adv Exp Med Biol* 608: 1-22.
- Goss, P., Allan, A.L., Rodenhiser, D.I., Foster, P.J. & Chambers, A.F. (2008). New clinical and experimental approaches for studying tumor dormancy: does tumor dormancy offer a therapeutic target?, *APMIS* 116(7-8): 552-68.
- Guarneri, V. & Conte, P.F. (2004). The curability of breast cancer and the treatment of advanced disease, *Eur J Nucl Med Mol Imaging* 31 Suppl 1: S149-61.
- Gupta, P.B., Onder, T.T., Jiang, G., Tao, K., Kuperwasser, C., Weinberg, R.A. & Lander, E.S. (2009). Identification of selective inhibitors of cancer stem cells by high-throughput screening, *Cell* 138(4): 645-59.
- Heppner, G.H. & Miller, B.E. (1983). Tumor heterogeneity: biological implications and therapeutic consequences, *Cancer Metastasis Rev* 2(1): 5-23.
- Hess, D.A., Meyerrose, T.E., Wirthlin, L., Craft, T.P., Herrbrich, P.E., Creer, M.H. & Nolte, J.A. (2004). Functional characterization of highly purified human hematopoietic repopulating cells isolated according to aldehyde dehydrogenase activity, *Blood* 104(6): 1648-55.
- Hirsch, H.A., Iliopoulos, D., Tsichlis, P.N. & Struhl, K. (2009). Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission, *Cancer Res* 69(19): 7507-11.
- Jiao, X., Katiyar, S., Willmarth, N.E., Liu, M., Ma, X., Flomenberg, N., Lisanti, M.P. & Pestell, R.G. (2010). c-Jun induces mammary epithelial cellular invasion and breast cancer stem cell expansion, *J Biol Chem* 285(11): 8218-26.

- Jin, L., Hope, K.J., Zhai, Q., Smadja-Joffe, F. & Dick, J.E. (2006). Targeting of CD44 eradicates human acute myeloid leukemic stem cells, *Nat Med* 12(10): 1167-74.
- Kakarala, M. & Wicha, M.S. (2008). Implications of the cancer stem-cell hypothesis for breast cancer prevention and therapy, *J Clin Oncol* 26(17): 2813-20.
- Kim, J.B., Ko, E., Han, W., Shin, I., Park, S.Y. & Noh, D.Y. (2008). Berberine Diminishes the Side Population and ABCG2 Transporter Expression in MCF-7 Breast Cancer Cells, *Planta Med.*
- Korkaya, H., Paulson, A., Iovino, F. & Wicha, M.S. (2008). HER2 regulates the mammary stem/progenitor cell population driving tumorigenesis and invasion, *Oncogene* 27(47): 6120-30.
- Lee, C.W., Raskett, C.M., Prudovsky, I. & Altieri, D.C. (2008). Molecular dependence of estrogen receptor-negative breast cancer on a notch-survivin signaling axis, *Cancer Res* 68(13): 5273-81.
- Lens, S.M., Vader, G. & Medema, R.H. (2006). The case for Survivin as mitotic regulator, *Curr Opin Cell Biol* 18(6): 616-22.
- Li, C., Heidt, D.G., Dalerba, P., Burant, C.F., Zhang, L., Adsay, V., Wicha, M., Clarke, M.F. & Simeone, D.M. (2007). Identification of pancreatic cancer stem cells, *Cancer Res* 67(3): 1030-7.
- Li, F., Tiede, B., Massague, J. & Kang, Y. (2007). Beyond tumorigenesis: cancer stem cells in metastasis, *Cell Res* 17(1): 3-14.
- Lindsay, J., Jiao, X., Sakamaki, T., Casimiro, M.C., Shirley, L.A., Tran, T.H., Ju, X., Liu, M., Li, Z., Wang, C., Katiyar, S., Rao, M., Allen, K.G., Glazer, R.I., Ge, C., Stanley, P., Lisanti, M.P., Rui, H. & Pestell, R.G. (2008). ErbB2 induces Notch1 activity and function in breast cancer cells, *Clin Transl Sci* 1(2): 107-15.
- Liu, M., Casimiro, M.C., Wang, C., Shirley, L.A., Jiao, X., Katiyar, S., Ju, X., Li, Z., Yu, Z., Zhou, J., Johnson, M., Fortina, P., Hyslop, T., Windle, J.J. & Pestell, R.G. (2009). p21CIP1 attenuates Ras- and c-Myc-dependent breast tumor epithelial mesenchymal transition and cancer stem cell-like gene expression in vivo, *Proc Natl Acad Sci U S A* 106(45): 19035-9.
- Liu, R., Wang, X., Chen, G.Y., Dalerba, P., Gurney, A., Hoey, T., Sherlock, G., Lewicki, J., Shedden, K. & Clarke, M.F. (2007). The prognostic role of a gene signature from tumorigenic breast-cancer cells, *N Engl J Med* 356(3): 217-26.
- MacDonald, I.C., Groom, A.C. & Chambers, A.F. (2002). Cancer spread and micrometastasis development: quantitative approaches for in vivo models, *Bioessays* 24(10): 885-93.
- Micalizzi, D.S., Christensen, K.L., Jedlicka, P., Coletta, R.D., Baron, A.E., Harrell, J.C., Horwitz, K.B., Billheimer, D., Heichman, K.A., Welm, A.L., Schiemann, W.P. & Ford, H.L. (2009). The Six1 homeoprotein induces human mammary carcinoma cells to undergo epithelial-mesenchymal transition and metastasis in mice through increasing TGF-beta signaling, *J Clin Invest* 119(9): 2678-90.
- O'Connor, D.S., Wall, N.R., Porter, A.C. & Altieri, D.C. (2002). A p34(cdc2) survival checkpoint in cancer, *Cancer Cell* 2(1): 43-54.
- Ozben, T. (2006). Mechanisms and strategies to overcome multiple drug resistance in cancer, *FEBS Lett* 580(12): 2903-9.
- Pantel, K. & Brakenhoff, R.H. (2004). Dissecting the metastatic cascade, *Nat Rev Cancer* 4(6): 448-56.
- Parkin, D.M., Bray, F., Ferlay, J. & Pisani, P. (2005). Global cancer statistics, 2002, *CA Cancer J Clin* 55(2): 74-108.
- Parkin, D.M. & Fernandez, L.M. (2006). Use of statistics to assess the global burden of breast cancer, *Breast J* 12 Suppl 1: S70-80.

- Patrawala, L., Calhoun, T., Schneider-Broussard, R., Zhou, J., Claypool, K. & Tang, D.G. (2005). Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2⁺ and ABCG2⁻ cancer cells are similarly tumorigenic, *Cancer Res* 65(14): 6207-19.
- Pece, S., Serresi, M., Santolini, E., Capra, M., Hulleman, E., Galimberti, V., Zurrida, S., Maisonneuve, P., Viale, G. & Di Fiore, P.P. (2004). Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis, *J Cell Biol* 167(2): 215-21.
- Pece, S., Tosoni, D., Confalonieri, S., Mazzarol, G., Vecchi, M., Ronzoni, S., Bernard, L., Viale, G., Pelicci, P.G. & Di Fiore, P.P. (2010). Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content, *Cell* 140(1): 62-73.
- Perez-Soler, R., Neamati, N., Zou, Y., Schneider, E., Doyle, L.A., Andreeff, M., Priebe, W. & Ling, Y.H. (1997). Annamycin circumvents resistance mediated by the multidrug resistance-associated protein (MRP) in breast MCF-7 and small-cell lung UMCC-1 cancer cell lines selected for resistance to etoposide, *Int J Cancer* 71(1): 35-41.
- Phillips, T.M., McBride, W.H. & Pajonk, F. (2006). The response of CD24^{-/low}/CD44⁺ breast cancer-initiating cells to radiation, *J Natl Cancer Inst* 98(24): 1777-85.
- Pisani, P., Bray, F. & Parkin, D.M. (2002). Estimates of the world-wide prevalence of cancer for 25 sites in the adult population, *Int J Cancer* 97(1): 72-81.
- Platt, V.M. & Szoka, F.C., Jr. (2008). Anticancer therapeutics: targeting macromolecules and nanocarriers to hyaluronan or CD44, a hyaluronan receptor, *Mol Pharm* 5(4): 474-86.
- Ponti, D., Costa, A., Zaffaroni, N., Pratesi, G., Petrangolini, G., Coradini, D., Pilotti, S., Pierotti, M.A. & Daidone, M.G. (2005). Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties, *Cancer Res* 65(13): 5506-11.
- Prince, M.E., Sivanandan, R., Kaczorowski, A., Wolf, G.T., Kaplan, M.J., Dalerba, P., Weissman, I.L., Clarke, M.F. & Ailles, L.E. (2007). Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma, *Proc Natl Acad Sci U S A* 104(3): 973-8.
- Pusztai, L. & Hortobagyi, G.N. (1998). High-dose chemotherapy: how resistant is breast cancer?, *Drug Resist Updat* 1(1): 62-72.
- Ragaz, J. (2009). Radiation impact in breast cancer, *Breast Cancer Res* 11 Suppl 3: S14.
- Riccioni, R., Dupuis, M.L., Bernabei, M., Petrucci, E., Pasquini, L., Mariani, G., Cianfriglia, M. & Testa, U. (2010). The cancer stem cell selective inhibitor salinomycin is a p-glycoprotein inhibitor, *Blood Cells Mol Dis* 45(1): 86-92.
- Ries, L.A.G., Melbert, D., Krapcho, M., Stinchcomb, D.G., Howlader, N., Horner, M.J., Mariotto, A., Miller, B.A., Feuer, E.J., Altekruse, S.F., Lewis, D.R., Clegg, L., Eisner, M.P., Reichman, M. & Edwards, B.K. (2008). *SEER Cancer Statistics Review, 1975-2005*. Bethesda, MD, National Cancer Institute.
- Sajithlal, G.B., Rothermund, K., Zhang, F., Dabbs, D.J., Latimer, J.J., Grant, S.G. & Prochownik, E.V. (2010). Permanently blocked stem cells derived from breast cancer cell lines, *Stem Cells* 28(6): 1008-18.
- Shafee, N., Smith, C.R., Wei, S., Kim, Y., Mills, G.B., Hortobagyi, G.N., Stanbridge, E.J. & Lee, E.Y. (2008). Cancer stem cells contribute to cisplatin resistance in Brca1/p53-mediated mouse mammary tumors, *Cancer Res* 68(9): 3243-50.
- Sheridan, C., Kishimoto, H., Fuchs, R.K., Mehrotra, S., Bhat-Nakshatri, P., Turner, C.H., Goulet, R., Jr., Badve, S. & Nakshatri, H. (2006). CD44⁺/CD24⁻ breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis, *Breast Cancer Res* 8(5): R59.

- Slamon, D. & Pegram, M. (2001). Rationale for trastuzumab (Herceptin) in adjuvant breast cancer trials, *Semin Oncol* 28(1 Suppl 3): 13-9.
- Sperr, W.R., Florian, S., Hauswirth, A.W. & Valent, P. (2005). CD 33 as a target of therapy in acute myeloid leukemia: current status and future perspectives, *Leuk Lymphoma* 46(8): 1115-20.
- Stahl, M., Ge, C., Shi, S., Pestell, R.G. & Stanley, P. (2006). Notch1-induced transformation of RKE-1 cells requires up-regulation of cyclin D1, *Cancer Res* 66(15): 7562-70.
- Stylianou, S., Clarke, R.B. & Brennan, K. (2006). Aberrant activation of notch signaling in human breast cancer, *Cancer Res* 66(3): 1517-25.
- Theodoropoulos, P.A., Polioudaki, H., Agelaki, S., Kallergi, G., Saridaki, Z., Mavroudis, D. & Georgoulas, V. (2010). Circulating tumor cells with a putative stem cell phenotype in peripheral blood of patients with breast cancer, *Cancer Lett* 288(1): 99-106.
- Tsimberidou, A.M., Giles, F.J., Estey, E., O'Brien, S., Keating, M.J. & Kantarjian, H.M. (2006). The role of gemtuzumab ozogamicin in acute leukaemia therapy, *Br J Haematol* 132(4): 398-409.
- Vassilopoulos, A., Wang, R.H., Petrovas, C., Ambrozak, D., Koup, R. & Deng, C.X. (2008). Identification and characterization of cancer initiating cells from BRCA1 related mammary tumors using markers for normal mammary stem cells, *Int J Biol Sci* 4(3): 133-42.
- Vazquez-Martin, A., Oliveras-Ferraros, C., Barco, S.D., Martin-Castillo, B. & Menendez, J.A. (2010b). The anti-diabetic drug metformin suppresses self-renewal and proliferation of trastuzumab-resistant tumor-initiating breast cancer stem cells, *Breast Cancer Res Treat.*
- Vazquez-Martin, A., Oliveras-Ferraros, C., Cufi, S., Martin-Castillo, B. & Menendez, J.A. (2010a). Metformin and Energy Metabolism in Breast Cancer: From Insulin Physiology to Tumour-initiating Stem Cells, *Curr Mol Med* 10(7): 674-91.
- Visvader, J.E. & Lindeman, G.J. (2008). Cancer stem cells in solid tumours: accumulating evidence and unresolved questions, *Nat Rev Cancer* 8(10): 755-68.
- Weiss, L. (1990). Metastatic inefficiency, *Adv Cancer Res* 54: 159-211.
- Wicha, M.S. (2006). Cancer stem cells and metastasis: lethal seeds, *Clin Cancer Res* 12(19): 5606-7.
- Woodhouse, E.C., Chuaqui, R.F. & Liotta, L.A. (1997). General mechanisms of metastasis, *Cancer* 80(8 Suppl): 1529-37.
- Woodward, W.A., Chen, M.S., Behbod, F., Alfaro, M.P., Buchholz, T.A. & Rosen, J.M. (2007). WNT/beta-catenin mediates radiation resistance of mouse mammary progenitor cells, *Proc Natl Acad Sci U S A* 104(2): 618-23.
- Wright, M.H., Calcagno, A.M., Salcido, C.D., Carlson, M.D., Ambudkar, S.V. & Varticovski, L. (2008). Brca1 breast tumors contain distinct CD44+/CD24- and CD133+ cells with cancer stem cell characteristics, *Breast Cancer Res* 10(1): R10.
- Wu, K., Jiao, X., Li, Z., Katiyar, S., Casimiro, M.C., Yang, W., Zhang, Q., Willmarth, N.E., Chepelev, I., Crosariol, M., Wei, Z., Li, A., Zhao, J., & Pestell, R.G. (2010). The cell fate determination factor dachshund reprograms breast cancer stem cell function. *J Biol Chem* in press.
- Yu, F., Yao, H., Zhu, P., Zhang, X., Pan, Q., Gong, C., Huang, Y., Hu, X., Su, F., Lieberman, J. & Song, E. (2007). let-7 regulates self renewal and tumorigenicity of breast cancer cells, *Cell* 131(6): 1109-23.



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Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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