Breast cancer stem cells and epithelial to mesenchymal transition, their putative role in tumor initiation, propagation, and metastasis

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Breast cancer stem cells (CSC) are small subset of tumor cells within the tumor, possessing distinct immunological phenotype; they initiate the tumor and sustain tumor growth. Epithelial to mesenchymal transition (EMT) is the loss of epithelial differentiation and gained the mesenchymal properties among some of tumor cells. Acquisition of mesenchymal phenotype allows these cells to infiltrate surrounding tissue, which ultimately enhance tumor propagation and progression. EMT occurrence is always co-existent with CSC subsistence. EMT induced by various factors is rich source of cancer stem like cells suggesting a possible biological similarity between CSC and EMT phenotypic cells. The inhibition of EMT occurrence and CSC elimination may have significant effect on cancer prognosis, which could suggest that these cells will be a target for cancer therapeutics. Prospective identification of new molecules and markers for these tumorigenic cells will facilitate the invention of new agents to eliminate cancers.

Keywords: cancer stem cells; epithelial to mesenchymal transition; Breast cancer, metastasis

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Introduction

Breast cancer is one of the most common human cancer all over the world, approximately it account for 25% of all cancers in females worldwide and 27% in advanced countries with the western lifestyle [1]. Breast cancers can be arise from any cell in the breast tissue.

Breast Cancer stem cell possess distinct immunological markers, they have CD44+/CD24−/low phenotype, they has been demonstrated by Al-Hajj et al. to have tumor-initiating capabilities in breast cancer. This tumorigenic phenotype has been associated with stem cell-like properties [2], with enhanced invasive characteristic [3], radiation, and chemotherapy resistance [4].

The concept of cancer stem cells has led to new theory about tumor genesis. CSC possess identical characteristics with normal stem cells in terms of their capacity for self-renewal, divide and proliferate, differentiation, and resistant to apoptotic stimuli. They can self-renew, causes recurrence, and metastasis. Meanwhile, they can proliferate to produce different types of cancer cells which constitute the bulk of the tumor, cancer stem cells has the ability to form new tumors, asymmetric division of CSC has been implicated as a main cause of breast cancer heterogeneity [5].

The CSC hypothesis posit that this minority of cells population can fuel and drive tumor growth and remain viable after conventional chemotherapy which eradicate the rapidly growing non tumorigenic cell which constitute the major bulk of the tumor, ultimately tumor recurrence is
Nowadays, it is believed that CSC derived from transformation of normal pluripotent stem cell rather than from more differentiated progenitor cells, this notion is supported by the fact that transformation of normal stem cell to CSC is due to successive and accumulative genetic stimuli and abnormalities, which can tookplace during the long life spans of normal stem cells. Moreover, CSC share the normal stem cells most of their essential biological features, such as self-renewal, division, and differentiation. The markers of CD44+/CD24-low phenotypic cell proposed as a marker for breast cancer stem cells, no wonder that normal breast epithelial cells with CD44+/CD24-/low Phenotype, express higher levels of stem cell associated genes.

Epithelial to mesenchymal transition (EMT) is the loss of epithelial differentiation and gain the mesenchymal phenotype. It was initially recognized as a feature embryonic development, which is of paramount importance for morphological development of the embryo. EMT can be induced by dissociation of cell-to-cell contact points and reorganization of the actin cytoskeleton, including the down-regulation and relocation of E-cadherin. This morphological, functional, and immunological transformation triggers by a wide scope of stimuli including growth factor signaling, tumor-stromal interactions and hypoxia. EMT phenomena may play an important role in stromal invasion of breast intraductal lesion and metastatic progression of breast cancer.

EMT is essential for embryonic evolution and normal wound healing, meanwhile, when it is deregulated can cause excess fibrosis (keloid) or enhance tumor invasiveness and remote metastasis. Vimentin is a universal mesenchymal marker, while E-cadherin is a marker of epithelial cells. The disassembly of the E-cadherin mediated adherent’s points, loss of contact inhibition, and increased cell motility are the sequential steps in epithelial to mesenchymal transition which can take place in the early stage of growing carcinomas as preliminary step to infiltrate the surrounding tissue or may occur late as initial step for lymphovascular permeation and ultimately distant metastasis. In conclusion, the EMT process is required in the progression of carcinomas, manifested by gaining of mesenchymal phenotype and loss of epithelial marker expression, enhancing cells motility, and stromal invasion.

Three novel notions have recently came out in breast cancer histogenesis; the role and of CSC in tumor formation, and the involvement of epithelial to mesenchymal transition in the tumor local invasion and distant metastasis together with the role of Telomerase to maintain the CSC immortal, and avoiding senescence. Figure 1 shows the possible interaction of those three concepts.

**Breast cancer**

Breast Cancers are heterogeneous disease with overt complexity and variability within its different histological subtypes giving rise to an extremely changeable clinical course, response to therapy and ultimate prognosis. The heterogeneity and complexity of breast tumor cells and its maintenance is not well understood. The possibility of every tumor cell has the capacity to proliferate independently has been hypothesized resulting in an extremely variable morphological features and histological variants having their unique particular clinical behavior and prognosis.

Breast cancer is one of the most common female cancer, representing about one quarter of all cancers in females all over the world and 27% in advanced countries with the western lifestyle. Breast cancer can also occur in men, it is more than 100 times more common in women than in men, which usually have had prognosis due to delays in diagnosis. Nowadays with the wide use of mammography as screening tool, more cases of pre-invasive breast lesions can be detected. The WHO Working Group agreed that more clinical data and genetic information are required for a better understanding of the biology of these neoplasms.
CSC (tumor-initiating cell or tumorigenic cell) are a small subset of cancer cells that can divide spontaneously, possess the capacity to self-renewal and to differentiate into the heterogeneous population of non-tumorigenic tumor cells that encompass the bulk of the tumor.

**Cancer stem cell theory**

One of the key questions is how and whether cancer stem cells are arising from normal tissue stem cells that maintain self-renewal characteristic but gain epigenetic and genetic stimuli needed for conversion to tumor initiating cells, or arise from more differentiated lineage of tissue cells that acquire self-renewal capacity. Its seems that both mechanisms might take place depending on the organ affected. Normal stem cells can live longer than differentiated cells. Their long life span makes them susceptible for subsequent mounting mutations. Thus, it is logical to hypothesis that CSC can derived from normal tissue stem cells. The crucial difference between CSC and normal tissue stem cells is the deregulation of cell division and differentiation [5].

Overall, resistance to apoptosis is one of the hallmarks of cancers cells. Which promote the survival especially for CSCs, since apoptosis is a cell defense mechanism in which it acts as intrinsic barrier to oncogenic stimuli [14]. Accordingly, CSC may have a unique mechanism to evade apoptosis to survive longer and self-renew [15].

The first cancer stem cells of CD34+/CD38− phenotype identified in acute myeloid leukemia [16]. Subsequently, accumulating evidence emerge recently have supported the subsistence of CSCs in a variety solid tumors, such as tumors of the breast, central nervous system, prostate, skin, GIT, head and neck, pancreas, and lung adenocarcinoma (Tab 1). A good number of cell antigens have used successfully for the detection and isolation of breast CSCs, including CD133, CD44, CD24, EpCAM, and ALDH1. Generally, more and more data is mounting in support of the CSC theory, and the benefit of stem cell features in understanding cancer biology.

Once cancer stem cell concept came out, two competing models describing the heterogeneity of cancers coming to the scene. The old model hypothesis that most of the cancer cells have the ability to proliferate collectively to form the tumors, in which, random changes responsible for tumor heterogeneity. In contrary, the newer CSC model hypothesis that most cancer cells lack the ability to proliferate and self-renewal capacity. Instead of only a specific subpopulation of tumor cells, which have stem cell characteristics can proliferate and differentiate to various type of tumor cell. Although the precise mechanism for cancer heterogeneity need to be explained [5].

**Breast Cancer stem cells**

Breast cancer was the first solid cancer from which CSC were detected and isolated. Al-Hajj et al. have been isolate CSC from mammary human tumor that can initiate breast cancer in NOD/SCID mice by successive transplantations, confirming their capacity for self-renewal. Subsequent identification of these cells proved that they were CD44+/CD24−/low phenotype. Few hundred of these cells can induce tumor when transplanted in recipient animal, while tens of thousands of different cancer cells harbored other markers fail to induce tumor. The CD44+/CD24−/low phenotypic CSC can be further subdivided based on epithelial cell adhesion molecule (EPCAM) expression. The Epcam+, CD44+/CD24−/low cells were capable to initiate breast cancer in NOD/SCID mice, while Epcam−, CD44+/CD24−/low cells fail to do that [17].

Recent studies shown that ALDH1 breast CSC marker can further subdivide the CD44+/CD24−/low subpopulation into smaller subsets of cells that are more cancerigenic: CD44+/CD24−/low ALDH1+ tumor cells were capable to initiate tumors from as low as 20 cells count, while CD44+/CD24−/low ALDH1+ cells were fail to generate tumor in the same cell density [24].

**Surface markers of breast CSC**

Cell markers currently used to identify breast CSC includes CD44, CD24, CD133, EpCAM, and ALDH1, either singly or in combination. Typically, the CD44 surface antigen is expressing in colon, breast, prostate, and pancreatic cancer stem cells [17,20,25]. In addition, CD133+ tumor cells with tumorigenic properties can initiate human glioblastoma, breast, colon, prostate, and pancreatic cancers in mice [5,20]. CD24 antigen has a more mysterious behavior since it expressed with pancreatic cancer stem cells [25], but negatively correlated in breast CSC.

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>CSC markers</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Breast cancer</td>
<td>CD44+/CD24−/low EpCAM+</td>
<td>[17]</td>
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<tr>
<td></td>
<td>ALDH1</td>
<td>[18]</td>
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<td></td>
<td>CD44+/CD49f/CD133+</td>
<td>[19]</td>
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<td>Haematological</td>
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<tr>
<td>Malignancy</td>
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<tr>
<td>Ca colon</td>
<td>CD133+,</td>
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<td></td>
<td>CD44+/Lin−/EpCAM+</td>
<td>[21]</td>
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<tr>
<td>Lung cancer</td>
<td>CD133+</td>
<td>[22]</td>
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<tr>
<td>Pancreatic cancer</td>
<td>CD44+/CD24+/EpCAM</td>
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<td></td>
<td>CD133+</td>
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**Table 1. CSC phenotype populations in various malignancies**

http://www.smartsctech.com/index.php/ccm
Al-Hajj et al. describe the breast CSC surface markers as CD44+/CD24−/low. The CD44 staining was almost exclusively membranous, while CD24 predominantly stained the cytoplasm. The CD44 staining was almost exclusively red membranous, with no or low brown cytoplasmic staining of CD24 [9] (Figure 2). Other publications have shown either membranous and/or cytoplasmic (Surowiak et al.). Cytoplasmic expression of CD24 may reflect discomposure of the protein distribution or degeneration in tumor cells [11]. Recently, Shipitsin et al. demonstrated that CD24 antigen is expressed on more differentiated tumor cells while CD44 antigen is expressed on progenitor cells with stem like properties. It has been found that breast CSC with CD44+/CD24− phenotype are further express genes that are implicated for tumor angiogenesis and tumor cell motility, and they are predominately estrogen receptor negative [26].

Coming to the conclusion that most of CD44+ tumor cells are basal-like and therefore are abundant in basal-like molecular sub variant of breast tumors, while CD24+ tumor cells are luminal-like and predominantly found in differentiated luminal molecular sub variant of breast cancers [27].

Using flowcytometry, CD133 is another marker for breast CSC identified from breast cancer stem cells isolated from cell lines of mice breast tumors [5]. CD133 is a well-known cell marker of CSC in many other organs encompasses brain, colon, breast, liver, and prostate. Likewise, it has been found that CD133+ subpopulation did not overlap with the CD44+/CD24− phenotypic cells, meanwhile, both subset of cells had a similar capacity for self-renewal, shared the expression of stemness genes [28].

Makki J et al. detected CD44+/CD24− phenotype CSC in only 74% of cases (123/167), suggesting that tumor initiating capacity is not entirely confined to those cells [9]. These finding suggests that there should be other generation of breast CSC that exhibit cell markers other than CD44.

Recently, Matthew and co-workers also describe a third population of CSC in ER negative breast cancers, they are xenograft-initiating cells express CD44+/CD49f+/CD133+/2+ cells that enhanced tumorigenicity, self-renewal in vivo, and potentially able to differentiate to diverse population of tumor cells [19]. The detection of CSC in normal breast tissue can predict the risk of developing triple-negative breast cancer [29], since this researcher detected plentiful CSC in the normal mammary gland tissue nearby to triple-negative breast cancer.

The significance of these surface molecules was not fully understood, they need more hard work to understand their functional significance, CD44 antigen assume to have an important role in tumor metastasis because a non-metastatic mice glioma cell line gained metastatic characteristics when a splice subpopulation of CD44 positive cells was ectopically over-expressed [30]. Whereas EpCAM molecule seem to be crucial for local invasion and migration of the mammary gland cancer cell line MDA-MB-231 [31].

Role of CSC in metastasis

Distant organ metastasis is the leading cause of mortality in most of cancer patients. Meanwhile, most of the cell in a tumor has no capability for distant metastasis. Tumor cells metastatic potential is multifactorial, responsible for overall tumor cell proliferation, angiogenesis, invasion, and survival [32]. Panayiotis et al. reported the existence of a subpopulation of tumor cells circulating in the peripheral blood having stem cell progenitor characteristics in patients suffering of remote breast cancer metastasis [33].

Metastasis is a complex process that involves not only the primary tumor cells but also homing and cell division at metastatic growth. It also requires the expression and the activity of several other genes on the cancer cells [3]. Recent gene expression profiling postulate that most cancers contains a subpopulation of cells has a metastatic potential and profile [34], including expression of metastasis- related genes and cells division properties at site of remote metastasis. In contrast to the traditional hypothesis of metastasis suggesting that metastatic cells are sparse and generate during end stages of tumor progression because of successive mounting mutations.

Does metastases generated directly from cancer stem cells. Mounting data have supported the notion of metastatic cancer stem cells. In patient with breast cancer,
The occurrence of high numbers of breast CSC in lymph node metastasis lesion supports the conclusion that the higher expression of CSC markers in metastatic sites indicating the paramount importance of CSC in tumor metastasis [9].

Apoptosis and regulation of CSC survival

CSC can evade apoptosis, may be, by using some intrinsic mechanism physiologically used by normal stem cells, this support the resistance of CSC to treatment modalities, since the chemo-radiotherapy initially act by enhancing cell death of tumor cells. There is accumulating data support that CSCs shows high resistance to stimuli that trigger apoptosis, in contrast to the remaining more differentiated tumor cells that encompass the tumor bulk, which can be easily targeted by anti-tumor modalities[15].

Detection and identification of breast cancer stem cells has paramount implications to understand the invasiveness of these cancers and the selection of anti-tumor therapies since CSC gain efficient ways to avoid and resist apoptosis, like over-expression of Bcl-2 and the various mechanisms of defective apoptotic signaling [15].

Self-renewal pathways in breast stem cells

Understanding the mechanism that organizes self-renewal capacity may disclose the scene of how deregulation of self-renewal pathways of normal breast stem cells may lead to cancer initiation. Dontu reported data that a key oncogenic pathways has implicated in regulation of normal stem [35]. Loss or deregulation of some physiological pathways that control the stem cell cycle proliferation implicated in the carcinogenesis and transformation of normal stem cells or their differentiated progeny to cancer stem cells. The following are some of these well-known pathways.

1. HER-2 overexpression (OE)

Human Epidermal Receptors-2 (HER-2) has a vital role in breast cancer genesis through regulation of stem cell proliferation, since it has been found that there is an important association between the expression of ALDH1 and HER-2 OE of the normal stem cell [18]. Recent research found that HER-2 over expression breast cancer as well as in normal breast ducts epithelial cells increases the proportion of stem cells, as reflected by ALDH expression, an early event in the development of Her-2 OE molecular subgroup of breast cancer may be the amplification and over expression of the HER-2 gene [36]. The remarkable therapeutic response of these aggressive tumors to Herceptine may be because these agents directly target the cancer CSC, it can decrease the number of stem cell lineage in trastuzumab-sensitive breast cancer cell line but not the resistant one [37].

2. Phosphatase and tensin homolog (PTEN)

PTEN is a human protein encoded by the PTEN gene, genetic alteration of this gene is an initial step in the initiation of several human cancers, deletion of the PTEN gene, a defect found in approximately 40% of human breast cancers [38]. It has been found that PTEN play an important role in regulation of self-renewal property of hematopoietic and neuronal normal stem cells; there is initial data prove that deletion of PTEN has similar effects on normal breast stem cell and CSC [36].

3. Wnt Signaling
They are kind of proteins act as signal transduction pathways that can pass signals from outside the cell to the inside through cell surface receptors, it was first identified for its role in carcinogenesis, but it is required for normal embryonic mammary development. Wnt signaling has also been shown to regulate the self-renewal and differentiation properties of different normal stem cells population. Its clinical significance has been demonstrated by induce a mutation that leads to several diseases, such as breast cancer and prostate carcinoma.

4. Notch Signaling

This pathway is concerning to conserved the cell signaling system, notch signaling found in all multi-cellular organism. It has been shown to have a vital role in breast cancer in rates as well as human mammary tumor, it play a critical role in cell fate determination of certain normal stem cells population like haemopoietic and neural stem cells.

5. Hedgehog Signaling and BMI-1

It is a signaling pathway (protein) that transmits information to embryonic cells required for proper development of embryo. Different parts of the embryo have different concentrations of hedgehog signaling proteins. The pathway also has roles in the adult.

Recent scientific researches that used transgenic animals (genetically modified organisms) found a vital role for hedgehog signaling in the development of the normal breast tissue and breast. Moreover, it has been found that this protein deregulated in certain variants of human mammary cancers. Polycomb complex protein (BMI-1) is a protein that in humans is encoded by the BMI1 gene has been reported as an oncogene by regulating p16and p19, which are cell cycle inhibitor genes. Hedgehog signaling can regulate the self-renewal property of normal breast stem cells as well as cancer stem cells through BMI-1 activation, Suling Liu and co-worker shown that hedgehog signaling protein is activated in mammary CSC of CD44+CD24−low phenotype, which has a vital roles in managing the self-renewal capacity of breast stem cells and breast CSC.

Breast CSC and tumor suppressors

Recent Clinical cancer studies has deeply investigate to identify the epigenetic and genetic changes that play a role in cancer development, which reflected to paramount rise in our understanding of the molecular and biochemical mechanisms that are implicated in malignant transformation and tumor initiation. There are definite evidences that several tumor suppressor genes play a critical role in breast carcinogenesis; these are including retinoblastoma tumor suppressor, BRCA1, BRCA2 and p53. The question now, can tumor suppressors genes be implicated in the evolvement of breast CSC? Most probably the tumor suppressors is implicated in the regulation of normal stem cell proliferation, plays a vital role in the maintenance of chromosome stability, DNA damage and repair, and activation of cell-cycle checkpoints, moreover, they are controlling cell cycle, and regulate apoptosis together with maintenance of cells differentiation. Loss or mutation of one of those proteins could be the initial step of normal tissue stem cells transformation to cancer stem cells. Deregulation of tumor suppressors could also occur at the level of multipotent progenitor’s lineage, which will grant the progenitors the self-renewal capacity leading to tumor initiation. Impairment or loss of expression of tumor suppressors can lead to accumulation of genetically unstable breast stem cells, which could be a target for further carcinogenic stimuli to be transformed to CSC. It is not well known whether the tumor suppressors do regulate the stem cell proliferation. Finally, the role of tumor suppressors in transformation of normal stem cell to CSC needs further investigation and consideration.

Future CSC-targeting therapies

Due to the rapid advance in the concept of CSC, new optimized therapeutic regimes to targeting CSCs efficiently are becoming vital for evaluating recent anti-cancer therapeutic modalities. It is widely accepted that the problem of drug resistance and tumor relapse is related to the survival of CSCs within the tumor. The successful treatment of a cancer needs a chemotherapeutic strategy that can eliminate cancer cells, which constitute the majority of tumor bulk as well as the cancer stem cells. It is widely accepted now that the traditional modalities of anti-cancers include...
chemotherapy, hormones, radiation and immunotherapy kills only continuously dividing cancer cells, which constitute the bulk of tumor thus, ultimately reducing tumor size leaving CSC behind, which finally result in recurrence of cancer and the emergence of more anaplastic cancer cells, which can resist most of anti-cancer modalities leading to patient death. An ideal anti-cancer therapy would kill rapidly growing cancer cells and, likewise, specifically, can targets and kill CSCs.

Specific antibodies attempts to target certain molecules at CSCs like the IL4-CXCR4 in CD133+ colonic CSCs, the preliminary results are promising, but there are only a few data about the probable side effects of these therapies agents. Understanding the mechanism and pathways are used by CSC allow the invention of new therapeutic modalities against CSC, but retains the normal stem cell lineage unaffected to regenerate the normal specific tissue.

**Epithelial to Mesenchymal Transition**

The process of epithelial to mesenchymal transition means that the epithelial cells undergo noticeable histological and morphological changes typically transition of epithelial cobblestone arrangement of epithelial cells to an elongated spindle like stromal cells. EMT process involves a dissociation of intercellular connection, re-arrangement of actin cytoskeleton, raised cell mobility and ultimately promote invasion, accompanied by deregulation and relocation of cell membrane E-cadherin molecule, together with deregulation and displacement of β-catenin molecule from the cell membrane to nucleus, and terminated by acquiring of one or more mesenchymal phenotype typically vimentin or fibronectin and E-cadherin. Vimentin is universal marker of mesenchymal tissue, while E-cadherin is the marker of epithelial cells.

EMT is an essential normal program during embryogenesis’ and wound healing after birth. On the contrary, when it deregulated can cause fibrosis and promote tumor invasion and metastasis. The dissociation of the E-cadherin contact point is a first step in EMT and may occur early or late in the growing carcinomas as an initial step toward stromal invasion, lympho-vascular permeation and remote organs metastasis. Vimentin staining was almost exclusively brown cytoplasmic (perinuclear), while E-cadherin predominantly stained the cell membrane a red colour (Figure 3).

During the processes of EMT, the adhesive epithelial cells loss their intercellular junctions and active cell contact inhibition, and converted into dissociated, mobile, and invasive mesenchymal phenotypic cells. However, mounting data has demonstrated that EMT is playing an important role in cancer propagation, progression, and distant organs metastasis. Progression of epithelial tumors (carcinomas) occurs through transitory local EMT emergence, to grant the tumor cells potent invasive and metastatic potential.

**EMT and cancer stem cell**

Progression of most epithelial tumors toward subsequent stage is associated with EMT changes include the loss of epithelial phenotype and gaining of mesenchymal differentiation, enhancing cell motility and promote invasion leading to tumor metastasis, and resistance to anti-cancer therapy. A data from new research have found that EMT plays a vital role in tumor metastasis as well as in tumor recurrence that could be closely related with the biology of CSC.

Morel et al. found that CD44+/CD24−/low breast CSC phenotype could be developed from non-carcinogenic ductal cells of CD44low/CD24+ phenotype. Further, they also found that some of CD44+/CD24−/low phenotypic CSC can undergo EMT process reflected by down-regulation of E-cadherin molecule and acquire of vimentin phenotype. The development of cells with EMT phenotype is implicated for the transformation of CD44low/CD24+ epithelial cell phenotype to CD44+/CD24−/low CSC phenotype. Noteworthy, epithelial cells positive for CD24 treated with EMT inducer (TGF-β), switching them to CD24− phenotypic cell after one-week incubation, concomitant with loss their E-cadherin expression to be mesenchymal phenotype.

Mani et al. also found that the induction of EMT phenotype in non-tumorigenic, immortal human breast cells, resulted in the acquisition of CD44+/CD24low phenotype concomitantly with the loss of E-cadherin and the acquisition of vimentin and increased capability to form mammosphere together with tumor initiating potential. Meanwhile, isolated CD44+/CD24low phenotypic cells from normal mammary gland and breast cancer cells expressed a mesenchymal phenotype such as vimentin and fibronectin.

Santisteban et al. demonstrated that development of cells with EMT phenotype by induction of immune response against ductal carcinoma lead to progressive increased in tumor size in vivo. Noticeably, tumor cells with mesenchymal phenotype had a CD44+/CD24low phenotype and it has the capacity to re-initiate tumor and promote resistance to anti-cancer therapy, which is concordant with breast CSC.

Gupta et al. recently observed that development of cells with EMT phenotype in transformed HMLER mammary tumor cells by shRNA-mediated de-regulation of E-cadherin
molecules produced high number of CD44+/CD24low population, these cells has 100-fold power to form mammosphere compared to cells with epithelial phenotype only [50]. Interestingly, increasing evidence has founded that tumor cells with EMT phenotype associated with CSC expression displayed a mounting resistance to anti-cancer therapy. These data obviously conclude that inducting of EMT could generate cells with stem-like characteristics. However, for the time being there are a few data available about the molecular mechanisms responsible for that processes [7].

By using different EMT-inducers, it has been shown that induction of EMT in immortal human breast cells is associated with acquiring of stem-like properties. In addition to that, normal stem breast cells, together with breast CSC express mesenchymal phenotype. These findings further support by what Morel et al. found.

The EMT properties help cancer cells to seperate and dissociate from the bulk of tumor, and then infiltrate into the surrounding stroma. This could be the preliminary step towards tumor propagation and promoting invasion and distant metastasis. It is believed now that tumor cells at the metastatic site which has mesenchymal phenotype can undergo opposite transformation, i.e., Mesenchymal-to-Epithelial Transition (MET) to recover the pathological characteristic of the primary tumors. This is a vital process by which the cancer cells at the metastatic site can grow to generate the metastatic tumor. Recent studies have shown that carcinoma of the colon and its metastatic growth exhibited a mixed epithelial-mesenchymal phenotype [7].

For carcinomas, the EMT considered a vital process in the growth of metastatic lesion, which induces cell-to-cell disruption and the acquiring mesenchymal phenotype, which enhance cellular motility. In many carcinomas (epithelial cells cancer), cells underwent EMT can be demonstrated at peripheral invading edge of the tumor to enhance cellular migration and ultimate remote metastasis [8]. The EMT process seems to control by physiological mechanisms such as the transforming growth factor β pathways. Recently, a study postulates that there may be a close relation and link between the EMT process and gaining of stemness characteristics [48]. Tumor cells undergo EMT changes could likely be the origin from which the metastatic cancer cells are derived, including metastatic CSC. CSC perhaps plays a role in generating specific niche for growth of metastatic foci. Interestingly, the first metastatic tumor cells can form a pre-metastatic niche, attracting different population of progenitor cells to site of new tumor growth [51].

Conclusions and Recommendations

Identification and isolation of CSC and EMT phenotypic cells in breast cancer research became one of the priorities in this field, since it is now well accepted that these cells are generating and driving the tumor as well as responsible for tumor heterogeneity, invasiveness, metastatic potential and resistance to anti-cancer therapeutic.

Makki et al. found that CD44+CD24-/low phenotype tumor cells seem to be related to CSC with certain levels of differentiation and confined to a distinct molecular subclass of breast cancer. Because, he failed to detect breast CSC in about 30% (44/167) of the cases, these findings suggest that tumor-initiating characteristic are not wholly limited to CD44+CD24-/low cells and other new biomarkers for CSC need to be invented. In order to translate the concept of CSC to clinical practice, we have to pay major attention to the prospective identification of breast cancer stem cells to recognize additional characteristic and new identification techniques.

Noteworthy that increased numbers of tumor cells of CSC and EMT phenotype in DCIS lesion is an initial step in tumor dissemination and propagation. This finding concludes that these cells are crucial for tumor initiation, invasiveness, and propagation [9].

The expression of breast CSC harboring CD44+CD24-/low phenotype was associated with poor prognosis and predicts lymph node metastasis, so these CSC are independent, negative prognostic factor. Their absence indicate favorable outcome, while their expression is a bad prognostic sign [9].

It is worthy to suggest that breast CSC of CD44+CD24-/low phenotype should be included in future validation studies as a prognostic marker in breast cancer. Estimating the frequency of breast CSC in different histological subtype of breast cancer can predict the clinical outcome and prognostic clues of each particular subtype of breast cancer [52].

A recent study has demonstrated a clear variation in the occurrence of EMT phenomena in different subtypes of breast cancers, as well as in various stages of the disease. It was more prevalent in DCIS lesion (Figure 4), triple negative breast tumors, and in metastatic lesions [9]. According to these findings, EMT phenomena may play an important role in stromal invasiveness and metastatic progression of tumors in addition to their putative role in therapeutic refractoriness; moreover, reversion of the process (MET) seems to occur in the invasive component and distant metastasis. Therefore, the stepwise EMT events provide a critical target for a new
anti-cancer therapy; meanwhile, inhibition of EMT may have a clinically important effect on cancer prognosis.

Accumulating data has found EMT occurrence is always co-existent with CSC subsistence of CD44+CD24-/low phenotype \(^{[9,47]}\) suggesting that EMT phenotypes induced a variety of factors are rich sources cell with stemness characteristic, which raise the possibility of biological similarities between CSC, and EMT-phenotypic cells.

New data suggest that the increased proportion and prevalence of tumor cells with CD44+/CD24−/low and vimentin+/E-cadherin- phenotype, in DCIS and metastatic lesion may play an important role in tumor invasiveness and aggressiveness, in addition to being a higher metastatic risk of the breast cancer \(^{[9]}\). We conclude that inhibition of EMT occurrence and CSC elimination may have a significant effect on disease outcome, which could raise the possibility that these cells will be new targets for antitumor agents and breast cancer treatment.

Finally, we can conclude that the currently used markers and technique to detect EMT and CSC tumor cells are not enough to identify these subtypes of tumor cells, because using these markers fails to detect breast CSC in about 30% of the cases and fails to detect the dissociated singly dispersed CSC and EMT cells in the stroma of the tumor \(^{[9]}\). The clinical significance on disease outcome and response to therapy must be further evaluated in more prospective studies.

We recommend further future work to identify and detect the expression of more proteins representing CSC and EMT markers in primary and metastatic breast lesions and inventing a new technique to demonstrate these distinct tumor cells, since it is difficult with the current markers and double IHC technique to identify the dissociated and isolated tumor cells harboring the CSC and EMT signature in formalin fixed paraffin embedded tissue, because these dissociated tumor cells share the same immunohistochemical expression with stromal histiocytes, endothelial cells, myofibroblasts and activated lymphocytes. These findings increase the need for new additional particular markers for these tumor cells, in order to invent a new therapeutic agent that could target tumor cells with CSC and EMT phenotypes at various stage of differentiation, sparing normal stem cells and reducing side effects.

**Conflicting interests**

The authors have declared that no competing interests exist.

**References**

18. Ginestier C, Hur M, Charafe-Jauffret E. ALDH1 is a marker of


