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REVIEW ARTICLE

A Leading Role of Stem Cells in Breast Malignant Cells

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ABSTRACT

A momentous evolution has been made in describing cellular hierarchy and the stem cell (SC) niche in the human mammary gland. Mammary stem and progenitor cells exist in two different states: Epithelial and mesenchymal. Several features of the mammary SCs predispose them to play a critical role in breast cancer (BC) initiation, progression, and metastasis. Signaling pathways contributing to the self-renewal, such as Wnt, Notch, Hh, and bone morphogenetic protein, have been shown to be linked with BCSCs. Furthermore, biomarkers connected with stemness, such as CD44, CD24, epithelial cell adhesion molecule, and ALDH1, have been identified and used to characterize these cells. In addition, many different miRNA families and micro environmental factors were shown to regulate a lot of cancer SCs (CSC) properties and maintain their stemness. All these findings have started a new era of BC research. In the present BC, SCs have become the targets of BC therapy, although the tests are mainly on the basic stage level. Since the CSCs are able to escape chemotherapy and are resistant to drugs, radiotherapy and apoptotic processes, the therapeutic targeting is mostly concentrated on the disruption of survival signaling pathways and the use of modern technology, like nanotechnology.

Keywords: Breast cancer stem cells, Breast cancer, Cancer stem cells, Epithelial-mesenchymal transition, Mammary stem cell, miRNA, Stem cell niche

INTRODUCTION

As most epithelia, mammary epithelium continuously replaces dead or damaged cells during the whole life of an animal and this process called tissue homeostasis is critical for adult tis sues maintenance. Typically, epithelial tissue homeostasis is maintained through the presence of stem cells (SC). They are functionally defined in connection with their ability to self-renew and differentiate into cell lineages of their original tissue.[1-3] Mammary SCs (MaSC) are capable of generating the complex bilayer system of the mammary epithelium composed of basal (myoepithelial) and luminal (secretory) epithelial cells. In addition, there are mesenchymal SCs (MSCs), representing the stromal (fat pad) part of this organ.[1]

According to current knowledge, scientists had made the model of SC mitotic division, which can be symmetric or asymmetric. During symmetric division, SC gives two daughter SCs and it allows for the expansion of SC population. When a SC undergoes asymmetric division, one SC is obtained maintaining the self-renewal properties, whereas the cell is called a progenitor cell. Progenitor cells have a more restricted potential in terms of their renewal and differentiation. Progenitor cells also have limited proliferation capacity and can undergo senescence.[1,2]

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Several features of MaSC make them plausible sites for breast cancer (BC) initiation. BC is a potentially life-threatening malignant tumor that still causes high mortality among women. Decreasing mortality rates have been achieved, that is, by efficient screening strategies.[4] Still, BC is ranked on the second place among cancer types regarding mortality.[5] It has been estimated that approximately 1.3 million females develop BC each year with around 465K expected to succumb to the disease.[6-8]

MaSC have been postulated to underline the cellular heterogeneity observed in human BCs due to their preserved replicative capacity and differentiation potential, resulting in prolonged life span and thus increased probability of harboring and accumulation of mutations.[9,10] The cancer SC (CSC) fraction typically constitutes 1–5% of the tumor size.[8,11] In the healthy human mammary gland, SC account for approximately 8% of the cells.[12] The concept of CSC has led to the development of new theoretical models explaining the cellular origin of cancer.[13,14]

One theory, called the stochastic theory, claims that every single cell can potentially become cancerous in the appropriate micro-environment. However, differentiated cells are probably unable to accumulate a sufficient number of mutations to become neoplastic because of their shorter life span. Second theory, called the hierarchy (CSC) theory, suggests that CSC are more likely to initiate the tumor, as they have longer life span, increased migratory, and proliferative potential and advanced DNA repair mechanisms. Since it is more probable that these two models coexist, a dynamic version of the CSC model has been developed, suggesting that within the tumor hierarchy, differentiated tumor cells may undergo dedifferentiation as a result of micro environmental influences. In addition to the generation of cells with stem-like properties, the tumor microenvironment also involved in the preservation of the established CSC subpopulation.[15,16]

Increasing evidence demonstrates that CSC plays a critical role not only in BC initiation but also in progression and metastasis.[13] Accumulating evidence indicates that the local recurrent and/or distant metastatic tumors, which constitute the major causes of lethality in the clinic, are related to the aggressive phenotype of a small fraction of CSCs, tumor-initiating cells (TICs) or cancer metastasis-initiating cells.[17] BCSCs are able to escape chemotherapy due to elevated expression of ABC transporters that enable them to efflux some chemotherapeutic drugs.[13]

They are resistant to apoptosis (they also express high levels of anti-apoptotic proteins, such as surviving and Bcl-2) and show drug resistance.[11] In addition, the activity of BCSCs can enhance and the ratio of side population can increase after radiation treatment. Furthermore, BC has capability to resist radiotherapy.[17-19] Therefore, it has been suggested that BCSCs might be responsible for tumor regrowth and the development of drug resistance.[2,13,17]

Identification of BCSCs represents a major step forward in elucidation of the BC tumor hierarchy and has started a new era of BC research. Still, in present, there is no uniform approach, which would allow for a quick and simple detection of BCSCs in solid tumors. Therefore, a lot of scientific studies are focused on targeting BCSCs in BC therapy in different ways, using the current knowledge about those cells. For example, BCSCs are characterized by the activation of stemness-related pathways, such as the Notch and Wnt pathways and by the expression of certain SC markers. Since CSC are highly resistant to chemotherapy, additional treatment of BC patients with BCSC-specific drugs and inhibitors, which target the Wnt or Notch pathway, respectively, will be required.[2]

THE CONCEPT OF SC HIERARCHY IN THE MAMMARY GLAND

The mammary epithelial tissue forms a highly organized branched bilayer ductal network consisting of basal myoepithelial cells and luminal (secretory) epithelial cells.[1,20] Distinct markers characterize luminal and basal cells. Luminal cells express cytokeratins 8/18 and 19, as well as other molecular markers, such as MUC1, GATA3, and CD24. Basal myoepithelial cells express CK14

(50 kDa), CK5 (58 kDa), and CK17 (46 kDa), as well as smooth muscle actin and vimentin.[21] Numerous scientific reports have provided evidence of existence of a much more complex mammary epithelial hierarchy, which is responsible for tissue growth and maintenance during periods of development and homeostasis.[20]

Mammary cell proliferation, turnover, and tissue regeneration are functions of MaSC.[21,22] To present the idea in a simplified model, progenitor cell lineages are derived strictly from bi-potent or multi-potent SCs. Then, they divide and differentiate into the epithelium of adult mammary gland composed of both matured luminal and basal cells [Figure 1a].[23] The scientists have identified different subpopulations of cells in human and mouse mammary gland, using cell sorting techniques.[20] Subsets of mammary epithelial cells (MEC) were characterized using different surface markers. Accordingly, CD24 and epithelial cell adhesion molecule (EpCAM) are known to be the luminal cell markers and CD49f and CD29 are the basal cell markers. This diversification is invariably used in classifying of luminal and basal MEC populations.

The perspective of MaSC isolation, which then were be able to give rise to an entire mammary epithelial tree on transplantation of a single SC[24,25] and the phenotypic identification of several mammary epithelial progenitor cell populations,[26,27] has enhanced our current understanding of the differentiation hierarchy.[28] Furthermore, *in vivo* genetic tracing experiments have shown that both cell types contribute to morphogenesis in puberty and pregnancy and ductal maintenance in the adult gland.[28]

To characterize MaSC, a clear distinction between normal SCs and tumor SCs must be made. Emerging evidence suggests that normal breast cells, as well as BC stem and progenitor cells, exist in two different states, epithelial-like and mesenchymal-like [Figure 1b].[27,29,30] Recent studies revealed that in the case of human BCSCs, epithelial-like SCs express aldehyde dehydrogenase ($ALDH^+$),

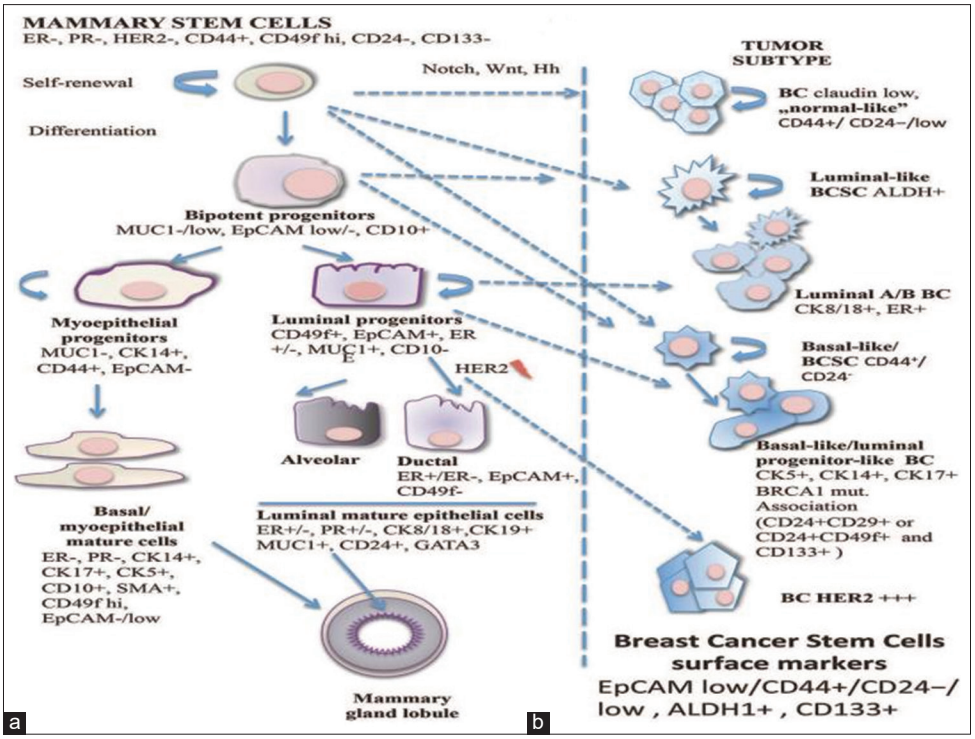

Figure 1: The simplistic draft of hierarchical model of human mammary gland stem cells (SC) (a) and correlation of SCs with breast cancer (BC) subtypes (b). Bi-potent or multi-potent SCs (with self-renewal ability) give rise to lineage-restricted bi-potent progenitor cells. These progenitors then divide and differentiate into the mature luminal (ductal and alveolar) and basal cells of the adult mammary epithelium. Cells are characterized with expression of different surface markers which allow for phenotypic identifying of the subpopulations. Normal mammary SCs (MaSC) must be distinguished from tumor SCs (BCSCs). Deregulation of MaSC self-renewal may contribute to preneoplasia of mammary gland

whereas mesenchymal-like SCs are characterized by $CD44^+/CD24^-$ surface expression.[29,31-33]

In particular, deregulation of conserved signaling pathways, such as Wnt, Notch, and hedgehog, is linked with oncogenesis. Breast tumors are divided into hypothetical subtypes according to different profiles and different origins of cells. We can find following subtypes: Normal-like/claudin low, luminal and basal like, and overexpressing HER2. Luminal progenitor's cells (A and B) are mostly associated with good prognosis, those with HER2 overexpressing, also with luminal features, but usually associated with poor survival. Basal-like (the most heterogeneous) origin from luminal progenitors cells and those tumors are the most aggressive and with tendency to exhibit triple-negative phenotype. In addition, those tumors are highly associated with BRCA1 gene mutations.

MASC AND BCSC MARKERS

The approaches to BCSC isolation at present include the following: Surface marker sorting, ALDH activity assay, flow cytometer sorting side population, etc.[8] CD44, CD24, and ALDH1 are the most commonly used biomarkers to identify the BCSC fraction.[31] Two proteins, CD44 and CD24, were found in 2003 to be useful markers to distinguish TICs from non-tumorigenic cells in BC.[2]

CD44 (hyaluronan-binding trans-membrane protein) is expressed in different isoforms and can have different glycosylation patterns.[34] Its smallest form (CD44s) is expressed in many cells, whereas its variant forms (CD44v) are particularly found in cancer cells. CD44v is involved in epithelial-mesenchymal transition (EMT), cellular migration, trans-endothelial migration, and extravasation and it supports many cellular activities required to initiate tumor growth and metastasis.[2,34] CD24 (heavily glycosylated membrane protein) downregulation may be required to prevent its interference with CD44-dependent invasiveness,[35] though the underlying mechanism is not clear since CD24 also has tumor-promoting effects.[2,36]

The gene expression profile associated with $CD44^+/CD24^-$ cells was demonstrated to correlate with a worse prognosis in BC[33] and approximately one-third of all circulating BC cells in the blood of BC patients is $CD44^+/CD24^-$.[37] $CD44^+/CD24^-$ phenotype of cell surface markers has an increased ability to form tumors in immunosuppressed mice than the bulk of the tumor cells.[38] Maycotte *et al*. had analyzed CD24 and CD44 expression in MCF7 and MDA-MB-468 cell lines using assay based on flow cytometer.

Analyzed cells showed different levels of autophagic flux ("autophagic flux" is defined as the activity of autophagic degradation, which comprises autophagosomes formation, transportation of substrates, and lysosomic degradation).[39]

CD24 expression was decreased in cells with low autophagic flux in both cancer cell lines. Similar results were obtained in cells expressing shRNA for ATG7 or BECN1, as these cells also showed low expression of CD24, whereas the expression of CD44 remained stable. Presented results indicate that cells with decreased autophagic activity have declined CD24 expression. These results suggest that autophagy can selectively regulate CSC maintenance in autophagy- dependent BC cells. It has been widely predicted that a quality control mechanism, like autophagy, is important for maintaining normal and CSC homeostasis.[7,38]

Palmer *et al*.[40] proposed a stem gene pluri potentiality signature as an indicator of the tumor grade in a variety of solid tumors, including BC. In addition to tissue samples, BCSC subpopulations have also been identified *ex vivo* within individual cultured BC cell lines. In triple-negative BC cell lines, $CD44^+/CD24^{-/low}$ BCSCs were further classified into two subcategories: The $CD44^{high}/CD24^-$ mesenchymal like basal B and the $CD44^{high}/CD24^{low}$ epithelioid basal A, which displayed stronger tumor-initiating properties.[15]

Recent data suggest that CD44 and CD24 may not be sufficient to distinguish the cancer cell subpopulation with CSC/TIC activity, so other proteins, like ALDH1 and EpCAM, may also be required for cancer cells to develop tumor-initiating potential.[2] Members of ALDH1 family ALDH1A1 and ALDH1A3 are thought to be the most important for SC activity in cancer cells.[41] Recently, ALDH1 expression has been linked to

the expression of RhoC,[15,42] a GTPase known to be involved in metastasis.

ALDH1-positive BC cells could be identified by the ALDEFLUOR assay and they showed stem-like and tumor-initiating activities.[15] In the abovementioned experiment of Palmer *et al*.,[40] distinct ALDEFLUOR-positive sub-groups with SC characteristics have been shown to exist in eight BC cell lines and a 413 gene-specific molecular signature characterizing these BCSCs was determined by microarray analysis.

EpCAM, a trans-membrane protein, was considered to be a cellular adhesion molecule until it was discovered that it is able to activate c-myc involved in maintenance of stemness.[36] The level of EpCAM expression may be critical for defining SCs. Recent reports demonstrated that BCSC activity is associated with low EpCAM expression, whereas luminal or basal cells showed either high or no expression of EpCAM, respectively.[43]

The aforementioned epithelial-like and mesenchymal like BCSCs have been shown to inter- convert from one type to another, presumably depending on the tumor phase and requirements.[31]

The use of CD49f as an additional marker for the detection of BC cells lacking CK8/18/19 expression has been shown to possibly enhance the detection of circulating tumor cells (CTCs) involved in EMT-associated processes, such as drug resistance and metastasis.[44] $CD44^+/CD24^-$ cells express EMT genes,[17] display a quiescent phenotype and are localized in the tumor periphery, possibly promoting tumor spreading. The characteristic pattern of surface markers expression ($CD44^+/CD24^{-/low}$) was found mostly in molecular subtype of breast tumors presenting low expression of claudin. It is accompanied by EMT-associated genes, such as N-cadherin, FoxC2, and Zeb.[17]

In contrast, ALDH1+ cells are situated within the tumor. They are typical epithelial cells, expressing mesenchymal-epithelial transition (MET) genes and high rate of proliferation, which can influence tumor progression. All these subpopulations are similarly expressing a large number of genes, which were confirmed by high-throughput gene expression profiling (microarray analyses). BCSCs are suggested to have hallmarks of both types of normal MaSCs, epithelial ($EpCAM^+/CD49f^+$), and mesenchymal ($EpCAM^-/CD49f^+$). According to research results, BCSCs with phenotype $ALDH1^+/CD44^+/CD24^-$ are more aggressive and exhibit big meta- static potential. In the immunosuppressed mice, it was possible to induce tumor growth using just a few $ALDH1^+/CD44^+/CD24^-$ cells.[31]

In human breast tumor cells, phenotype $CD44^+/CD24^{low}$ is connected with basal-like tumors, in particular with inherited BRCA1 BC. In addition, the cells are expressing the CD49f marker and their status is $CK5/14^{high}$ $EGFR^{high}$ and ER^{low}, PR^{low}, and $HER\text{-}2^{low}$. It is worth noting that basal-like tumors are often linked to poorer prognosis. The occurrence of the $CD44^+/CD24^{low}$ phenotype was found to be lower in tumors of luminal type and particularly HER-2+ tumors, irrespective of ER status.[11]

Results of a different study demonstrated the presence of BCSC subtypes in a CTCs population, in peripheral blood samples taken from 30 patients. In total number of 1439 CTCs, 35% of the CTCs in 2/3 patients displayed the $CD44^+/CD24^{-/low}$ phenotype, while 17.7% CTCs selected in seven patients revealed phenotype $ADLH1^{high}/CD24^{-/low}$.[45]

β1 integrin subunit (CD29) has also been implicated in the phenotypic characterization of BCSCs. It has been shown that BRCA1 mutant cancer cell lines contain CD24+CD29+ or CD24+CD49f+ cells, with increased proliferation and colony-forming ability.[15]

In BCTCs epithelial markers expression is routinely detected and therefore, many isolation techniques are based on the use of specific antibodies, such as EpCAM and MUC1. For example, for EpCAM identification, the most popular tests are Cellsearch™ system (Veridex LLC, Raritan, NJ, USA) approved by the US Food and Drug Administration, the herringbone chip, the AdnaTest BC detection kit, fluorescence-activated cell sorting analysis, and the microfluidic technology. Apart from the peripheral blood, BCSCs have also been isolated directly from the primary or metastatic tumors of BC patients.[31]

Other techniques used for SC isolation are 3D cultivation in cell cultures spheroids. SCs are

detectable by light microscopy as small and light cells and have the ability to maintain DNA staining (using BrdU) due to their low proliferative activity.[46] However, it was shown that only 15% of [3H] thymidine-positive cells are also positive for one of the two SC markers p21CIP1 or Musahi-1 (MSi-1).[47]

The next marker worth mentioning is CD133 (prominin-1). Hematopoietic progenitors and adult SCs normally express this trans-membrane glycoprotein. It is a well-established melanoma and brain CSC marker. In addition, the expression of CD133 has been also detected in BCSCs and has been associated with resistance to chemotherapy in BC biopsies.[48] Furthermore, distinct $CD44^+/CD24^-$ and $CD133^+$ subpopulations with CSC characteristics have been detected in BRCA1 breast tumors, while CD44pos CD49fhi CD133/2hi cells were characterized by xenograft initiating capacity in estrogen receptor (ER)–negative BC.[15]

Co-expression of stem (ALDH1) and EMT (TWIST) markers has been demonstrated in CTCs from patients with early and metastatic BC. The majority of CTCs expressing the SC marker CD133 also co-expressed the EMT marker N-cadherin and vice versa. The expression of CD133 in CTCs of BC patients has been suggested to promote chemo resistance.[15] Basal-type breast tumors with elevated SLUG expression were shown to overexpress stem-like genes, including CD133.[20]

Additional studies revealed that BC overexpressing SLUG display increased proportions of $CD44^+/CD24^-$ CSCs, suggesting that transcriptional programs induced by SLUG promote stemness.[49]

Activation of some genes is proposed to be associated with SC phenotypic characteristics, for example, Sox2 gene (a transcription factor involved in the maintenance of pluri potency of undifferentiated embryonic SCs).[15]

Activation of this gene is typical for early steps of BC development and characterizes tumor with basal-like phenotype. Increased expression of Sox2 is analyzed as prognostic predictor of BC. Furthermore, mutations in p53 are representative for BC with SC-like patterns. It is suggested that loss of p53 function promotes dedifferentiation and is positively selected during tumor progression.[15,50]

THE ROLE OF MICROENVIRONMENT IN BC PROGRESSION: SC NICHE

SC niche refers to a microenvironment in which SCs reside. The anatomical niche for SC is composed of different compartments.[51] Signals are from surrounding cells (stromal cells, a specific type of fibroblast which interacts with the stem/progenitor cells through surface receptors, gap junctions, cytokines, growth factors and, hormones, etc.) and extracellular matrix survival.[1]

Since mammary gland is an endocrine-responsive organ, many hormonal factors are analyzed also in connection with SCs, for example, the biological influence of E2 and P on the compartment of stem and progenitor cells is largely unknown. However, it is assumed that the SCs are ER negative, whereas the progenitor cells are ER positive.[2] The role of BRCA1 gene in human ER^- stem/progenitor cell differentiation into ER^+ luminal epithelial cells has been revealed in the latest scientific findings.[11]

ER^-SC transition into ER^+ progenitor cells is precluded by BRCA1 deletion. Studies demonstrated that women with heterozygous mutations in the BRCA1 gene are more susceptible to breast and ovarian cancers and the tumors formed were mostly of basal-like phenotype, showing characteristic deficiency of ER, PR and HER-2 receptors.

As mentioned above, deregulation of the micro environmental homeostasis of normal SC is suggested to contribute to their neoplastic transformation.[52] The activation of the EMT program has been associated with the acquisition of SC traits by normal and neoplastic cells.[15] Transcription factors involved in EMT (e.g., Snail, Twist and Zeb) have also been found to induce SC properties in human mammary carcinoma cells.[15] Environmental cues from signaling molecules, which induce EMT in BC such as IL-6, can promote pluripotency in BC cells through a positive feedback loop including NF-kB, Lin28, and Let-7 miRNA.[15]

MIRNA AND SCS IN BC

MicroRNAs are negative regulators of genes, repressing expression at the posttranscriptional

level.[53] They also regulate various properties of CSC, including self-renewal, differentiation, proliferation, and fate determination, by affecting several key signaling pathways at the molecular level. Many different miRNA families have already been connected with suppressing/promoting cancer cells. For example, miR-125a is known tumor suppressor in bulk tumor cells of BC origin;[53,54] however, it has been shown that miR-125a plays a different role in breast epithelial SC, which is cancer promotion.[53] MicroRNA profiling of BCCSs indicated that miR-200c, miR-203, and miR-375 expression was significantly inhibited, whereas the expression of miR-125b, miR-100, miR-221, and miR-222 was most notably enhanced.[55] Expression analysis of miRNAs in both normal mouse and human mammary tissue has revealed three conserved clusters of miRNAs, miR-200C-141, miR-200b-200a-429, and miR-183-96-182, which appear to be downregulated in MaSC and putative BCSCs.[56,57] In humans, miR-93 level was significantly higher in luminal progenitor cells than in the MaSC-enriched population and overexpression of this miRNA biased these cells toward a luminal fate.[58]

MiR-200 family serves as a key mediator of CSC due to its prominent role as an EMT regulator. These family members are downregulated in BCCSs due to epigenetic alternation, in comparison with non-tumorgenic cancer cells.[59] Downregulation of miR-200 expression expands the SC compartment and promotes BC progression. The tumor suppressor p53, which can activate miR-200c by direct binding to miR-200c promoter sites, is reported to regulate both EMT and CSCs.[60] Similar results were obtained in the case of miR-22, a strong inhibitor of miR-200 promoter demethylation, which is connected with tumor invasiveness and of EMT and cancer stemness toward metastasis.[61]

In addition to miR-200 family, miR-21 and MiR-302/369 have also been proposed to regulate EMT and CSC. In BC, the depletion of miR-21 expression leads to reversal of EMT and decreased CSC numbers through inactivation of AKT/ERK pathway.[60] MiR-302/369 cluster members can directly target EMT genes, such as transforming growth factor-beta (TGF-β) receptors or the RhoC and the downregulation of miR302/369 promotes the switch of fibroblasts into somatic SCs.[60]

miRNAs can also regulate the BC cell interactions with other cells by affecting certain genes, for example, Tac1 gene, linked to BC, and regulates BC cell interaction with the MSCs. Three miRNAs miR-130a, miR-206, and miR-302a have been shown to regulate Tac1 expression and their action against Tac1 may affect quiescence of BC cells in the marrow cavity.[11]

SIGNALING PATHWAYS REGULATING MASC AND CONTRIBUTING TO THE ETIOLOGY OF BC

Wnt (wingless), Hh (hedgehog), Notch, and bone morphogenetic protein (BMP)/TGF-β signaling pathways contribute to the self-renewal of stem and/or progenitor cells in a variety of organs. When deregulated, these pathways can contribute to oncogenesis.[59]

The Notch pathway has been shown to play a particular role in MaSC expansion[62,63] and promotes BC progression by supporting EMT.[11,64] Overexpression of the Notch pathway components has been linked to decreased survival of BC patients.[65] In a large proportion of BCs, epigenetic mechanisms that activate Notch signaling were related to the role of miR-146a, which targets NUMB, a negative regulator of Notch.[59] Inhibition of Notch1 with specific antibodies significantly reduced the $CD44^{+}CD24^{-/low}$ subpopulation (BCSCs) and diminished the incidence of brain metastases from BCC.

β-Catenin, a downstream target of Wnt signaling pathway, has been identified as a crucial survival signal for MaSC and a balance modulator between differentiation and stemness in adult SC niche in the mammary gland.[59] Overexpression of Wnt in mouse mammary glands can also lead to increased mammary tumor formation. Such tumors contain cells of both basal/myoepithelial and luminal phenotypes, suggesting an origin from a common precursor.[11,59]

In the hedgehog pathway, Patched (PTCH) trans-membrane protein is a receptor for the hedgehog family of signaling molecules (Sonic-Shh, Indian-

Ihh and Desert-Dhh)[59] and has been connected to early embryonic tumorigenesis.[11] PTCH constitutively represses Hh pathway activity through its interaction with a trans-membrane protein Smoothened (SMO).[59] Overexpression of these pathway components, that is, Shh, Ptch1 and Gli1, has been found in majority of human BCs. Furthermore, studies demonstrated that EMT stimulation by TGF-β co-occurs with BCSC formation.[66]

BCSCs with $CD44^{+}/CD2^{4-/low}$ phenotype show increased expression of many tory cell types. In one of the experiments, when MDA-MB-231 cells (model of BC) were injected to athymic mice, the change in TGF-β actions was observed. The cancer-promoting actions (tumorgenic and metastatic) of TGF-β were counteracted by BMP7 or BMP2/7 heterodimer,[59] which diminished Smad signaling pathway activity and increased cancer cell invasiveness. In addition, the activity of pro-survival and anti-apoptotic pathways is often increased in CSCs. Typically, for example, JAK/STAT pathway is highly activated.[59]

WAYS OF TARGETING CSCS: PHARMACOLOGICAL AGENTS

Although targeting BCSCs brings hope for future treatment of BC and is widely tested on the basic research level, a disproportionally limited number of clinical trials evaluating the effect of treatment on the expression of BCSC biomarkers are in progress.[31] Among the tested treatment approaches are those regulating the activity of signaling pathways.[67] The targeting of BCSCs involves the disruption of BCSC survival signaling pathways (i.e., Notch, HER2, hedgehog, Wnt, PI3K/Akt/mTOR, interleukin 8, and TGF-beta).[31] Targeting Notch signaling has become a promising field in the treatment of SCs in BC. By inhibiting the Notch pathway, the CSC population can be reduced along with improved responses to chemotherapy.[68]

Several inhibitors of Wnt signaling molecules are under investigation with reference to several cancers.[69] For example, inhibition of the Notch signaling pathway by γ-secretase inhibitors (GSI) has been shown to reduce the pool of BCSCs.[15,62] GSI and other drugs that interfere with the Notch pathway are currently under consideration as new options to treat BC.[65] Because there is a link between the Notch and Her2-dependent pathways,[70] blocking either of them was found to affect CSC survival. Hence, Her2 inhibitors, such as trastuzumab, may be potential additional drugs suitable for targeting CSC.[71]

Several scientific groups have exploited cyclopamine (SMO signaling inhibitor), to inhibit the Hh cascade, thereby inhibiting the growth, invasion and metastasis of breast, prostatic, pancreatic, and brain malignancies both *in vitro* and *in vivo*.[72] PKF118-310, an inhibitor of Wnt signaling pathway, was recently reported to eliminate BCSCs in a HER2 overexpressing mouse model. Vismodegib, GDC-0449, a hedgehog inhibitor, can block tumor growth in tamoxifen resistant BC xenografts.[31] Everolimus (RAD001), an inhibitor of PI3K/Akt/ mTOR pathway, halted tumor growth of SC in primary BC cells and cell lines and was particularly effective when administered in combination with docetaxel.[73]

The resistance of BCSCs to chemotherapeutic drugs leads to the reconstitution of the initial tumor cell population and disease progression.[15] Conventional therapies targeting the tumor bulk have proven insufficient for the eradication of CSC. For example, conventional therapies based on mitotic interference of taxanes (paclitaxel and docetaxel)[74] do not target the subpopulation of quiescent CSC in a tumor. Bhola *et al*.[75] reported that paclitaxel increased IL-8 expression by autocrine TGF-β signaling and enriched CSC. Interestingly, Gupta *et al*. reported that SAL, a polyether antibiotic widely used in veterinary medicine, is a potent agent able to selectively target BCSCs and to inhibit mammary tumor growth *in vivo*.[43] Since autophagy promotes the maintenance of BCSCs,[76] SAL can inhibit autophagy tor of potassium ionophore in Wnt signaling. Another therapeutic approach is blocking the ABC transporters expressed in most CSC.[13]

For instance, tyrosine kinase inhibitors (TKIs) act by binding to ATP and preventing it from binding to the ATP-binding site of several oncogenic tyrosine kinases. It has been reported that some TKIs,

such as nilotinib (Tasigna), can efficiently reduce the activity of ABCB1 and ABCG2 transporters. Apatinib (YN968D1) was tested on BC cell lines and in xenograft models of BCs overexpressing ABCG2 and/or ABCB1. In combination with paclitaxel, it significantly increased the activity of paclitaxel in the animal models. The therapeutic use of ABC transporters inhibitors has failed so far because of the toxicity issues.[13]

One of the most recent innovative approaches in BC therapy is the recruitment of normal SCs for the eradication of tumor cells. It has been pointed that MSCs have "tumor tropism," which means that they show the ability of migration not only toward the sites of inflammation or injury but also importantly to the tumor microenvironment. Other tested options include the following: targeting of CSC metabolic pathways, the use of miRNAs, the use of small inhibitors as salinomycin, cancer immunotherapy, drugs involved in the treatment of noncancerous diseases and nanotechnology (Nanodrugs can easily accumulate within tumor sites due to their enhanced vascular permeability).[31]

CONCLUSIONS

Scientific findings from BC studies have revealed that the SC content in breast tumor correlates with its invasiveness and the outcome of the disease. The resistance of BCSCs to chemotherapeutic drugs and other conventional BC therapies has led scientists to move toward establishment of novel therapeutic approaches. Current knowledge about BCSC characteristics and regulators still allows only for evaluation of those therapies on an experimental level of preclinical studies. The most efficient cancer treatment protocols remain to be established on the basis of simultaneous targeting of BCSCs and bulk tumor cells. Therefore, there is still a great need for profound studies, which would extend our knowledge about SCs and the interplay between these cells and tumor microenvironment. Beholding at the practical aspects of BCSC usage one of the biggest challenges but still, need to be resolved is the isolation of their population from the patients' blood

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