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BREAST CANCER STEM-LIKE CELLS: CLINICAL IMPLICATIONS AND THERAPEUTIC STRATEGIES

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Abstract

Breast cancer is the most frequently diagnosed cancer in women, being also the leading cause of cancer death among female population, including in Romania. Resistance to therapy represents a major problem for cancer treatment. Current cancer treatments are both expensive and induce serious side effects; therefore ineffective therapies are both traumatic and pricy. Characterizing predictive markers that can identify high-risk patients could contribute to dedicated/personalized therapy to improve the life quality and expectancy of cancer patients. Moreover, there are some markers that govern specific tumor molecular features that can be targeted with specific therapies for those patients who are most likely to benefit. The identification of stem cells in both normal and malignant breast tissue have lead to the hypothesis that breast tumors arise from breast cancer stem-like cells (CSCs), and that these cells influence tumor's response to therapy. CSCs have similar self-renewal properties to normal stem cells, however the balance between the signaling pathways is altered towards tumor formation In this review, we discuss the molecular aspects of breast CSCs and the controversies regarding their use in the diagnosis and treatment decision of breast cancer patients.

Keywords: cancer stem-like cells, breast cancer, signaling pathways, targeting strategies

Introduction

Malignant neoplasms are the second cause of death, after heart diseases, and it is estimated to rank first beginning with 2010. Worldwide, 12.7 million people were diagnosed with some type of cancer in 2008, 56% of the cases resulting in death [1]. Breast cancer is the most frequently diagnosed cancer in women, being also the leading cause of cancer death among female population, including in Romania according European Cancer Observatory. If diagnosed at early stages, the overall five-year relative survival among US women is 98%, but drops to 84% and 23% when the disease has spread to regional lymph nodes or distant organs [2]. Early detection through mammography and improved treatments has increased the survival of these patients in westernized countries [3], however some breast cancer patients fail to respond to conventional treatments, leading to tumor recurrence. This can be caused by baseline resistance due to intrinsic factors, or acquired during therapy.

Breast cancer prognosis is dependent on the number of lymph nodes involved, tumor size, histological grade, and hormone receptor status. Even so, even after an accurate classification, tumors can respond to treatment in a surprising way, and the prognosis can vary. In order to

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explain these observations, other factors such as Her2 gene amplification, EGFR family members, cell cycle fraction analysis, p53 mutations, presence of circulating tumor cells and lymphovascular invasion are being investigated for prognostic and predictive value. Therefore, therapy selection requires the consideration of not only the clinical status of the patient but also the molecular characteristics of the tumor. Current systemic treatments of breast cancer include cytotoxic, hormonal, and immunotherapeutic agents, medications that are administered in adjuvant, neoadjuvant, and metastatic settings. Molecular analysis classifies breast tumors into four major subtypes: luminal A and B, HER2-overexpressing and basal-like tumors, treatment regimens being dependent on the patient breast tumor molecular subtype. With the advancement of new chemotherapeutics, hormone and biological agents and the integration of systemic therapy with surgery and radiation therapy, treatment plan decision is proving to become more complicated.

The CSCs concept

Despite early detection and molecular classification of breast tumors have improved breast cancer patients outcome, approximately 30% of early-stage breast cancer cases relapse. In general, cancer reappears at distant sites, thereby strengthening the hypothesis that breast cancer is a systemic disease. Currently, the research is focused on finding and characterizing specific markers that can identify high-risk patients and have the potential to be developed as a targeting strategy for future therapies.

Recent molecular studies have emphasized a series of molecular particularities that have been involved in therapy response and disease relapse [4-6], however tumor heterogeneity complicates the study and treatment of breast cancer. Several cell populations exist within a single tumor; moreover there are differences within the same cell populations [7]. Two models have been proposed to describe tumor heterogeneity (Figure 1): in the non-hierarchical model (Figure 1A), cells undergo a clonal evolution, all cells having equal chances to acquire genetic lesions and develop a malignant phenotype, while the hierarchical model (Figure 1B) proposes the cancer stem-like cells (CSCs) concept, in which a subset of cells act as multipotent progenitors that drive tumor growth. Recent isolation of subpopulations of tumor cells that have stem cells related cell behavior supports the CSC hypothesis.

Advances in stem-cell technology have made possible the identification of stem cells in normal and malignant breast tissue. "Primitive" stem-like tumor-initiating cells have been previously identified within breast tumors [8], therefore suggesting that CSCs are responsible for breast tumors heterogeneity [9]. More and more reports provide evidence that breast tumors arise from breast cancer stem-like cells (CSCs) [10], and that these cells influence tumor response to therapy [4-6]. CSCs are slowly proliferating cells, quiescent in G0 phase for long periods of time; hence they may escape conventional treatments that mainly target actively proliferating cells. CSCs have similar self-renewal properties to normal stem cells, however the balance between the signaling pathways is altered towards tumor formation [11]. CSCs are not necessarily transformed normal stem cells, but rather a mix between the two models (Figure 1C) [12] could explain the genetic instability and plasticity of tumor cells. CSCs may arise from restricted progenitors or differentiated cells by acquiring stem cell-like properties and further undergo clonal selection to generate different subtypes of breast cancers (Triple negative, Her2-gene amplified, luminal).

CSCs identification and functional analysis

CSCs can be identified based on functional activity (self-renewal, serial tumor propagation) and phenotypic markers ($CD44^+/CD24^-$, aldehyde dehydrogenase-1 (ALDH) activity) [13]. CD44 has been positively associated with stem cell-like characteristics and CD24 expression is related to differentiated epithelial features [14].

Immunohistochemistry staining is the only standardized accepted diagnostic tool and this method has been previously used to detect CSCs in breast cancer patients [15]. However, immunohistochemistry analysis cannot asses CSCs functionality, therefore this detection method needs to be correlated with functional assay of CSCs activity.

The 'gold standard' methods for assessing CSC activity experimentally are *in vivo* tumor formation and serial transplantation assays. Studying CSCs in isolated systems, such as cell cultures, has its limitations, it has been suggested that breast CSCs require a particular niche in which to grow for maintenance [16]. There is an active crosstalk between tumors and the microenvironment, microenvironmental effects can influence the induction of the epithelial-to-mesenchymal transition (EMT) of breast cancer cells [11], a process that is associated with CSCs enrichment of tumors [17].

In vivo transplantation methods are technically challenging, lengthy, expensive, with ethical implications and impractical in clinical trial settings. Alternative *in vitro* methods such as mammosphere assays and identification of cell surface markers such as CD44+/CD24-have been previously utilized in pre-clinical studies and in pre-surgical window trials.

CSCs signaling pathways and targeting strategies

Understanding the molecular mechanisms that govern tumor resistance is imperative for individualized medicine as well as for future treatment developments. Several studies have showed that breast CSCs are associated with resistance to standard radiation and chemotherapy [18,19], moreover these cells are enriched following treatment driving tumor recurrence and metastases [20].

Several mechanisms have been described to be responsible for CSCs resistance phenotype the most characterized being increased drug efflux transporters, increased DNA repair machinery and increased antiapoptotic

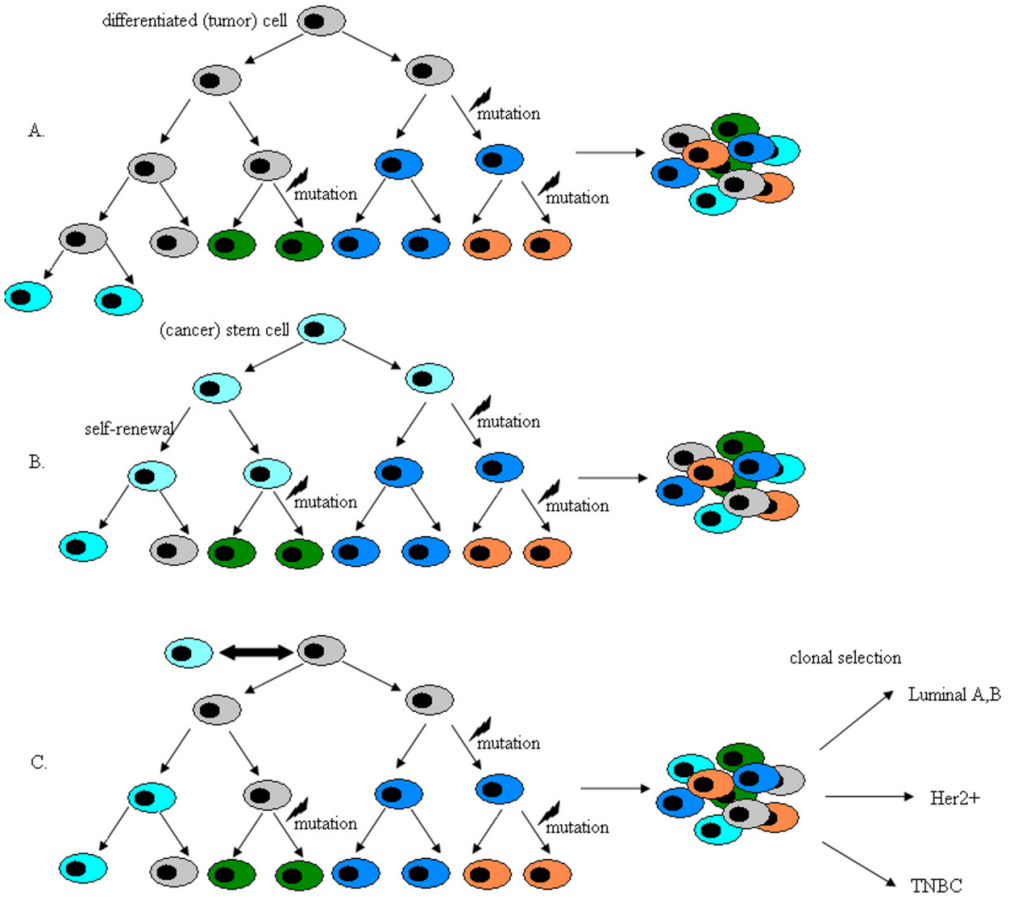

Figure 1. Models of tumor evolution that can explain breast tumor heterogeneity. A. Clonal evolution: all cells have the ability to undergo mutations and generate different clones, B. CSCs model: only a subset of cells that present self-renewal ability can drive tumor growth, C. Mixed model of clonal evolution of CSCs: differentiated cells can acquire stem cells features that upon subsequent mutations generate different clones. Dominant clones determine the breast cancer subtype.

proteins expression [21]. Potential ways to target these mechanisms have been described, which include inducing cell differentiation to inhibit the self-renewal proprieties, promote apoptosis, targeting resistance mechanisms or the CSC niche.

Several groups, (including ours), have used transcriptomic analysis to investigate and predict the tumor response to treatment in order to identify patients with high risk of treatment failures. These studies generated a tremendous amount of data; hundreds of genes have been found to be over or down regulated after treatment, moreover, the data among groups is often inconsistent. According to the theory that treatment resistance is given by the CSCs presence within the tumor mass, these studies need to be reassessed to take into consideration the significance of CSCs populations. CSCs abundance within tumors can be associated and varies with ER, PR, Her2 expression and molecular subtype (Table I). Studies have showed that not all tumors present CSCs [15,22-29], but their presence is associated with poorer outcome [27]. To our knowledge, there are very few gene expression studies that assessed the gene signature of CSCs within the tumors [20,30], but these studies compared CSCs signatures with those of tumor cells, thus important information regarding the CSCs plasticity to tumor cells and vice versa could have been lost. CSCs signaling pathways described so far are common both to normal stem cells and cancer biology and it has not brought us closer to clarify whether CSCs arise from normal stem or cancer cells. Moreover, there are no defined drugs that specifically target CSCs in the clinic.

Aberrant Notch, Hedgehog, Wnt, EGFR and Nf-kB pathways have been described to maintain malignant stem cell activity [31]. In a recent study [32], we have identified stem cells pluripotency signaling pathways to be regulated as response to treatment. We have identified several canonical pathways to be involved in treatment response, among which some that are known to be involved in stem cells pluripotency. When we measured this activity by mammosphere assay, we observed that the treated cells formed smaller mammospheres, but the number of CSCs was little affected if at all when compared to untreated cells, which sustains CSCs enrichment and tumor re-bulking theory.

Table I. Distribution of CD44+/CD24− phenotype according to breast cancer expression of ER,PR, Her2 receptors.

n*	CD44+/CD24− phenotype (%)										Ref.
	ER		PR		Her2		Subtype				
	-	+	-	+	-	+	Luminal		Her2+	TNBC	
							A	B			
132	12.1	13.6	12.1	13.6	16.6	9.1	16.6	28.1	17.6	44.8	[28]
121	4.4	7.2	1.6	10.5	7	4.4	-	-	-	-	[22]
57	21.1	78.9	28.1	71.9	-	-	-	-	-	-	[23]
156	13.1	24.1	17.2	18.7	27.9	7.4	1.6	21.1	3.1	10.9	[24]
94	17	8	19	2			8			23	[29]
818	1	4.27	1.5	4.27	5.6	0.4	4.76		0.36	0.61	[25]
108	25.2	19.7	21.7	21.1	23.8	19.2	-	-	-	-	[26]
38					36.8	7.8	26.7			60	[27]

*n=number of patients

Several similarities have been observed between CSCs and epithelial mesenchymal transition (EMT) state. Both normal and malignant breast stem cells can adopt either EMT or MET (mesenchymal to epithelial transition) states, which are inter-convertible. The preferences for one or the other states at a given point is regulated by complex signals that originate in the microenvironment, these in turn, activate signaling cascades within the CSC population. Each of the two states have distinct characteristics: CSCs with mesenchymal morphological features that express CD24-/CD44+, EpCAM-/CD49f+ are considered in the EMT state, these cells being largely quiescent and present increased invasiveness. CSCs with dynamic self-renewal activity and expression of ALDH and EpCAM+/CD49f+ are in MET state, while cells that express both CD24-/CD44+ and ALDH may be in transition between these states. The existence of multiple states further complicates the study of CSCs and the development of effective therapeutic strategies. Targeting Notch pathway with γ-secretase inhibitors (DAPT, MK-0752, RO4929097) has been showed to significantly reduce CSCs activity both *in vitro* and *in vivo* settings being currently tested in phase I/II clinical trials [33, 34]. Moreover, Notch inhibitors are tested in combination with Hedgehog inhibitors GDC-0049, as these two pathways interact with each other to maintain self-renewal [35]. Transgenic mice lacking specific ABC transporters showed increased drug sensitivity, however, the use of ABC drug transporters like BCRP and ABCG2 increases the risk of neurotoxic effects [36].

Due to the length of time and funds it takes to study a new drug and get it approved for clinical use, researchers are lately revisiting old drugs that are currently used in other pathologies but are known to target pathways altered in CSCs. For example, Metformin (used for type 2 diabetes treatment) combined with conventional therapy has been showed to further reduce tumor growth in triple negative breast cancer by specifically targeting CSCs [37]. Also retinoids and rexinoids, strong inducers of differentiation, are currently used in some types of leukemia and has been showed to induce breast CSCs differentiation [38]. PARP inhibitors are being tested to target the CSCs DNA repair machinery [39], so far results are not clear.

So far, compounds that target CSCs have been identified mostly throughout technologies employing automated drug screening. Salinomycin, a recently described CSCs specific drug has been found after screening around 16000 compounds [40]. This method is expensive, laborious and time consuming. Moreover, some of the identified drugs are facing with the challenge of selective CSCs targeting in comparison with normal stem cells as these agents are targeting pathways that are vital in development (Notch, Wnt, Hedgehog), thus limiting their clinical applicability. Therefore, a robust analysis of the basic science must precede clinical trials in order to find drugs that could specifically target CSCs.

Overall, standard therapies in combination with CSC-targeted therapies may potentially provide a more effective treatment strategy by de-bulking the tumor mass and preventing recurrence [31].

Conclusions

There is a common belief that targeting CSCs provides a promising approach for cancer prevention and treatment [41-43], therefore the CSCs model became the foundation of preventive and therapeutic strategies in cancer. It has been showed that CSCs have important implications for early detection, prevention, and treatment of breast cancer [44-46], but the clinical and prognostic impact of these markers in breast cancer remains a controversial

issue, demanding additional efforts to elucidate the role of CSCs in breast cancer management. Alterations in the stem-cell self-renewal pathways may be responsible for both hereditary and sporadic breast cancers, making them attractive targets for the development of cancer preventive strategies. Furthermore, because CSCs are highly resistant to radiation and chemotherapy, the development of future anti-cancer therapies may require the active targeting of this cell population. Therefore, two main questions remain: are CSCs ready for the clinic, but also, is the clinic is ready for CSCs?

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