Breast Cancer RESEARCH

REVIEW

Three interrelated themes in current breast cancer research: gene addiction, phenotypic plasticity, and cancer stem cells

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Abstract

Recent efforts to understand breast cancer biology involve three interrelated themes that are founded on a combination of clinical and experimental observations. The central concept is gene addiction. The clinical dilemma is the escape from gene addiction, which is mediated, in part, by phenotypic plasticity as exemplified by epithelial-to-mesenchymal transition and mesenchymal-to-epithelial transition. Finally, cancer stem cells are now recognized as the basis for minimal residual disease and malignant progression over time. These themes cooperate in breast cancer, as induction of epithelial-to-mesenchymal transition enhances self-renewal and expression of cancer stem cells, which are believed to facilitate tumor resistance.

Introduction

Modern biomedical science is embodied in the principle of One Health, or the recognition that exploration of evolutionarily conserved genes and proteins *in vivo* and *in vitro* using animal models and cell lines, respectively, is a viable and robust means of understanding human health and disease [1]. The One Medicine concept was first articulated by the German physician Virchow in the 1860s [2]. Experimental cancer research is founded on fundamental discoveries made using primary and transplanted mouse breast cancer models [3]. To this armamentarium, modern investigators have added cultured and xenografted human cell lines. While critical for discerning fundamental cancer mechanisms, model systems present both opportunities and challenges for translational medicine practitioners. These opposing prospects are exemplified in three primary themes within the current breast cancer literature, which are briefly reviewed and integrated here: gene addiction; phenotypic plasticity, exemplified by epithelial-to-mesenchymal transition (EMT); and cancer stem cells (CSC). In particular, the EMT phenomenon illustrates the interactions among these three themes.

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Historical context

Most of the fundamental concepts underpinning gene addiction, phenotypic plasticity, CSC, and EMT are deeply embedded in the history of breast cancer research. As with many scientific questions, early investigations into these concepts were obscured by inconsistent terminology and the resulting inability to link findings identified independently by numerous researchers. For example, the hypothesis that cancer originates from an embryonic stem cell was first proposed in the late 1800s by Connheim and Virchow [4]. Connheim called the cells blastema, which Virchow erroneously proposed arose from the connective tissue [4].

The recognition of cellular plasticity can primarily be credited to the modern developmental biologists who described this epithelial-to-mesenchymal transition and coined the concept of EMT [5,6]. Medical scientists have only recently embraced EMT as a clinically significant phenomenon in cancer biology. For example, descriptions of carcinosarcomas in the mouse mammary gland noted in 1906 would be called EMT tumors 100 years later [7]. Similarly, the famed Spanish morphologist Cajal observed 'pear-like cells, not attached to each other' in human breast cancer in the late 1800s that surely now would be noted as EMT [5]. In contrast, the concept of gene addiction in breast cancer is less than a decade old. Nevertheless, integration of gene addiction with phenotypic plasticity, EMT tumorigenesis, and CSC provides the key to an integrated mechanistic understanding of current breast cancer biology.

Gene addiction as the driver for cancer induction and cancer phenotypes

The reliance of cancer cells on expression of specific genes (gene addiction) has provided important mechanistic

insight into the phenomena of plasticity, CSC, and EMT. The concept of gene addiction was primarily based on genetically engineered mouse (GEM) experiments in which conditional transgenic overexpression of oncogenes rapidly induced tumors [8,9]. High intralesional oncogene levels were always coupled with neoplasia, while switching off the oncogene expression led to tumor regression [10,11]. Prominent examples of such addiction in mouse breast cancer models include tumors driven by *MYC*, *ERB2* (*HER2-neu*), and *RASGRF1* (*Ras*) – oncogenes that are also upregulated in a substantial fraction of human breast cancers [12,13]. With the recognition of key roles for other genes in modifying cancer phenotypes, cancer gene addiction has been broadened and recently divided into two categories: oncogene addiction, and non-oncogene addiction (NOA) [14]. The genes linked to NOA do not initiate cancer *per se* but instead play critical roles in cancer development and progression. The NOA genes encode a large variety of molecules involved in almost all critical signaling pathways. Notably, NOA genes are usually downregulated or lost in cancer, and thus fall under the broad classification of tumor-suppressor genes (TSGs).

It is important to recognize that cancer cell addiction to both oncogenes and NOA genes has a profound effect on the histomorphology of cancers [15], resulting in genotype-specific tumor phenotypes (Figure 1). For example, loss of cadherin-1 (*CDH1*, also termed E-cadherin), a NOA gene, is associated with human lobular breast cancer [16] and leads to an identical pattern (phenocopy) in mouse mammary gland tumors (Figure 1A,B). In mice, overexpression of the *Her2/Neu* oncogene results in solid nodular tumors (Figure 1C) that mimic human comedo carcinoma [17], while enhanced expression of the *Myc* oncogene leads to hyperchromasia, an increased nuclear:cytoplasmic ratio, and enlarged nucleoli (Figure 1D). These phenotypes are readily recognized by an experienced pathologist.

The NOA concept includes the loss, mutation or downregulation of TSGs, such as *BRCA1*, *BRCA2*, *PTEN*, *TP53* (*p53*), and *RB1* (*Rb*) [14]. The traditional TSGs serve as gatekeepers (by preventing excess cell proliferation) or as caretakers (by repairing DNA to maintain genome integrity). More recently, a TSG class has been identified that inhibits metastasis [18,19]. Loss of function for many TSGs has been linked to unique morphological phenotypes for subsets of mammary gland tumors. For example, *BRCA1* is associated with medullary breast carcinomas [20], *Pten* deletion is linked to adenomyoepithelial differentiation in murine mammary tumors (Figure 1E), *TP53* mutations are associated with EMT in cancers of the breast and other organs [21], and *Rb1* mutations induce neuroendocrine tumors in most organs, including the breast [22]. Specific molecular signatures can therefore be linked to particular structural phenotypes. Furthermore, the most important molecular determinants in defining the ultimate anatomy of malignant breast tumors rests within the original genetic errors that are expressed in preneoplastic or non-invasive neoplastic lesions such as ductal carcinoma *in situ* in humans as well as mammary intraepithelial neoplasia in mice [23].

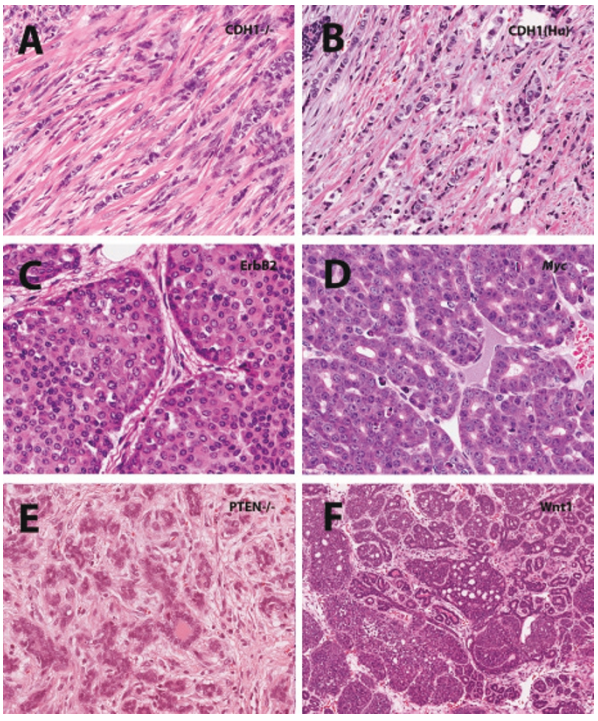

Figure 1. Breast tumor phenotypes are often associated with a specific gene addiction. (A) Tm((CDH1$^{-/-}$)x(p53$^{-/-}$)) mice develop cadherin-1 (Cdh1)-deficient mammary tumors characterized by a single-file pattern of neoplastic epithelium infiltrating a dense stroma [43]. **(B)** Lobular carcinoma of the human breast is a CDH1-deficient tumor that displays a similar growth pattern, with cords of neoplastic epithelium coursing through dense connective tissue. **(C)** Tg(cNeu) mice that overexpress the *ErbB2* oncogene develop breast cancers having a solid, nodular growth pattern with relatively uniform oval nuclei and abundant red–orange cytoplasm. **(D)** Typical neoplastic mammary epithelial cells in Tg(cMyc) mice exhibit a high nuclear:cytoplasmic ratio along with large pleomorphic nuclei having coarse hyperchromatic chromatin and prominent nucleoli in response to overexpression of the *c-Myc* oncogene. This tumor has a glandular pattern that is rarely seen in Tg(cNeu) mice. **(E)** Adenomyoepithelioma from a Tm(Pten$^{-/-}$) mouse that features small gland-like spaces surrounded by a highly cellular stroma in response to ablation of the *Pten* tumor suppressor gene. Note the reddish polar cytoplasm characteristic of the tumor cells. **(F)** A complex Type P tumor in a Tg(Wnt2) mouse illustrating the intricate growth patterns associated with uncontrolled overactivity in the canonical Wnt pathway. Note the central ductal structure with various neoplastic masses at the periphery. All figures were digitally captured using whole-slide imaging of hematoxylin and eosin-stained slides using the 10x or 20x objective; figures have been cropped to a similar size for ready comparison.

Genotype-specific phenotypes have been extended to include entire pathways [24]. An example of this capacity is the combined members of the Wnt signaling pathway [25,26]. In the canonical Wnt pathway, the many Wnt ligands bind their receptors to stabilize β-catenin, allowing it to enter the nucleus to regulate the transcription of target genes that control cell fate and maintain the pluripotency of adult stem cells. Uncontrolled canonical Wnt signaling is a hallmark of cancer. In the mouse mammary gland, tumors resulting from disruption of the Wnt pathway (Wnt tumors) exhibit complex and heterogeneous phenotypes comprised of intermingled epithelial and mesenchymal derivatives (Figure 1F).

Linking the expression of specific oncogenes to neoplasm development provided significant impetus to the notion of personalized molecular therapy. The basis of this concept is that tumors will regress if the initiating oncogene(s) is targeted with the appropriate drug. This premise was soon dispelled, however, by evidence in the mouse that certain oncogene-derived tumors frequently escape their oncogene addiction [27]. Although sobering, this escape from oncogene addiction has also provided an opportunity to explore one of the central problems in clinical oncology – the drug-resistant tumor. How do addicted tumors become drug resistant?

Additional acquired mutations in neoplasms from various tissues represent an obvious answer to this question. For example, human leukemias resistant to the tyrosine kinase inhibitor imatinib are associated with a mutant *ABL1* (*c-Abl*) proto-oncogene [28]. Mouse tumors with *Myc* mutations that were oncogene independent frequently have an activated *Hras* (*K-ras*) gene [29]. However, other mechanisms of enhanced malignancy, metastasis, and tumor resistance have emerged recently that also deserve consideration – namely, phenotypic plasticity and CSC.

Gene addiction, epithelial-to-mesenchymal transition, and phenotypic plasticity in breast cancer

Epithelial-to-mesenchymal transition is a common breast cancer phenotype

Escape from oncogene addiction was observed in GEMs that developed mammary tumors despite having lost expression of the oncogenic transgene. The neoplastic mammary gland epithelium formed tumors with many histomorphologic appearances. One category first described in mouse models is the carcinosarcoma, now classified generically as an EMT tumor [7,30]. These masses are of epithelial origin but contain a substantial spindle cell component (Figure 2).

During the first half of the twentieth century, these mixed neoplasms were believed to be artifacts of transplanting tumor tissue or cultured tumor cells into allogenic mice [30]. More recent research indicates that EMT tumors represent intraneoplastic dedifferentiation to a more embryonic state, where tumor cells have lost their pure epithelial attributes and gained some mesenchymal properties [31]. Contrary views to this premise have been expressed [32].

During both embryogenesis and tumor formation, EMT is characterized by transformation of plump epithelium into fusiform (spindle-shaped) mesenchyme [31]. The EMT process has been described in detail in both mouse mammary development and tumorigenesis (see reviews in [33]). The process encompasses gradual disruption of epithelial architecture, resulting in discontinuity of basement membranes, loss of cellular cohesion, altered apico-basal polarity, and assumption of spindloid cellular profiles. In addition to having altered morphology, spindle cells in mixed tumors express both epithelial (for example, cytokeratins) and mesenchymal (for example, fibronectin, smooth muscle actin, vimentin) cytoskeleton components, although the extent of marker expression tends to vary from region to region (Figure 2) [7,34]. The morphologic continuum observed in such tumors probably reflects the ability of neoplastic mouse mammary epithelium to divide into daughter cells with distinct phenotypes: epithelioid and spindloid [29]. Of note is that the proportion between the two components has serious implications in proliferation and invasiveness of many cancers, especially those of the mouse mammary gland [34]. In human breast cancer, however, there is little agreement about exactly what constitutes EMT tumorigenesis [7,35]. Currently, the presence of EMT in human breast cancers is largely established by specific EMT signatures on gene expression arrays with little attention to phenotype.

The occurrence of EMT within human breast tumors has been linked with malignant transformation and enhanced local and vascular invasiveness, and may reflect the partial dedifferentiation of tumor cells [36]. Interestingly, it appears that metastatic tumor cells undergo EMT to invade the vasculature and then rapidly restore an epithelial phenotype via a reverse mesenchymal-to-epithelial transition (MET) to implant successfully at distant sites [37-39]. Active, bidirectional phenotypic plasticity in human EMT is therefore associated with enhanced malignancy, distant dispersal, and poor prognosis [40].

The biological behavior of human and mouse EMT tumors in the mammary gland is not always identical. Mouse EMT tumors that arise spontaneously or develop from tissue culture transplants are locally aggressive but do not metastasize [7]. In contrast, EMT in the human breast is associated with widespread metastasis and is a strong indicator of a poor prognosis [40]. Several explanations for this divergence between species have been postulated. The most obvious is that the morphologic similarity between EMT tumors from humans and from

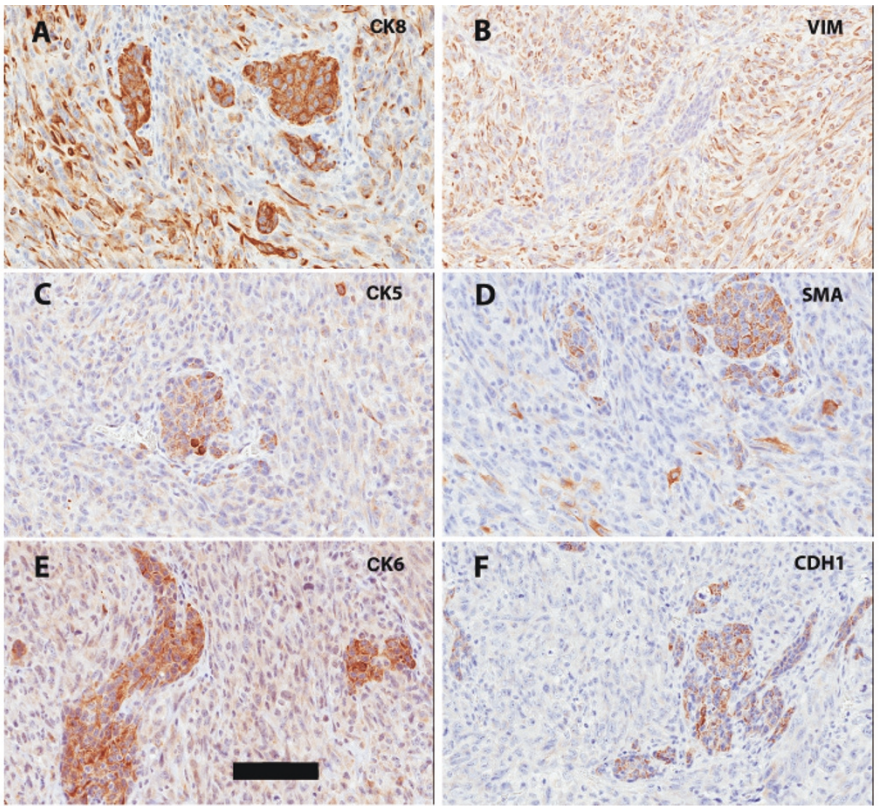

Figure 2. Immunohistochemical phenotypes of epithelial-to-mesenchymal transition tumors in the mouse mammary gland in Tm(Stat1$^{-/-}$) mice. Immunohistochemistry for various breast cell markers in serial sections of a mouse epithelial-to-mesenchymal transition (EMT) mammary tumor showing both epithelial and spindle cell components. Left: Cytokeratin (CK) staining for three epithelial markers: **(A)** luminal CK8/18, **(C)** basal CK5, and **(E)** progenitor CK6 cells. Right: Staining for two mesenchymal markers, **(B)** vimentin (VIM) and **(D)** smooth muscle actin (SMA), and **(F)** the epithelial junctional complex marker cadherin-1 (CDH1). (A), (B) The images are arranged to highlight the dual CK–VIM staining pattern of EMT tumors. The presence of (C), (D) basal cell antigens and (E) progenitor cell CK in conjunction with (F) loss of CDH1 characterizes the malignant breast epithelial population. All images were captured from whole-slide images acquired with the Aperio ScanScope XT (Aperio, Carlsbad, CA, USA) using the 20x objective. All markers were detected using an indirect immunoperoxidase procedure with diaminobenzidine as the chromagen and hematoxylin as the counterstain. (E) Bar = 100 μm. The Tm(Stat1$^{-/-}$) mice were kindly provided by Dr RD Schreiber [109].

mice may represent a random happening, rather than an indication of shared molecular pathways. Supporting this premise is the fact that the transcriptional repressor *SNAI1* (*SNAIL*), a major regulator of EMT, is overexpressed in highly metastatic human breast cancers [41] but is decreased in metastasis of mice [34]. Another possibility is that mouse EMT spindle tumors represent a terminally differentiated lesion, and thus are not capable of metastasis [42]. If the latter proves true, development of therapeutic agents to induce terminal differentiation may represent a viable therapeutic strategy for human breast cancer patients.

CDH1 non-oncogene addiction defines the phenotype of breast tumors

Repression of CDH1 *is the non-oncogene addiction that drives epithelial-to-mesenchymal transition*

One of the key molecular examples of NOA in human breast carcinomas is the association between the EMT phenotype and altered cell-to-cell adhesion due to reduced *CDH1* expression (Figure 2F) [39,42]. Like many of the NOA genes, *CDH1* interacts with multiple signaling pathways. Because of its apparent central role in human and mouse EMT tumorigenesis, *CDH1* perturbations will be discussed in detail as a prototype for NOA in mammary gland neoplasia.

CDH1 is a major component of adherens and tight junctional complexes. Loss of heterozygosity for *CDH1* was first described in a unique breast cancer subtype, the lobular carcinoma, which is characterized by single files of epithelial tumor cells infiltrating dense fibrous stroma (Figure 1B). Provocatively, GEM with null mutations of both *Cdh1* and *Trp53* targeted to the mammary gland developed breast tumors that are an exact phenocopy of human lobular carcinoma (Figure 1A,B) [43]. Reduced *CDH1* expression may reflect gene inactivation via mutation [16], but more often results from epigenetic down-regulation by *CDH1* promoter hypermethylation [44] or

from dysregulated *CDH1* transcriptional repression [42,45].

Numerous molecules have been implicated in transient *CDH1* repression. Prominent examples include ligands that act via serine/threonine kinase receptors, such as transforming growth factor beta, or tyrosine kinase receptors, such as epidermal growth factor, fibroblast growth factor, hepatocyte growth factor, insulin-like growth factor, platelet-derived growth factor, and vascular endothelial growth factor. Expression of *CDH1* is also controlled by many transcription factors, including some members of the forkhead box (for example, *FOXC1*, *FOXC2*), basic helix–loop–helix (for example, *TWIST1*), and zinc finger (for example, *SNAI1* (*SNAIL*), *SNAI2* (*SLUG*), *ZEB1*) transcription factor families [46,47], as well as by certain cytokines, such as IL-6 [48] and chemokine (C-X-C motif) ligand-12 (formerly termed stromal cell-derived factor-1) [49].

These factors and the signaling pathways they affect often operate synergistically with each other, and with other molecules. Activation of NOTCH1, an important promoter of tumor angiogenesis, functions together with *SNAI1*-induced *CDH1* repression to promote breast cancer metastasis [50], while vascular endothelial growth factor cooperates by boosting *SNAI1* expression [51]. Reduction of *CDH1* levels by fibroblast growth factor-1 drives malignant transformation and EMT by inducing matrix metalloproteinase-3, the activity of which promotes increased tumor cell motility [52]. Inhibition of CDH1 and SNAI1 is mediated by feedback from other proteins, such as downregulation of SNAI1 levels by glycogen synthase kinase-3 beta, which is in turn regulated by the canonical Wnt pathway [53,54] or by small inhibitory microRNAs [55]. Some of these molecules are useful prognostic markers in human breast cancer, including C-X-C chemokine receptor type 4, which binds chemokine (C-X-C motif) ligand-12 [49], and SNAI1 [41]. Many of these same factors are controlled via the hedgehog signaling pathway [56], suggesting that hedgehog may also impact *CDH1* expression in breast cancer.

Suppression of *CDH1* is also associated with steroid hormone-responsive pathways. For example, estrogen receptor (ER)-positive tumors may develop an enhanced metastatic potential and become resistant to estrogen blockers (for example, tamoxifen) because C-X-C chemokine receptor type 4-mediated signaling increases mitogen-activated protein kinase activity, which ultimately represses *CDH1* [57,58]. Androgen receptor signaling, when combined with DNA modifications made by histone deacetylase-1, downregulates *CDH1* in neoplastic breast epithelial cells, thereby impelling EMT and metastasis [59]. The impact of *CDH1* in EMT is accompanied by reduced *ERα* (that is, *ESR1*) transcription, which is regulated by SNAI1 [60] and SNAI2 [61]; these two transcription factors are regulated reciprocally by ERα. Interestingly, ERα expression in EMT is also found in the cytoplasm rather than exhibiting an exclusive localization to the nucleus as occurs in normal breast tissue [62]; the functional implications of this displacement are unknown.

The influence of steroid hormones in breast cancer is not limited to the neoplastic epithelium. Carcinoma-associated fibroblasts express aromatase, an enzyme that augments *in situ* estrogen production, thereby providing carcinoma-associated fibroblasts with better tumor-promoting capabilities that are not found in normal fibroblasts in non-neoplastic breast tissue [63]. This capability probably renders the neoplastic microenvironment more suitable for the survival and expansion of the breast carcinoma cells.

EMT in the context of malignant transformation obviously involves other mechanisms besides CDH1 represssion. One common finding is cadherin switching, where CDH1 is replaced by another type (usually CDH2 (also called N-cadherin) or CDH11), resulting in increased cellular mobility [64,65]. Altered cadherins in tumors can be used as markers for EMT [64]. Furthermore, other junctional complex components involved in epithelial differentiation, such as claudins, occludins, and plakophilins, are also altered in breast cancer, especially along the invasive front [66]. As with CDH1, these other junctional proteins are also repressed by transcription factors of the basic helix–loop–helix and zinc finger families [42,45]. EMT may also result from disrupted interactions between integrins on neoplastic cells and their ligands within the basal lamina and/or extracellular matrix [67]. Alterations in cell–stromal interactions promote local invasion and facilitate metastasis. Additionally, mutations of the tumor suppressor gene *Trp53* in GEM result in EMT [68], possibly via interference by *Twist1* [69]. Mutations of the human homolog gene *TP53* are a common feature in breast carcinoma in humans but are not always associated with EMT [70].

Finally, current thinking in the research community is that a recently identified breast tumor genotype, claudin^{low}, develops EMT as a prominent and reproducible feature [71,72]. The expression level of various claudins in primary human breast cancers is usually higher than that of their regional lymph node metastases, suggesting that claudin reductions may contribute to tumor progression [73]. To our knowledge, the relationship between the claudin^{low} genotype and altered expression of *CDH1*, or other molecules that can modify *CDH1* levels, remains to be clarified. Nonetheless, claudin^{low} tumors are associated with the core molecular signature of EMT – for example, downregulation of *CDH1* with concomitant upregulation of Goosecoid (*GSC*, a homeobox gene), *SNAI2*, transforming growth factor beta 1 and *TWIST1* – suggesting

a probable interaction between the pathways regulating *CDH1* and claudin [47].

In summary, *CDH1* is considered a central figure in clinical EMT tumorigenesis. As documented above, however, extensive, complex molecular interactions between *CDH1* and the other key molecules are difficult to evaluate, let alone validate. The complexity coupled with the early state of NOA investigations guarantees conflicting notions about which are the key molecules driving the process and obscures mechanistic understanding of the entire process. The pathology community will have to continue searching for evidence that the EMT–MET paradigm is more than a molecular illusion.

Restoration of CDH1 *promotes mesenchymal-to-epithelial transition*

Metastatic seeding is the rate-limiting event for carcinoma progression [39]. The current speculation is that EMT is required for initial tumor embolization, but that restoration of the original epithelial phenotype via MET is necessary for invading cancer cells to form viable metastases [74]. The MET process is hence reciprocal to EMT and includes both the reacquisition of an epithelial phenotype with the ability to form glands [74] and also the restoration of the molecular complement indicative of epithelial differentiation, such as *CDH1* expression [39].

The molecular mechanisms of MET in breast cancer metastases remain unknown. Recent reports suggest that MET is induced in breast carcinoma cells by many signaling events: binding of signal transducer and activator of transcription-5 to its tyrosine kinase receptor, JAK2 [38]; reduced expression of phosphoglucose isomerase/autocrine motility factor [75]; and inhibition of SRC homology phosphotyrosyl phosphatase-2 (PTNP11 in humans), a vital promitogenic and prosurvival transducer in the epidermal growth factor receptor signaling pathway [76]. All three pathways induce *CDH1* expression and downregulate expression of mesenchymal markers (for example, fibronectin, vimentin). The signal transducer and activator of transcription-5/JAK2 pathway also reduces levels of SNAI1, an important *CDH1* repressor [38]. In spite of these known complex molecular relationships, the presence of MET has rarely been documented in the clinical setting. It is not clear whether MET occurs in all metastatic clinical situations or whether the microarray and molecular data may reflect associations rather than actual mechanisms.

These proposed events remain mostly conjectural through guilt by association or documentation in rare tumor types. The popular reviews of the metastatic process present cartoons showing individual cells that undergo EMT and migrate through the connective tissue to invade the vasculature and be carried to a distant site where the reverse MET process occurs to ensure colonization. Not many pathologists have seen this phenomenon in traditional diagnostic or research samples [16]. The actual visualization of these events has been provided by Condeelis and coworkers, with intravital microscopy showing tumor cells migrating through the tissue with an escort of macrophages [77,78]. Most direct observations of intravascular malignant cells in standard microscopy, however, reveal these cells on rafts of fibrin clots [79,80] or as non-invasive intravascular emboli [81]. In other words, the clusters of cells that are molecularly able to invade and metastasize are probably small microscopic subsets that do not necessarily represent the entire neoplasm. This observation poses a problem in validating gene expression microarray signatures related to EMT because the overall tumor genetic make-up probably masks these rare events.

Cancer stem cells in breast cancer

In spite of the speculations of Virchow and Connheim, the stem cell hypothesis became lost in the field effects hypothesis [4], and some medical textbooks of the 1950s taught tumor biology in the terms of dedifferentiation of mature cell populations. However, experimental proof eventually emerged that most cancers were clonal [3]. In mammary tumor biology, Joe Leighton and Barry Pierce provided evidence of a rare stem cell population in mouse mammary tumors that gave rise to neoplasms [3]. The inherent plasticity of the CSC in the context of the mammary gland was discussed in the 1970s by Beatrice Mintz [3]. The ultimate demonstration of plasticity was given by Gail Martin using blastocyts injected with teratocarcinoma cells to produce normal healthy mouse pups [82].

Tumor heterogeneity has become an important theme of breast cancer research, suggesting that tumors consist of multiple clonally derived subpopulations [57,83,84]. Large subpopulations of tumor cells are either capable of expansion or are terminally differentiated, while only a small subset of primitive, pluripotent CSC is capable of self-renewal, asymmetrical mitoses, and multilineage-specific differentiation [83].

Human breast CSC are usually associated with a $CD44^{+}/CD24^{-/low}$ signature [83]. Expression of these surface molecules is affected by numerous genetic and epigenetic factors [85], however, and new markers (for example, aldehyde dehydrogenase-1, multidrug-resistance proteins) are regularly being linked to self-renewability [83,86,87]. Accordingly, a definitive molecular phenotype for breast CSC has not been determined.

The origin of breast CSC also remains uncertain. Competing hypotheses are that CSC arise by de-differentiation of proliferation-competent epithelial cells, or by oncogenic mutations in normal stem cells [88]. At least four genetically distinct breast cancer subtypes have

been defined by microarray profiling [89], and at least two types of stem cells have been identified within these subtypes – basal progenitors and luminal progenitors [90]. At present, the former population is considered to be a better candidate for breast CSC [83]. Claudin^{low} tumors, however, have been hypothesized to arise from an even earlier breast epithelial precursor than either basal-like or luminal progenitor cells [71,72]. Furthermore, some evidence suggests that the CSC are present in the early premalignant stages [91].

Comparable stem cell subsets have been defined in mouse mammary tumors, which share many conserved genes with their human counterparts [92]. A recent study in developing (prenatal and early postnatal) mice suggests that different cohorts of cultured, poorly differentiated breast epithelial cells exhibit distinct patterns of cytokeratin (CK) expression. In particular, CK5 occurs in basal cells, CK6 marks multipotent progenitor cells in the basal layer, CK8 is found in luminal cells, and CK14 is confined to myoepithelial cells; small populations are positive for both CK6 and CK14 [93] (Figure 2). The CK6^{+} breast epithelium population has been both proposed [94] and denied [95] as another marker for mammary gland stem cells. Importantly, unique subpopulations of CSC appear capable of giving rise to different breast cancer variants in both humans and mice [92]. If some or all of the above-mentioned CK patterns are eventually confirmed, these intermediate filaments could be used as molecular indicators for functionally unique breast epithelial cell types.

Lastly, breast CSC are presumed to thrive only in specific microenvironmental niches [57] due to the availability of the many supporting factors that affect stem cell numbers and function. For example, the populations of both normal stem cells and CSC are characterized by phosphatidylinositol 3-kinase activation resulting from HER2 amplification [96] in conjunction with decreased PTEN expression and decreased canonical Wnt/β-catenin interactions [97]. Signaling via Sonic Hedgehog [98] or NOTCH [99] also promotes self-renewal of cultured human mammary stem cells. The presence of transforming growth factor beta augments CSC motility and invasiveness [100]. The tumor microenvironment is also important in CSC biology because many factors that support cancer growth, hormone resistance, immune evasion, and metastasis are produced by stromal cells, such as chemokine (C-X-C motif) ligand-12 by mesenchymal stem cells [83,101] and carcinoma-associated fibroblasts [102].

Phenotypic plasticity is linked to cancer stem cell generation and competence

Local invasiveness and metastasis are governed by numerous factors, including the heterogeneity of cell subsets within the primary tumor [57,83,84], the interaction of tumor cells with the microenvironment [103], and the unique combination of cell signaling factors affecting the migrating metastatic cells (seeds) and the colonization sites (soil) [104]. The tendency to undergo EMT is one such factor, as this process generates multiple epithelial subsets with divergent states of stemness relative to more differentiated cells [105].

Several lines of evidence support the link between EMT and CSC in breast cancer. First, EMT induction endows normal and transformed mammary epithelial cells with stem cell properties, including the ability to self-renew and efficiently initiate tumors [106]. Second, neoplastic breast epithelium undergoing EMT exhibits a CD44^{+}/CD24$^{-/low}$ genotype that is consistent with the proposed molecular signature of breast CSC [72,107]; this signature has also been linked to the claudin^{low} genotype [72]. Examples include the induction of CSC through EMT-driven activation of the Ras-mitogen-activated protein kinase pathway [85] as well as via overexpression of SNAI1 and TWIST, key regulators of EMT [46].

Existing data suggest that the EMT program can trigger generation of breast CSC. One intriguing possibility is that EMT foments inherently different CSC subtypes that are responsible for the initiation and progression of breast cancers with divergent patterns of progression and metastasis. The divergent patterns are exemplified by the ability of one oncogene (*Myc*) to generate mouse mammary tumors with different phenotypes [108]. Given the obvious phenotypic complexity of human breast cancer, considerable work correlating EMT and phenotypic plasticity, specific gene addictions, and CSC biology will be required to produce innovative therapies to attack this dreaded disease.

Conclusion

Therapeutic approaches to breast cancer increasingly rely on personalized approaches that identify and attack specific genes, proteins, or pathways. Such targeted treatments depend on the concept of gene addiction (to overexpressed oncogenes and/or reduced non-oncogenes) as a driving force in tumor initiation and progression. Drugs aimed at particular genes have shown some promise, but tumor plasticity – perhaps residing in the rare cancer-initiating stem cells – frequently leads to drug resistance and relapse, preventing a patient moving from temporary remission to permanent cure. Ultimate success in breast cancer therapy will rely on our recognition, understanding, and control of CSC and the gene addictions that control phenotypic plasticity of breast cancers.

Abbreviations

CK, cytokeratin; CSC, cancer stem cells; EMT, epithelial-to-mesenchymal transition; ER, estrogen receptor; GEM, genetically engineered mouse;

IL, interleukin; MET, mesenchymal-to-epithelial transition; NOA, non-oncogene addiction; TSG, tumor-suppressor gene.

Competing interests
The authors declare that they have no competing interests.

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