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EMT and Cancer Stem Cells contribute to Breast Cancer Heterogeneity

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Abstract

Breast cancer is the most common cancer in women, and accounts for ~30% of new cancer cases and 15% of cancer-related deaths. Tumor relapse and metastasis are primary factors contributing to breast cancer related deaths. Therefore, the challenge for breast cancer treatment is to sustain remission. A driving force behind tumor relapse is breast cancer heterogeneity (both inter-tumor, between different patients and intra-tumor, within the same tumor). Understanding breast cancer heterogeneity is necessary to develop preventive interventions and targeted therapies. A recently emerging concept is that intra-tumor heterogeneity is driven by cancer stem cells (CSCs) which are capable of giving rise to a multitude of different cells within a tumor. Studies have highlighted linkage of CSC formation with epithelial to mesenchymal transition (EMT). In this review, we summarize current understanding of breast cancer heterogeneity, links between EMT and CSCs, regulation of EMT by Runx transcription factors, and potential therapeutic strategies targeting these processes.

Keywords

Breast cancer; Tumor heterogeneity; Cancer stem cell; Epithelial to mesenchymal transition

Introduction

World-wide about 30% of new cancer cases are breast cancer, making this the most common cancer in women. Approximately 1 in 8 women in the U.S. will experience invasive breast cancer during their lifetime. In 2017, about 252,000 new invasive breast cancer cases were diagnosed in the U.S. (Siegel, Miller et al. 2016). In the past few decades, significant

advances in early detection and treatment of breast cancer have greatly improved the overall 5-year survival rate for patients from 35% in 1960's to 89% in 2016 (Miller, Siegel et al. 2016). Despite this progress, breast cancer remains the second leading cause of cancer-related death in American women. About 40,000 women in the U.S. died from breast cancer in 2017 (Siegel, Miller et al. 2016), and worldwide, half-a-million women die from breast cancer each year (Siegel, Miller et al. 2016).

Tumor relapse and metastasis are the primary reasons for poor survival among breast cancer patients, despite successful resection or chemo- and radio-therapy. A major cause of breast cancer relapse and metastasis is the existence of cancer stem cells (CSCs), which are highly resistant to chemo- and radio-therapy and capable of metastasis (Velasco-Velázquez, Popov et al. 2011). Several studies have reported that breast cancer cells utilize components of the developmental epithelial-to-mesenchymal transition (EMT) program to generate CSCs, thereby facilitating metastasis and drug resistance (Ye, Brabletz et al. 2017).

Breast cancer is not a single disease. There are distinct breast cancer subtypes associated with different clinical outcomes. Breast tumors exhibit both inter- and intra-tumor heterogeneity as a result of both genetic and non-genetic alterations (Polyak 2007). Inter-tumor heterogeneity is defined by the differences between patients, which are reflected in staging systems and pathological classifications (e.g., Basal like, Luminal A/B) of breast cancer (Skibinski and Kuperwasser 2015). Intra-tumor heterogeneity reflects the coexistence of breast cancer cells that differ in phenotypic characteristics or metastatic potential within the primary tumor, and between a primary tumor and its secondary tumor at a metastatic site. The origin of intra-tumor heterogeneity can be explained by genetic and epigenetic alterations, and/or divergent adaptive responses (Prasetyanti and Medema 2017).

Here we review the contributions of EMT and CSCs to breast cancer heterogeneity. New insights are presented into molecular mechanisms that link CSCs and EMT in breast cancer, and therapeutic strategies that have been developed for targeting breast cancer stem cells are explored.

Breast Cancer Origin and Inter-tumor heterogeneity

Breast cancer can be either ductal or lobular, with the ductal type comprising the majority of cases (40–75%) (Bombonati and Sgroi 2011). Ductal breast carcinoma progression is further divided into 4 progressive stages based on histology: flat epithelial atypia (benign lesion), atypical hyperplasia (precancerous lesion), ductal carcinoma in situ (DCIS), and invasive ductal carcinoma (IDC, which is highly aggressive and metastatic) (Bombonati and Sgroi 2011). However, patients exhibit considerable variations in clinical responses to treatment even in the tumors at the same stage, indicating the need for a new classification method (Polyak 2007). Thus, there is a requirement for a more refined classification that will be mechanistically informative and provide increased effectiveness for tumor diagnosis and identification of therapeutic targets method (Polyak 2007, Rivenbark, O'Connor et al. 2013). Based on gene expression profiling, as well as on the expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-related protein (HER2), breast cancer is characterized into 6 distinct molecular subtypes, summarized in

Table 1. This table includes four major subtypes: Luminal A, Luminal B, Basal-like, Her2 enriched, and two unusual subtypes: Claudin-low and normal-breast like (Sørlie, Perou et al. 2001, Sørlie, Tibshirani et al. 2003, Prat, Parker et al. 2010, Eroles, Bosch et al. 2012).

Understanding inter-tumor heterogeneity is key for developing targeted therapy and preventive interventions. Inter-tumor heterogeneity has been proposed to reflect the different cells-of-origin (Burrell, McGranahan et al. 2013). Therefore, the origin of this heterogeneity likely reside in the developmental hierarchy of the normal mammary gland, which contains both luminal and basal cell lineages (Skibinski and Kuperwasser 2015).

It has long been speculated that accumulation of specific mutations in a particular cell type of the normal mammary epithelium generates transformed multi-potent cells that give rise to a specific breast cancer subtype (Smalley and Ashworth 2003). Initially it was proposed that the molecular features of each subtype mirrored the characteristics of the putative normal cell type of their origin. For example, mammary stem cells have been postulated to be the cell of origin for basal-like breast cancer because they share features such as expression of basal cytokeratin and low expression of hormone receptors (Polyak 2007). While Luminal A tumors were thought to be derived from relatively well-differentiated cells of the ER+ lineage, Luminal B tumors had been proposed to develop from less differentiated luminal progenitors (Polyak 2007). This hypothesis has been recently challenged using *in vivo* lineage tracing (Visvader and Stingl 2014, Skibinski and Kuperwasser 2015). In this method, particular cell types from the mammary gland such as mammary stem cells, luminal progenitors and basal progenitor cells, have been identified using different cell surface markers (Summarized in Fig. 1). Comparison of the gene expression signatures of these lineages with breast cancer subtypes suggests that one lineage may give rise to multiple subtypes (shown in Fig. 1) (Lim, Vaillant et al. 2009). These authors also showed that luminal progenitors likely serve as the origin of both luminal and basal-like breast cancers; whereas the basal progenitor signature is most closely aligned with the expression profile of the Claudin-low subtype (Lim, Vaillant et al. 2009). These findings were confirmed by studies of the origin for BRCA-1 associated breast cancer. Multiple strategies have all demonstrated that BRCA-1 associated basal-like breast cancer is derived from luminal progenitor cells (Lim, Vaillant et al. 2009, Molyneux, Geyer et al. 2010, Proia, Keller et al. 2011, Bai, Smith et al. 2013).

The question remains as to why luminal progenitor cells can give rise to both luminal and basal breast cancer subtypes. One explanation is that the luminal progenitor population itself is heterogeneous (e.g., estrogen receptor expression) (Booth and Smith 2006, Shehata, Teschendorff et al. 2012). Another hypothesis is based on the striking finding that 80% of basal-like breast tumors carry p53 mutations (Network 2012). Early loss of p53 may cause genome instability and thereby allow the acquisition of secondary mutations. The cells with p53 and secondary mutations may gain a competitive advantage over neighboring clones with regard to proliferation, migration and invasion, which are also features of basal-like breast cancer (Skibinski and Kuperwasser 2015). Currently the origin of Her2 positive breast cancer remains unclear. Better understanding of the etiology and biology of each subtype will enhance the precision of diagnosis and treatment of women with different forms of breast cancer.

Breast Cancer Intra-Tumor Heterogeneity

It has also long been observed that tumors contain distinct clonal cell populations that differ in karyotype and chemo-resistance (Shapiro, Yung et al. 1981, Yung, Shapiro et al. 1982). More recently, it has been determined that within a single tumor, there are multiple clones with distinct genetic and epigenetic profiles, as well as different somatic mutations identified by deep-sequencing technology (Anderson, Lutz et al. 2010, Gerlinger, Rowan et al. 2012). This phenomenon has been described as intra-tumor heterogeneity (Marjanovic, Weinberg et al. 2013, Prasetyanti and Medema 2017). Intra-tumor heterogeneity is not limited to the differences in malignant cancer cells. In fact, a tumor is a complex structure containing different clones of tumor cells as well as other cell types, such as infiltrating immune cells, stromal cells and endothelial cells (Lu, Weaver et al. 2012, Junttila and de Sauvage 2013).

Both intrinsic and extrinsic factors influence intra-tumor heterogeneity. The intrinsic factors exist at both genetic and epigenetic levels which contributes to differences of tumor cells response to treatment (Prasetyanti and Medema 2017). Cancer cells usually inherit, acquire and sustain aberrations in their genome, such as point mutations, translocations, deletions and amplifications (Vogelstein, Papadopoulos et al. 2013). Those mutations reflect the degree of genome instability, which is a hallmark of cancer (Hanahan and Weinberg 2011). Among those mutations, some are designated driver mutations that induce activation of oncogenic pathways and/or obstruction of tumor suppressors (Stratton, Campbell et al. 2009). Intensive efforts have led to the identification of 40 mutation driver genes in breast cancer patients (Pereira, Chin et al. 2016). Significantly, epigenetic heterogeneity is often observed in tumors (Dawson and Kouzarides 2012). Drugs that target epigenetic enzymes, which alter chromatin structure and function, are rapidly being developed and undergoing clinical trials (Simó-Riudalbas and Esteller 2015, de Lera and Ganesan 2016).

The spectrum of extrinsic factors strongly influences tumor heterogeneity. Tumors are constantly under selective pressure as a consequence of the dynamic tumor microenvironment, applied therapies, and attacks from the immune system (Colak and Medema 2014, McGranahan and Swanton 2017). These pressures act as the extrinsic factors for intra-tumor heterogeneity. For example, cancer cells that survive within a hypoxic tumor microenvironment caused by poor vascularization commonly maintain a mesenchymal phenotype and exhibit high hypoxia-inducible factor (HIF) expression, thereby inhibiting cell differentiation (Castaño, Fillmore et al. 2012). Furthermore, therapies can act as selection mechanisms that influence the evolution of tumor cells (Kreso and Dick 2014, McGranahan and Swanton 2017). Treating luminal breast cancer with an aromatase inhibitor induces remodeling of the clonal population through acquisition of new mutations or the enrichment of existing mutations (Miller, Gindin et al. 2016). Therefore, the combination of genetic/epigenetic alterations and the tumor microenvironment can generate intra-tumor heterogeneity and support tumor progression by conferring a competitive advantage to subsets of cancer cells (Prasetyanti and Medema 2017).

Cancer Stem cells: a major force of tumor plasticity

Intra-tumor heterogeneity may also be explained by the cancer stem cell (CSC) theory. Cancer stem cells (CSCs) are defined by competency to form new tumors, self-renewal, and differentiation into non-stem like cancer cells (Shibue and Weinberg 2017). When CSCs are injected into immunocompromised mice, they have the ability to generate tumors with high efficiency compared with bulk, unfractionated tumor cells (Alison, Lim et al. 2011). Thus, CSCs have been implicated in initiating and promoting primary tumor growth, and in driving the metastases at distal sites (Al-Hajj, Wicha et al. 2003, Abraham, Fritz et al. 2005, Sheridan, Kishimoto et al. 2006, Ginestier, Hur et al. 2007, Liu, Wang et al. 2007).

Cancer stem cells were first isolated from AML leukemia based on the expression of cell-surface markers (Lapidot, Sirard et al. 1994), and later, from solid tumors such as breast (Al-Hajj, Wicha et al. 2003), brain (Singh, Hawkins et al. 2004), colon (O'Brien, Pollett et al. 2006, Ricci-Vitiani, Lombardi et al. 2006) and pancreas (Hermann, Huber et al. 2007). The Wicha group first isolated breast cancer stem cells (BCSCs) in 2003 using cell surface markers for CD24^{low}/CD44^{high} Lineage^{negative} (Al-Hajj, Wicha et al. 2003). They showed that within this population, as few as 200 cells were able to initiate tumor formation in immunocompromised mice (Al-Hajj, Wicha et al. 2003). It is now clear that BCSCs exist in two distinct development states and can reversibly transition between them due to cell plasticity (Liu, Cong et al. 2014). The first state is the mesenchymal-like state in which BCSCs express the CD24⁻/CD44⁺ cell surface marker profile. They are mainly quiescent with low proliferation. The location of this population is commonly at the tumor-invasive edge adjacent to the tumor stroma. The second population is the epithelial-like state that express a de-toxifying enzyme, aldehyde dehydrogenase (ALDH). These BCSCs are highly proliferative, and localized at the center of the tumor (Liu, Cong et al. 2014). BCSCs expressing CD24⁻, CD44⁺, and ALDH⁺ show the greatest tumor-initiating capacity (Liu, Cong et al. 2014).

BCSCs are also associated with chemo-resistance and breast cancer relapse. Conventional chemotherapies target highly proliferative breast cancer cells, which kill most of the breast cancer cells and may be effective initially, but over time the surviving BCSCs cause tumor relapse (Li, Lewis et al. 2008). The mechanisms of chemo-resistance in BCSCs can be divided into two major groups: intrinsic resistance, which is attributable to genetic alteration, and extrinsic resistance, which is linked with the influences from the tumor microenvironment (Rebucci and Michiels 2013). The genetic alterations in BCSCs include aberrant expression of the protein that detoxifies the chemotherapy agents. For example, ABC transporters are usually expressed at high levels in BCSCs, and can cause efflux of chemotherapeutic drugs from the cells. Once cells acquire this phenotype, they can survive chemotherapy (Hirschmann-Jax, Foster et al. 2004). BCSCs also generally have high ALDH expression, which metabolizes chemotherapeutic agents, such as cyclophosphamide, and thereby eliminates the toxic effects from the chemotherapy (Crocker and Allan 2012). Another major factor involved in chemo-resistance of BCSCs is the tumor microenvironment (Crowder, Balikov et al. 2014). In an hypoxic environment, activation of the expression of HIF factors promotes new blood vessel formation and a quiescent

phenotype in BCSCs (Mimeault and Batra 2013). This phenotype contributes significantly to chemo-resistance in BCSCs.

Breast cancer stem cells have been associated with metastasis. Gene expression profiles of BCSCs shows an invasive gene signature with increased metastatic potential (Liu, Wang et al. 2007). The CD44⁺/CD24^{-/low} BCSC phenotype has also been found in cancer cells that have disseminated into the bone marrow of breast cancer patients (Balic, Lin et al. 2006). In a mouse xenograft model, human breast cancer cells metastasized to the lung express high levels of the stem cell marker CD44, strongly suggesting a metastatic role of BCSCs (Liu, Patel et al. 2010). It has been proposed that BCSCs may enter the circulation and become circulating tumor cells (CTCs), with potential metastasize to distal organs and seed metastatic lesions (Battle and Clevers 2017). Some CTCs have high expression levels of BCSC markers (Baccelli, Schneeweiss et al. 2013). Moreover, from liquid biopsy samples of luminal breast cancer patients, CTCs with a BCSC signature are enriched upon disease progression, while the CTCs with a bulk tumor signature do not change (Baccelli, Schneeweiss et al. 2013).

Epithelial Mesenchymal Transition: Development, cancer and metastasis

The concept of epithelial-mesenchymal transition (EMT) was first described almost 50 years ago by Elizabeth Hay (Hay 1968). EMT is an evolutionally conserved morphogenetic program during which epithelial or epithelial-like cells undergo a series of biochemical changes allowing them to acquire a mesenchymal phenotype (Thiery 2002). During the EMT process, polarized epithelial cells lose the expression of tight junction molecules such as E-cadherin and acquire mesenchymal properties, such as enhanced migration, invasiveness, and elevated resistance to apoptosis. EMT is precisely regulated by the interplay of signaling pathways, transcription factors and miRNAs. Several families of transcription factors, for example, Zeb, Snail and Twist, are activated by a variety of signaling pathways, including TGF- β , NOTCH and WNT (Nieto 2002, Yang, Mani et al. 2004, Liu, El-Naggar et al. 2008, Lamouille, Xu et al. 2014). In turn, these transcription factors initiate the EMT program by silencing E-cadherin expression at the cell surface. The loss of E-cadherin is a fundamental hallmark of EMT (Kalluri and Weinberg 2009). Furthermore, mesenchymal-like cells commonly express Vimentin, a cytoskeletal protein necessary for migration (Kalluri and Weinberg 2009). It has been suggested that EMT is not an all-or-nothing process from purely epithelial to purely mesenchymal phenotypes, but rather is a multi-stage process, with one or more intermediate stages (Grigore, Jolly et al. 2016). These intermediate phenotypes have been referred to as partial EMT (Shibue and Weinberg 2017).

There are three different types of EMT, which are necessary for diverse functions: 1. EMT that is required for the formation of mesodermal and neural tube tissue during embryogenesis; 2. EMT associated with tissue regeneration and organ fibrosis; 3. EMT that contributes to the pathogenesis of cancer metastasis (Kalluri and Weinberg 2009, Thiery, Acloque et al., Kovacic, Mercader et al. 2012).

EMT drives essential components of physiological control for embryonic development. During gastrulation, complete EMT is obligatory to generate fully committed mesenchymal cell types that are competent to form the early mesoderm or endoderm (Viebahn, Lane et al. 1995, Thiery, Acloque et al. 2009). In contrast, in the mammary gland, partial and reversible EMT is observed during morphogenesis (Nakaya and Sheng 2013). During puberty, mammary epithelial stem/progenitor cells that reside in the terminal end buds of the breast initiate elongation and migration, thereby driving branching morphogenesis (Micalizzi, Farabaugh et al. 2010). These epithelial cells transiently acquire mesenchymal features, including loss of apical-basal polarity (Ewald, Brenot et al. 2008, Ewald, Huebner et al. 2012), and elevated expression of the EMT transcription factors Snai1 and Twist (Kouros-Mehr and Werb 2006, Foubert, De Craene et al. 2010). The cells in the terminal end buds are regulated by a number of extracellular factors known to induce EMT, including epidermal growth factor (EGF) and hepatocyte growth factor (HGF). In the mouse mammary gland, overexpression of HGF causes hyperplastic branching morphogenesis, while inhibition of HGF signaling blocks budding of side branches (Rosário and Birchmeier 2003). Branching morphogenesis is an exceptionally plastic process with an incomplete EMT program, as both the epithelial and mesenchymal lineages are essential for normal mammary gland function. Recently two transcription factors, Elf5 and Ovol2, have been shown to be the gatekeepers of mammary epithelial differentiation by inhibiting EMT at terminal end buds (Chakrabarti, Hwang et al. 2012, Watanabe, Villarreal-Ponce et al. 2014). Elf5 is the master regulator for transforming luminal progenitor cells into alveolar cells during pregnancy and lactation (Oakes, Naylor et al. 2008, Choi, Chakrabarti et al. 2009). Therefore, a partial EMT state, gaining partial mesenchymal features with maintenance of some epithelial characteristics, is critical during mammary gland development.

Beyond functioning in development, EMT also plays a major role in many types of cancer. Almost 80% of human cancer deaths derive from epithelial tissues including tumors of the breast, lung, pancreas, prostate, colon, ovary, kidney and liver (Ye and Weinberg 2015). Hyperplasia or early stage tumors arising in these tissues continue to express the epithelial marker E-cadherin, whereas highly aggressive primary tumors cells usually exhibit high motility and invasiveness which are the features of mesenchymal cells (Calabrese, Tavaré et al. 2004, Choi, Lee et al. 2013, Cheng, Chang et al. 2014). Cancer cells have the capability to utilize the EMT process to initiate invasion and metastasis (Chaffer, San Juan et al. 2016).

In breast cancer, an EMT signature is enriched in basal-like and Claudin-low subtypes compared with Luminal A/B subtypes (Prat, Parker et al. 2010). Because tumor progression is positively associated with acquisition of mesenchymal features, this may be an explanation for why basal and Claudin-low breast cancers are more aggressive. Depletion of activators of EMT, such as Twist, Snail and Zeb in human or mouse breast cancer cells, greatly inhibits metastasis induced by mammary fat pad or tail vein injection (Yang, Mani et al. 2004, Guo, Keckesova et al. 2012, Zhang, Corsa et al. 2013, Roy, Gonugunta et al. 2014, Tran, Luitel et al. 2014). For instance, depleting Snail in MMTV-PyMT mice completely abolished 95% of lung metastasis (Tran, Luitel et al. 2014). Consistently, activating EMT in human breast cancer cells can enhance metastatic dissemination (Tran, Luitel et al. 2014). Therefore, EMT has been defined as a critical component of the metastatic process.

To understand regulation of EMT, early studies focused on the genes that support activation of EMT (Yang, Mani et al. 2004, Eger, Aigner et al. 2005). More recent studies have revealed cohorts of genes that sustain the epithelial phenotype and thereby suppress EMT (Hong, Messier et al. 2017, Duhamel, Goyette et al. 2018). Emerging evidence supports a requirement for the RUNX1 transcription factor to establish and sustain the mammary epithelial phenotype (van Bragt, Hu et al. 2014, Hong, Messier et al. 2017). Expression of EMT-related genes and phenotypic properties following RUNX1 knockdown in mammary epithelial cells is consistent with its role in suppressing tumor growth (van Bragt, Hu et al. 2014, Hong, Messier et al. 2017). Other members of the Runx transcription factor family share a canonical genome binding sequence, but have distinct mechanistic actions in regulating EMT. RUNX2 is a positive regulator of EMT in breast, thyroid and prostate cancer, consistent with its tumor promoter activities (Baniwal, Khalid et al. 2010, Ching, Baniwal et al. 2011, Niu, Kondo et al. 2012). RUNX3 supports a series of phenotypic functions that impact the epithelial to mesenchymal transition in gastrointestinal and lung cells (Voon, Wang et al. 2012, Yang, Fu et al. 2017). Increased mechanistic distinctions for Runx family factors in specific cell types will be biologically and clinically informative.

Although EMT processes are well documented in many *in vitro* cancer cell models and even *in vivo* animal experiments, the existence of EMT during tumor progression and its relevance in metastasis have remained matters of controversy. One of the key concerns is the lack of convincing histological evidence of EMT in clinical samples (Thiery, Acloque et al. 2009). Two recent reports raise debate about whether EMT is dispensable for invasion and metastasis (Fischer, Durrans et al. 2015, Zheng, Carstens et al. 2015). Fisher et al. used a spontaneous breast to lung metastasis mouse model and labeled fibroblast-specific protein 1 (Fsp1) as a marker for EMT. They observed that many Fsp1 negative cells metastasize to lung, indicating that EMT is not necessary for metastasis (Fischer, Durrans et al. 2015). In another study, Zheng et al. knocked out either Snail or Twist in a spontaneous pancreatic ductal adenocarcinoma (KPC) model and observed no difference in metastasis by tracing α -smooth muscle actin as a mesenchymal marker (Zheng, Carstens et al. 2015). However, there is some question regarding whether Fsp1 or α -smooth muscle actin are proper EMT markers, as they are rarely induced upon activation of EMT (Aiello, Brabletz et al. 2017, Ye, Brabletz et al. 2017). Furthermore, using the same KPC mouse model, Krebs et al. showed that depletion of Zeb1 suppresses stemness, invasion and metastasis, indicating that EMT is necessary for metastasis *in vivo* (Krebs, Mitschke et al. 2017). Thus, the discrepancy of the role of EMT in metastasis requires further studies.

Two studies discussed above demonstrated that EMT is key to chemo-resistance (Fischer, Durrans et al. 2015, Zheng, Carstens et al. 2015). Several other studies also demonstrated that induction of EMT confers resistance to chemotherapy and radiotherapy (Creighton, Li et al. 2009, Oliveras-Ferraro, Corominas-Faja et al. 2012). One hypothesis is that the EMT activator Twist can bind to the promoter and activate expression of the ABC transporters, which is responsible for efflux of drugs out of the cell (Saxena, Stephens et al. 2011). The underlying molecular mechanisms of EMT-induced chemo-resistance remain unresolved.

It has been postulated for decades that EMT is related to the generation of CSCs. In 2008, Mani et al. first demonstrated that a CD44^{high}/CD24^{low} population was generated from the

bulk population upon EMT induction by either TGF- β or transcription factors (Mani, Guo et al. 2008). This subpopulation exhibits a gene expression profile similar to mammary stem cells and is able to initiate tumors quite efficiently in mouse (Mani, Guo et al. 2008). Later, multiple studies confirmed the link between EMT and breast CSCs (Thiery et al. 2009; Scheel et al. 2011; Chaffer et al. 2013). Mechanistically, a number of pathways and transcription factors that are known to induce EMT, including Notch, hedgehog, WNT, TGF- β and NF κ B, are also capable of regulating cancer stem cells, which suggests EMT is a major cause of cancer stem cell formation (Fig. 2) (Scheel and Weinberg 2012).

Conclusions and Future Direction

A rapidly evolving concept is that the cancer stem cell (CSC) and epithelial to mesenchymal transition (EMT) phenotypes play important roles in breast cancer progression, heterogeneity, metastatic competence, resistance to therapies, and relapse. Emerging evidence indicates that EMT and BCSCs are likely related to a high risk for breast cancer recurrence and poor prognosis. Understanding these processes of breast cancer pathogenesis will therefore lead to effective treatment options.

Traditional therapies against breast cancer too often result in failure and cancer relapse. There are growing interests and strategies in targeting CSCs based on their unique characteristics. These strategies include targeting specific BCSC surface markers, modulation of signaling pathways of EMT and CSCs, inhibiting ABC transporters to reduce drug efflux, induction of apoptosis and differentiation in BCSCs, and targeting components of the microenvironment. All of these provide necessary signals for BCSC maintenance and self-renewal.

Further investigations into BCSC and EMT biology need to take advantage of additional technological advances for the visualization, isolation, and better characterization of their phenotypes. Robust biomarkers and elucidation of the genes and the signaling pathways that are altered in breast tumor cells will be required. Improving our understanding of these cellular states may help to determine potential targets for novel therapies to prevent metastasis and relapse, and improve clinical outcomes.

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Figure 1. Schematic model of mammary epithelial hierarchy and potential relationship with breast tumor subtypes

Cell surface markers used for the isolation of epithelial cell populations from the mouse mammary gland are indicated. The four major tumor types are shown linked to their closest probable normal epithelial cell type of origin. Basal-like subtype could originate through mutation of p53 and BRCA1 in the luminal progenitor cells.

Figure 2.
EMT and Cancer Stem Cells share similar pathways

Features of the molecular subtypes of breast cancer. Information gathered from (Eroles, Bosch et al. 2012) and (Kennecke, Yerushalmi et al. 2010)

Table 1

Molecular Subtype	Luminal A	Luminal B	Basal Like	HER2 Positive	Claudin Low	Normal Breast Like
Frequency	50–60%	10–20%	10–20%	15–20%	12–14%	5–10%
ER/PR/HER2	ER+PR+HER2–	ER±PR±HER2+	ER–PR–HER2–	ER–PR–HER2+	ER–PR–HER2–	ER±PR±HER2–
Proliferation	Low	High	High	High	High	Low
Histologic grade	Low	Intermediate	High	High	High	Low
Prognosis	Good	Intermediate	Poor	Poor	Poor	Intermediate
P53 mutation	Low	Intermediate	High	High	High	Low
Top metastatic site	Bone	Bone, Liver	Lung	Lung	Unclear	Unclear