Phenotypic Plasticity: Driver of Cancer Initiation, Progression, and Therapy Resistance

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Our traditional understanding of phenotypic plasticity in adult somatic cells comprises dedifferentiation and transdifferentiation in the context of tissue regeneration or wound healing. Although dedifferentiation is central to tissue repair and stemness, this process inherently carries the risk of cancer initiation. Consequently, recent research suggests phenotypic plasticity as a new paradigm for understanding cancer initiation, progression, and resistance to therapy. Here, we discuss how cells acquire plasticity and the role of plasticity in initiating cancer, cancer progression, and metastasis and in developing therapy resistance. We also highlight the epithelial-to-mesenchymal transition (EMT) and known molecular mechanisms underlying plasticity and we consider potential therapeutic avenues.

Stem Cells and Differentiation

All stem cells are defined by the key properties of self-renewal (the ability to generate more of themselves) and differentiation potential (the ability to divide asymmetrically and generate more differentiated progeny) (reviewed in Reya et al., 2001). Adult tissue stem cells typically have a more restricted potential, and they can produce only a limited number of cell types. However, tissue stem cells persist throughout adult life in organs that continually or periodically regenerate, such as the skin, intestine, mammary gland, and the hematopoietic system. Because of their long life, tissue stem cells have an enhanced potential to acquire the necessary oncogenic hits for tumor formation, and they are the suspected cells of origin for many cancers, including breast cancer (Visvader, 2011).

Development from a fertilized egg to a mature organism is thought to proceed in a fundamentally hierarchical manner (Marjanovic et al., 2013). Each stem cell asymmetric division produces a progressively more differentiated cell type, beginning with the zygote and ending with all of the terminally differentiated cells of the body. At the branch points of the hierarchy are stem cells and/or multipotent progenitor cells, which, during asymmetric division, generate lineage-committed progeny that no longer possess self-renewal (also termed transit amplifying cells). In most tissues, the progeny cells eventually give rise to post-mitotic, terminally differentiated cell types. The classic and best-studied example of a developmental hierarchy is the hematopoietic system (Reya et al., 2001). Long-term hematopoietic stem cells reside in the bone marrow and generate transit-amplifying progenitors and progressively more differentiated cell types, including lymphocytic and myelocytic cells. The

strength of the hematopoietic paradigm has influenced the belief that solid tissues are similarly organized.

However, certain phenomena have challenged the concept of differentiation as a permanent or unidirectional process. These phenomena suggest that many "terminally differentiated" cells retain the potential to change fate. Here, we use the term "plasticity" to refer generally to a broad set of such phenomena including dedifferentiation (the loss of lineage commitment and reacquisition of stem cell features) and transdifferentiation (direct fate switching to another differentiated cell type) (Cunha et al., 1995; Booth et al., 2008; Bonfanti et al., 2012; Schwitalla et al., 2013; Tetteh et al., 2016).

Phenotypic Plasticity: A Historical Perspective

Plasticity has a long history. The early literature often described dedifferentiation and transdifferentiation in the context of regeneration or wound healing. A well-described example of transdifferentiation is the regeneration of the amphibian retina by pigment epithelial cells that specifically respond to tissue damage (Okada, 1980). Similarly, as Godlewski (1928) first reported in 1928, dedifferentiation of epidermal cells to generate chondrocytes and skeletal muscle cells occurs in the regenerating axolotl limb (Rose, 1947). However, generally, these observations were limited to "lower" vertebrates such as amphibians, which have a capacity for tissue regeneration far exceeding that of mammals. Recently, however, it has become clear that mammalian cells can also be induced to dedifferentiate or transdifferentiate. Typically, investigators achieve "reprogramming" of mammalian cells by introducing one or more transcription factors into a differentiated cell type. Davis et al. (1987) performed the earliest

example of this type of reprogramming with MyoD, which induced conversion to myoblasts when ectopically expressed in fibroblasts. Then came the seminal discovery that a combination of four transcription factors, OCT4, SOX2, KLF4, and MYC (OSKM), could "reprogram" adult human or mouse fibroblasts to an embryonic stem-like state (Takahashi and Yamanaka, 2006; Takahashi et al., 2007). The reality of induced pluripotency has led to an extensive re-evaluation of the permanence of the differentiated state. Lately, investigators have demonstrated that fibroblasts and other cell types could be transdifferentiated or "directly reprogrammed" to cardiomyocytes, neurons, and pancreatic neuroendocrine cells, among other cell types (Zhou et al., 2008; Vierbuchen et al., 2010; Szabo et al., 2010; Ieda et al., 2010; Efe et al., 2011; Kim et al., 2011; Tanabe et al., 2018). For example, the generation of pancreatic β-cells has been reported from hepatocytes or pancreatic αδ-cells (Cozar-Castellano and Stewart, 2005; Sapir et al., 2005; Zhou et al., 2008). In these cases, introduction of genes could induce a shift in the developmental fate of cells in liver and convert them into pancreatic-like cells in the absence of a stem cell intermediate.

All of these examples involved transient or permanent expression of one or more transcription factor in the original cell type, which appeared to transition into a different cell type without proceeding through an intermediate multipotent stage. These studies proved that differentiation states are changeable, metastable entities, and the studies demonstrated that specific transcription factors could shift cells from one state to another.

Intrinsic versus Extrinsic Plasticity

It is useful to distinguish plasticity induced by forced expression of transcription factors, sometimes termed "intrinsic plasticity," from plasticity induced by changes in the microenvironment, termed "extrinsic plasticity" (Bonfanti et al., 2012; Marjanovic et al., 2013). The strongest evidence for extrinsically triggered dedifferentiation comes from recent lineage-tracing studies in diverse settings such as the lung (Tata et al., 2013) and hair follicle (Rompolas et al., 2012). Investigators have definitively mapped the fates of differentiated cells and their progeny with genetic markers following ablation of a particular cell population within the tissue. In both cases, the non-ablated, differentiated cell populations underwent dedifferentiation to regenerate the ablated cells. Therefore, plasticity has a regenerative function *in vivo*. In addition, extrinsic cues and certain pathologic states may trigger transdifferentiation. For instance, in a mouse model of calcifying atherosclerosis, adoption of an osteogenic or chondrogenic phenotype by vascular smooth muscle cells preceded calcification of the vessel intima (Speer et al., 2009). In some of these cases, the induction or expression of certain TFs regulates the switch between hierarchy and plasticity.

Plasticity may also be triggered artificially by experimental manipulation. *Ex vivo* cell culture often fails to recapitulate most aspects of the tissue microenvironment, and such cell culture often results in dedifferentiation. In 2D cultures, mammary epithelial cells (MECs) stochastically acquire stem-like traits upon short-term culture *in vitro* (Chaffer et al., 2011; Keller et al., 2012), and long-term MEC culture leads to widespread epigenetic changes and the adoption of an uncommitted ectodermal stem cell phenotype (Holst et al., 2003; Garbe et al., 2009; Keller et al., 2012; Roy et al., 2013; Breindel et al., 2017).

However, culturing MECs within 3D matrices that recapitulate the biological and mechanical properties of *in vivo* tissue preserves lineage identity and functionality *ex vivo* (Sokol et al., 2016). Similarly, articular chondrocytes growing in monolayer culture lose the ability to express cartilage proteins, but this behavior can be reversed if the chondrocytes are grown in soft agar, which is more mechanically similar to cartilage (Benya and Shaffer, 1982). These findings underscore the importance of instructive structural inputs that alter cellular differentiation potential.

Transplanting cells from their native microenvironment to a different site *in vivo* can also trigger dedifferentiation or transdifferentiation because of inductive signals present in the recipient tissues (Booth et al., 2008; Boulanger et al., 2007; Bonfanti et al., 2012) (Figure 1). For example, Bonfanti et al. (2012) showed that thymic epithelial cells could generate hair follicle multipotent stem cells when transplanted into the inductive microenvironment of the dermis. Booth et al. (2008) and Boulanger et al. (2007) showed that neuronal and lymphoid cells could generate mammary structures when transplanted into the inductive microenvironment of the mammary fat pad. In adult mammary glands, both luminal and myoepithelial lineages contain long-lived unipotent stem cells displaying extensive renewing capacities (Van Keymeulen et al., 2011). This multipotency is associated with embryonic development and hybrid signatures of both basal and luminal markers (Wuidart NCB 2018; Lilja et al., 2018). Expression of p63 in adult luminal progenitors can also reprogram these cells into an intermediate hybrid multipotent-like state before the formation of mature basal cells (Wuidart et al., 2018) (Figure 1). Likewise, expression of active Notch1 in basal cells reactivate an embryonic multipotent program in adult basal cells before giving rise to luminal cells (Lilja et al., 2018).

However, all the molecular signals operative in these de- or trans-differentiation processes are not clear, nor is it clear if all progenitor types will be equally amenable to modification by an instructive environment (Lu et al., 2012).

Plasticity is relevant to the understanding of tumorigenesis and pathogenesis. Cancer is a highly diverse disease, exhibiting heterogeneity both between different tumors (intertumor heterogeneity) and between cells among a single tumor (intertumor heterogeneity). It is becoming increasingly clear that tumors hijack the normal differentiation programs of the normal tissues in which they develop as part of the mechanism by which tumor diversity is generated. Therefore, to understand cancer pathogenesis, we require a clearer picture of cancer development. In this review, we discuss the role of phenotypic plasticity during cancer initiation, progression, and resistance to therapy, and we review the relevant factors that dictate the switch from hierarchy to plasticity in normal tissues and in cancer.

Plasticity and the Origins of Cancer

The cell of origin (also referred to as the tumor precursor cell or the tumor-initiating cell) refers to the original cell that receives the first oncogenic hits and undergoes clonal expansion in the earliest stage of tumor progression. The identity of the cell of origin can have a substantial impact on the behavior and progression of the resulting tumor because, in many cases, the characteristics of the tumor precursor cell are passed on epigenetically to the tumor cells (Gupta et al., 2005; Ince et al., 2007). Conversely, the characteristics of the tumor cell of origin are not
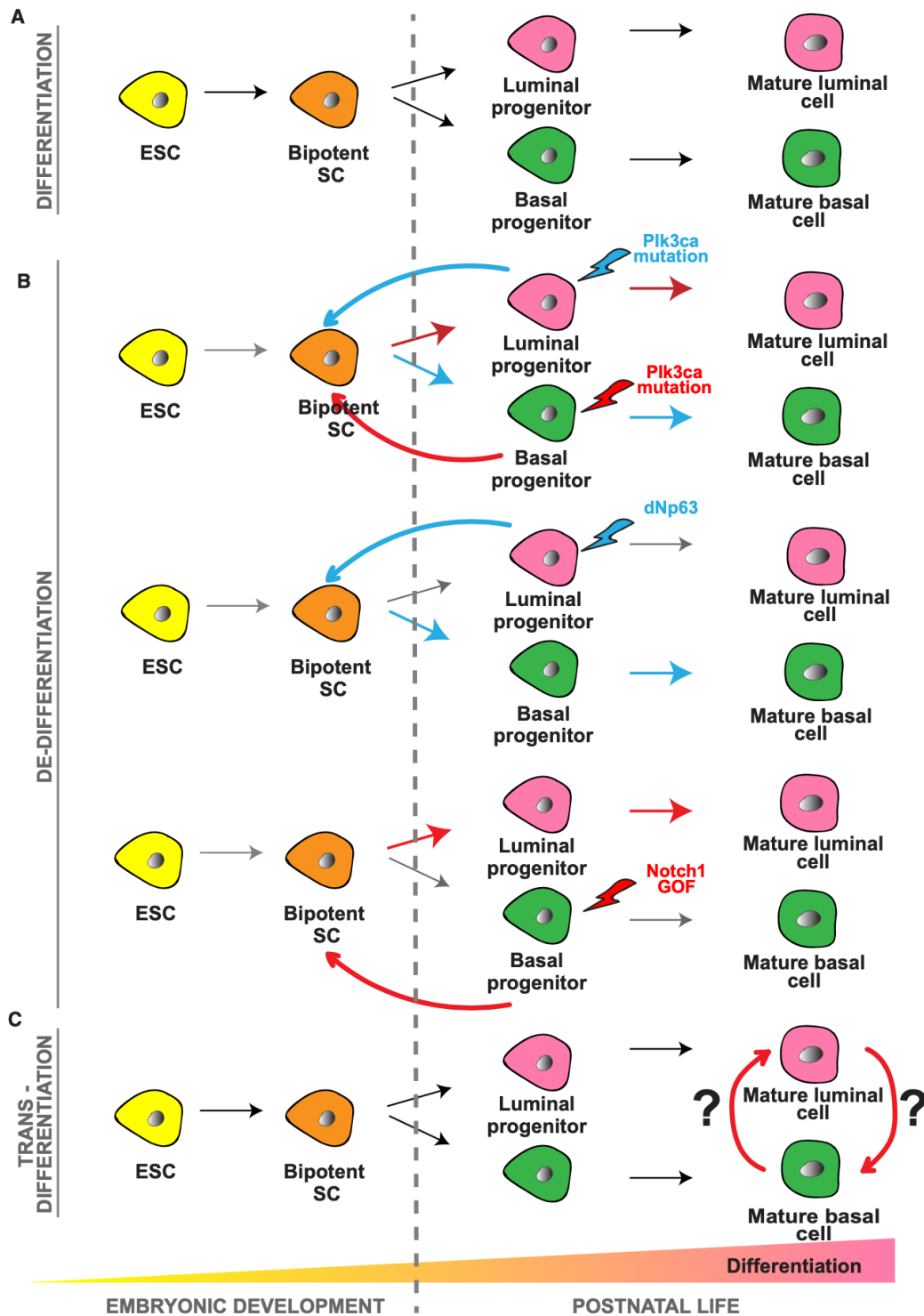
Figure 1. Types of Differentiation that Are Induced during Cellular Plasticity
Types of epithelial differentiation and plasticity seen in the mammary gland and how it relates to more primitive states of multipotency seen during embryonic development.

necessarily equivalent or even similar to the characteristics of the cancer stem cell (CSC) (Visvader, 2011). Moreover, although in many breast tumors the cell of origin is suspected to be a long-lived tissue stem cell, this supposition is not universally true. Even when the cell of origin is a stem cell, it is by no means guaranteed that the resulting cancer cells will resemble their original precursor or that the stem cell program will survive neoplastic transformation intact. Therefore, CSCs, tissue stem cells, and cells of origin are distinct concepts.

Approaches for Identifying the Cell of Origin

Identifying the cell of origin seems straightforward in principle, but identification can be quite challenging to accomplish experimentally because (1) transformation of the original precursor cell cannot usually be observed directly, and (2) the influence of the cell of origin on the tumor phenotype is not always overt. In the case of breast cancer, intrinsic subtypes have been intensely studied from a biological perspective, with the two main subtypes being luminal and basal-like; but how they are generated in the first place has only started to be defined (Prat and Perou, 2011). In principle, both genetic and epigenetic influences can act at early stages of cancer progression to determine the overall phenotype of the tumor. First, there is epigenetic influence imparted by the features of the tumor cell of origin. In addition, mutations, copy number aberrations, or other derangements in key developmental regulators, such as transcription factors, can drive tumor phenotype. Both forces collude to generate intertumor diversity in breast cancer.

To identify the cell of origin of breast cancer, investigators have used two main approaches. The first approach involves isolating normal cell subsets by FACS and either comparing them to the tumor subtypes or using lentiviral vectors to transduce these cells *ex vivo* with a combination of oncogenes that will lead to tumorigenesis. Interestingly these studies revealed that the global gene expression profiles of basal-like tumors were most similar to the luminal progenitor profile in normal tissues (Lim et al., 2009). Further, transformation of luminal progenitor cells led to tumors with both luminal and basal features (Keller et al., 2012). In contrast, transformation of human cells with an EpCAMlow/CD49fhigh immunophenotype, thought to contain basal and myoepithelial (ME), stem and/or bipotent progenitor cells, gave rise to aggressive tumors with squamous differentiation and other metaplastic features (Keller et al., 2012). These tumors were molecularly most similar to the claudin-low intrinsic subtype, which displays high expression of MaSC-associated genes and mesenchymal markers. Metaplastic breast cancer is rare in humans; therefore, these tumors may represent the rare transformation of basal and ME progenitors or stem cells (Prat and Perou, 2011).

A complementary approach is to direct conditional expression of oncogenes (or deletion of tumor suppressor genes) to specific mammary epithelial subpopulations to initiate tumorigenesis in a defined cell population. Molyneux et al. (2010) employed a mouse model in which loss of the BRCA1 tumor suppressor was targeted to either KR14-expressing basal and ME or to β-lactoglobulin (Blg)-expressing luminal cells on a p53-heterozygous background. This approach revealed that targeting BRCA1 loss to luminal cells recapitulated the basal-like phenotype of human BRCA1-associated breast tumors. KR14-driven BRCA1

loss also led to tumor formation; however, histology was that of malignant adenomyoepithelioma, which is not usually seen in BRCA1-associated human cancer.

Together, these studies enshrine progenitor cells as the likely cells of origin, but recent findings have demonstrated that plasticity is relevant to understanding the origins of tumors and their heterogeneity. Solid cancers are highly diverse, exhibiting heterogeneity both between different tumors (intertumor heterogeneity) and between cells within a single tumor (intra heterogeneity). It is becoming clear that tumors reactivate and/or hijack developmental differentiation programs of the tissues in which they originate as part of the mechanism by which tumor diversity is generated. To evaluate plasticity during tumor initiation in breast cancer, investigators have used a genetic approach (Van Keymeulen et al., 2015). The oncogenic PIK3CA mutation was activated, with or without p53 deletion, using K5CreER in basal cells of the mammary gland and K8CreER in luminal cells. Surprisingly, activation of PIK3CA mutation in basal cells induced the formation of luminal estrogen receptor (ER) and progesterone receptor (PR)-positive tumors, whereas its expression in luminal cells gave rise to luminal ER$^+$PR$^+$ tumors or basal-like ER$^-$PR$^-$ tumors. Interestingly, oncogenic PIK3CA mutation activated a multipotent genetic program in normally lineage-restricted populations at the early stage of tumor initiation, influencing future tumor heterogeneity (Van Keymeulen et al., 2015). Similar observations were made in BRCA1-associated hereditary breast tissues. Recent work with mice and humans demonstrated that lineage restriction is dysregulated in preneoplastic BRCA1 cells and tissues, in which there is an overexpansion of luminal progenitor cells that fail to differentiate and aberrantly express basal epithelial cell markers (Lim et al., 2009; Molyneux et al., 2010; Proia et al., 2011). The cause of this defect appears to be aberrantly increased protein stability of Slug in the BRCA1 tissues. In normal tissues, Slug represses luminal differentiation in basal cells, and it is important for the mammary stem cell phenotype (Proia et al., 2011; Guo et al., 2012; Nassour et al., 2012; Phillips et al., 2014). In BRCA1 mutant tissues, however, Slug is aberrantly stabilized, and it accumulates in luminal cells, a phenomenon that likely explains why the tumors are basal-like (Proia et al., 2011). Therefore, in these cases the causal role of the specific mutation incurred in the cell-of-origin also explains the origin of the breast cancer molecular subtypes. Hence, certain gene mutations bias the cell-of-origin to adopt a different cell fate, and this fate is reflected in the tumor phenotype.

Epithelial-to-Mesenchymal Transition-Induced Plasticity and Tumor Initiation

Phenotypic plasticity during tumor initiation is also driven by activation of the developmental differentiation program—the epithelial-to-mesenchymal transition (EMT). This is the process by which cells acquire plasticity and gain the properties of stem cells. In EMT, cells of a differentiated epithelial phenotype lose apicobasal polarity, become motile, and express markers characteristic of mesenchymal cells (Figure 2) (Thiery et al., 2009; Puisieux et al., 2018). EMT is intimately linked with an undifferentiated or stem-like state, including the capacity for extended self-renewal and the acquisition of a stem-like gene expression program (Mani et al., 2008; Morel et al., 2008).
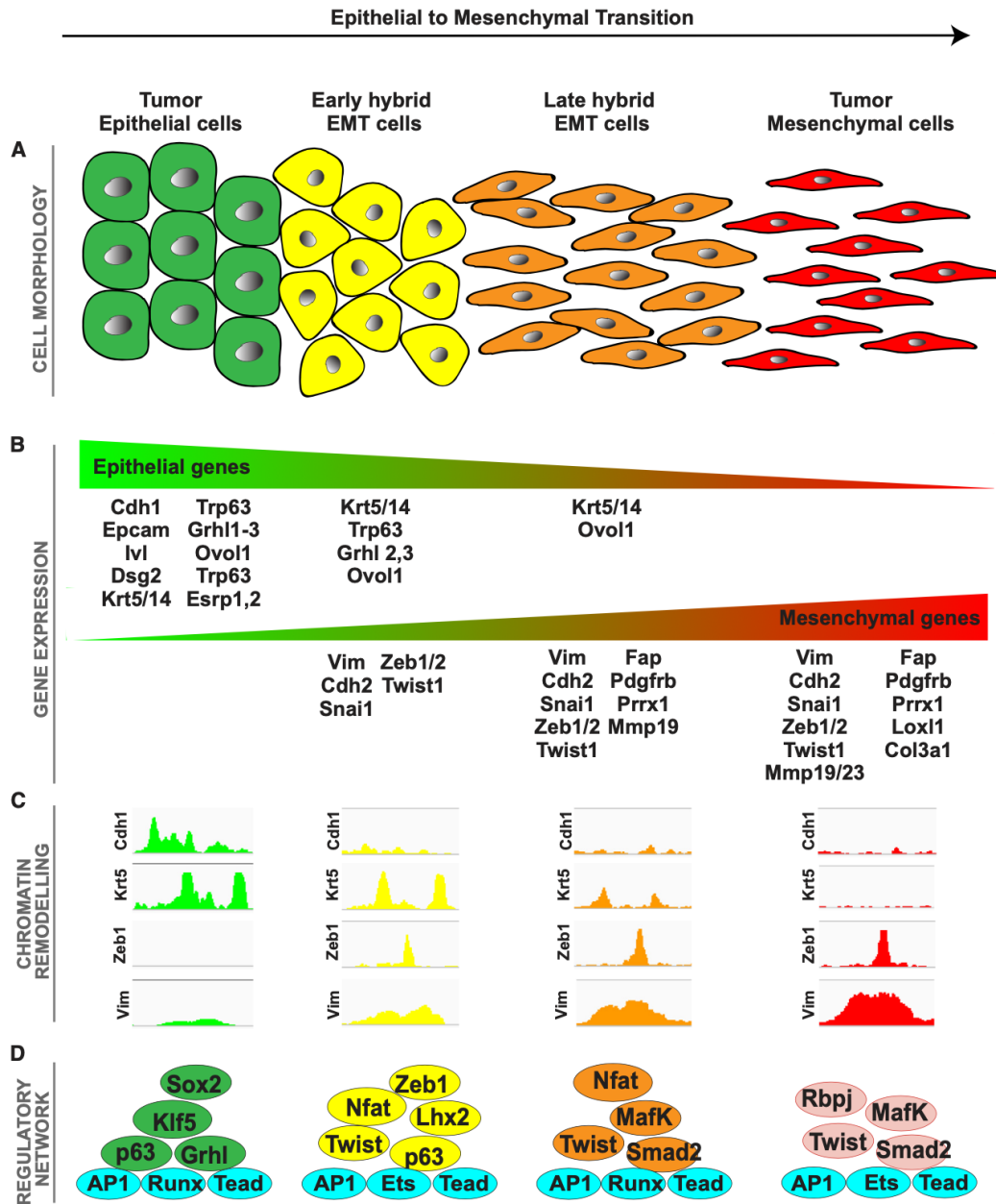
Figure 2. Tumor Transition States Occurring during EMT
(A–D) Changes in cell morphology (A), gene expression (B), chromatin remodeling (C), and transcription factors (D) involved in the regulation of different tumor transition states occurring during EMT.

EMT-induced plasticity has been evaluated during tumor initiation in colon cancer. Using a mouse model with of an inducible and conditional stable allele of β-catenin in IECs, inflammatory signaling through nuclear factor κB (NF-κB) caused dedifferentiation of post-mitotic intestinal epithelial cells leading to the generation of tumor-initiating cells *in vivo* (Schwitalla et al., 2013). In this model, β-catenin was highly expressed, and in

colorectal cancer, this has been strongly correlated with EMT (Brabletz et al., 2018).

However, not all epithelial tumors activate EMT programs with the same frequency, and the dedifferentiation process that takes place leads to re-expression of primitive cell transcriptional programs and cellular metaplasia. In addition, although acquisition of metaplastic and mesenchymal traits is a prominent feature

of some cancers, those traits are rarely observed in other cancers, a circumstance that may reflect intrinsic properties of their cells of origin. Recently, a genetic model of skin cancer was employed to activate oncogenic Kras mutation with simultaneous deletion of p53. Combined with lineage tracing, investigators showed that skin squamous cell carcinomas (SCCs) were derived from interfollicular epidermis (IFE). IFE (K14CreER) displayed a well-differentiated phenotype, whereas skin SCC derived from hair follicle (HF) stem cells (Lgr5CreER) gave rise to tumors with wide range of EMT, from well-differentiated to totally mesenchymal or sarcoma-like tumors with increased metastatic potential (Latil et al., 2017). Interestingly, transcriptional and epigenomic profiling revealed that IFE and HF tumor-initiating cells possessed distinct chromatin landscapes and gene regulatory networks. Thus, this profiling demonstrated, for the first time, that accessibility of key epithelial and mesenchymal TF in the cancer cell of origin primes and dictates the tumor phenotype and EMT (Latil et al., 2017).

Plasticity and Tumor Progression and Metastasis

The EMT is the most widely studied example of phenotypic plasticity, and its role in tumor progression and metastasis is well established (Brooks et al., 2015; Liu et al., 2013). Metastasis is responsible for most cancer patient deaths (Lambert et al., 2017). When tumors spread to distant sites, life expectancy decreases significantly, and, despite important advances, treatment options are limited for patients with metastatic disease. To successfully form metastasis, tumor cells should acquire certain plasticity, thus enabling the invasion of the underlying mesenchyme, intravasation into the blood circulation, and, finally, extravasation and colonization of distant organs (Lambert et al., 2017). The hypothesis that EMT and its reverse process, mesenchymal to epithelial transition (MET), promote the invasion-metastasis cascade has been accepted for over a decade (Brabletz et al., 2018). However, recent studies have challenged the indispensability of full mesenchymal transition in the metastatic process (Fischer et al., 2015; Zheng et al., 2015a). The concept of hybrid epithelial and mesenchymal phenotype has acquired increasing importance for our understanding of the EMT process and its implications for metastasis (Jolly et al., 2015, 2016; Nieto et al., 2016).

Hybrid EMT and Partial Cell-State Transitions

Recently, investigators have identified several transition states occurring during EMT in skin SCC and in mammary tumors (Pastushenko et al., 2018). The different tumor cell subpopulations associated with different EMT stages from epithelial to completely mesenchymal states, passing through intermediate hybrid states, presented similar tumor-propagating cell capacity. However, the tumor cell subpopulations displayed different cell plasticity and invasiveness. Intravenous injection of different subpopulations revealed a strong increase in metastatic potential of early hybrid EMT states. The quantification of YFP+ circulating tumor cells (CTCs) confirmed this observation: the vast majority of CTCs exhibited EpCAM-CD106-CD51-CD61 phenotype that was associated with co-expression of both epithelial and mesenchymal markers. Interestingly, all tumor cells independently of their degree of EMT could revert to the epithelial state. However, the increase in metastatic capacity of the hybrid states did not correlate with the ability of tumor cells to undergo

MET. Thus, other mechanisms beside MET contribute to the higher metastatic potential of these hybrid epithelial and mesenchymal populations (Pastushenko et al., 2018).

In a pancreatic cancer model, driven by Pdx1-cre-mediated activation of mutant KRas and p53, Zeb1 was a key factor for phenotypic plasticity, formation of precursor lesions, invasion, and, notably, metastasis. In this model, depletion of Zeb1 suppressed stemness, colonization capacity, and, particularly, phenotypic/metabolic plasticity of pancreatic tumor cells (Krebs et al., 2017). In a mouse model of breast cancer, 6% of the tumors expressed Twist1, and most of the Twist1+ cells coexpressed several other EMT TFs (Snail, Slug, Zeb2), lost ERα and luminal marker K8, and exhibited a partial EMT phenotype (E-cadherin+/vimentin+) (Xu et al., 2017). Interestingly, compared with tumors that expressed Twist1, Twist1 knockout tumor cells had largely decreased the expression of the different EMT-inducing TFs, the frequency of CTCs, and the incidence of lung metastasis (Xu et al., 2017). Snail has also been reported to have a key function in tumor growth, invasion, and metastasis in human breast cancer cell lines (Olmeda et al., 2007), mouse skin carcinoma cells lines (Olmeda et al., 2008), and gastric cancer (Shin et al., 2012), among others. Overexpression of Slug and Snail in head and neck SCC cell lines repressed miR-101, subsequently activating EZH2, and inducing EMT, migration, and invasion of cancer cells (Zheng et al., 2015b).

Several lines of evidence suggest that hybrid epithelial and mesenchymal states also exist in human cancers. Tumor cells co-expressing both E-cadherin and vimentin were found in invasive breast cancer (Yamashita et al., 2015). Interestingly, the subset of tumors co-expressing these two markers exhibited the worst disease-free survival (DFS) and overall survival (OS) among all breast cancer patients analyzed. We were able to detect different degrees of EMT in lung, breast, and esophagus SCC patient-derived xenografts (PDX), thus demonstrating that EMT is not a binary phenomenon in human cancers (Pastushenko et al., 2018). Computational modeling that considered mutual inhibitory loops between several microRNAs (miRNAs) and EMT transcription drivers showed that a hybrid EMT state could potentiate the progress of developmental programs and increase metastatic potential (Jolly et al., 2015; Tian et al., 2013; Nieto et al., 2016).

The presence of tumor cells in the circulation has been associated with metastasis in multiple cancers (Aceto et al., 2015). When analyzing the EMT phenotype of CTCs, most studies found an association between the presence of hybrid and mesenchymal CTCs with clinical prognosis (Yu et al., 2013; Wu et al., 2015; Hyun et al., 2016; Lecharpentier et al., 2011; Satelli et al., 2015; Zhao et al., 2017). In hepatocellular carcinoma patients, the presence of hybrid and mesenchymal CTCs correlated with more advanced clinical stages and metastasis (Boral et al., 2017). In breast cancer patients, therapy or disease progression was accompanied by an increase in mesenchymal CTCs (Yu et al., 2013). Breast cancer patients with brain metastasis also exhibited CTCs with a higher EMT score.

Despite existence of a growing body of evidence linking EMT to disease progression, recent evidence supports the notion that a partial cell-state transition in the absence of a full EMT is sufficient to drive invasive progression. For example, by upregulating expression of secreted proteases that degrade basement

membrane, SMARCE1 is sufficient to drive the invasive progression of early stage and *in situ* tumors (Sokol et al., 2017). SMARCE1 upregulates protease expression by forming a SWI/SNF-independent complex with the transcription factor ILF3. This association, which occurs in invasive cells that have undergone a partial EMT, directs the genomic localization of SMARCE1 to genes encoding for proteases and other matrix-remodeling factors. An increasing body of evidence suggests that EMT occurs through different transition states and that cells presenting hybrid EMT state display increased metastatic potential. Future studies should focus on understanding the precise molecular mechanisms controlling the transition through EMT or stabilization of tumor cells in specific state.

Plasticity, Stress, and Resistance to Therapy

The primary cause of adult cancer deaths is metastasis of epithelial tumors that are resistant to therapy. Carcinoma cells acquire both of these critical malignant traits—metastasis and drug resistance—when they undergo de-differentiation. Experimental induction of EMT or de-differentiation in cancer cell lines and mouse models is sufficient to promote invasion and metastasis (Thiery et al., 2009; Mani et al., 2008). De-differentiation is also sufficient to promote resistance to a wide spectrum of chemotherapy drugs; often, de-differentiation increases the IC_{50} dose of a chemotherapy drug by ~10-fold (Gupta et al., 2009; Thiery and Sleeman, 2006). Consistent with these findings in experimental models, in clinical samples, high tumor grade (Polyak and Weinberg, 2009), invasiveness (Savagner et al., 2005; Yang et al., 2009), and survival within the circulation (Tester et al., 2000) all correlate to poor response to chemotherapy (Blanco et al., 2002).

Targeting Key Determinants of Therapeutic Resistance

Although an increasing number of treatment options exist, in modern cancer medicine, the development of therapeutic resistance is a major challenge and the cause of treatment failure and disease recurrence. The differentiation state of a tumor is a key determinant of therapeutic resistance (Arienti et al., 2016; Chang, 2011; Haslehurst et al., 2012; Del Vecchio et al., 2014; Kurrey et al., 2009; Housman et al., 2014). Overexpression of certain transcription factors associated with EMT or metaplasia causes resistance to traditional chemotherapy such as radiation and chemotherapy drugs (Dong et al., 2017; Haslehurst et al., 2012; Kurrey et al., 2009). Conversely, inhibition of transcription factor expression increases therapeutic efficacy of these treatments.

The downstream mechanism responsible for resistance to therapy is related to the multiple mechanisms that control target genes. Radioresistance and chemoresistance are achieved by promoting the acquisition of a de-differentiated state (Kurrey et al., 2009; Del Vecchio et al., 2014) by increasing expression of stemness-related genes. This de-differentiated state causes metabolic changes that impair pro-drug activation and drug uptake (Feng et al., 2014, 2017; Del Vecchio et al., 2014). For example, experimental induction of Snail or Twist1 causes constitutively active Perk kinase signaling and activation of its downstream target, NRF2. Nrf2 is a master transcriptional regulator of the antioxidant response, a key mediator of therapy resistance (Feng et al., 2014; Del Vecchio et al., 2014). In addition, overexpression of Slug antagonizes cell death triggered by cancer therapies and promotes cell survival by repressing the pro-apoptotic protein PUMA (Wu et al., 2015).

Currently, two classes of clinical interventions have been suggested that could prove useful for targeting plasticity in cancer. The first class of intervention would either block or reverse de-differentiation to prevent cancer cells from becoming metastatic and drug-resistant, for example, by neutralizing secreted factors that promote EMT or by inhibiting the expression of transcription factors that induce EMT. The second class would block a signaling pathway used by EMT cells to invade, survive in the circulation, or resist therapy. Although, in principle, both of these EMT-targeting strategies could inhibit tumor malignancy, neither on its own would be toxic to cancer cells. Because these EMT-targeting therapies lack cancer cell toxicity, the cancer cells might eventually develop resistance.

These considerations suggest that it is important to destroy cancer cells that have undergone an EMT, and not just to block or reverse EMT. Although this goal is attractive, in practice it has been difficult to find chemical compounds that selectively kill cancer cells that have undergone an EMT; on the contrary, such cells are almost invariably highly resistant to any chemical treatment.

Plasticity and Tumor Stemness

In established cancers, cancer stem cells or "tumor stemness" is the ability of tumor cells to both self-renew and to produce other cell types that constitute the tumor. Activation of EMT programs has been associated not only with acquisition of mesenchymal traits, but with the expression of stem cell markers and an increased ability to form mammospheres, a property associated with mammary epithelial stem cells (Mani et al., 2008). Investigators have proposed that some properties commonly attributed to CSCs, such as invasiveness and metastatic potential, may be acquired by activation of the EMT program. Indeed, in breast cancer patients, CTCs commonly express EMT markers, a property that suggests EMT may enable these cells to leave the primary tumor site, intravasate into the vasculature, and travel to distant sites (Aktas et al., 2009).

Stochastic cell-state transitions may also generate cells with the properties of stem cells and/or CSCs. Recently, Chaffer et al. (2011) reported that a subpopulation of basal-like mammary epithelial cells retained the capacity to spontaneously generate stem-like cells *in vitro*, and the same population could generate CSC-like cells following oncogenic transformation. The transformed cells were enriched for CSC markers, and they exhibited enhanced tumorigenicity in xenotransplantation assays. Moreover, similar transitions have been observed in cultured breast cancer cell lines, in which non-CSCs isolated by fluorescence-activated cell sorting (FACS) regenerated the CSC population at a rate that was too rapid to be explained by sorting impurities (Gupta et al., 2011). Because the *in vitro* tissue culture microenvironment is presumably more or less homogeneous, these transitions are more likely to occur randomly instead of in a directed manner. Gupta et al. (2011) attempted to model these transitions as a Markov process, in which the cells stochastically transition between luminal-like, basal-like, and stem-like states at characteristic frequencies. Markov modeling accurately predicted the collective cell-state transition behavior of FACS-purified luminal, basal, and stem cells (Gupta et al., 2011). Markovian cell-state transitions may also occur in

non-cancerous mammary cells (Phillips et al., 2014). As a caveat, investigators have not yet explored the *in vivo* prevalence of stochastic transitions between non-CSCs and CSCs in breast cancer. Recently, however, several groups have reported *in vivo* evidence of stochastic interconversion between CSCs and non-CSCs in other cancer types, including Wnt-driven intestinal tumors (Schwitalla et al., 2013).

Two major types of phenotypic plasticity exist in cancer: initiating plasticity and maintaining plasticity. Initiating plasticity is generated by the influence of the cell of origin and the specific driver mutations that occur during tumorigenesis. These two forces collaborate to generate the tumor phenotypes that are varied even within the same tissue. Conversely, maintaining plasticity is a result of genetic evolution and hierarchical and plastic interconversion between cellular phenotypes. Maintaining plasticity is also problematic from a therapeutic perspective. Plasticity significantly muddles the analysis of tumor phenotype because many common modalities used to study tumors at the genomic and molecular level (such as exome sequencing and microarrays) rely on bulk tissue, and these methods typically cannot resolve heterogeneous or rare subpopulations within a tumor. From a therapeutic standpoint, maintaining plasticity is also problematic because the presence of multiple types of cancer cells within a single tumor vastly increases the chance that a given therapy will fail to kill some of the malignant cells. Hence, great efforts have been taken to understand the origin of cellular diversity within breast and other tumors.

Molecular Mechanisms Underlying Plasticity

Cellular differentiation states are dynamically regulated in normal cells and tissues via the activation or inactivation of specific transcriptional factors. The factors that promote cellular plasticity during development and wound healing overlap with those that generate phenotypic plasticity and cellular heterogeneity in different types of cancers, because both groups of factors participate in aberrant activation of developmental programs (Table 1). For example, Notch and Wnt development pathways that play key roles in cell fate decisions, tissue patterning, and morphogenesis during development, can also contribute to the regulation of differentiation and self-renewal of CSC in different molecular subtypes of breast cancer (Brooks et al., 2015). Notch signaling is essential to maintain melanocyte precursor homeostasis and interestingly, is low or undetectable in normal adult melanocytes (Bedogni, 2014) and a gradually increasing expression pattern of Notch can be observed from nevi, to primary melanoma lesions, to metastatic melanoma. Notch1 activation confers a metastatic phenotype to primary melanoma *in vivo*, whereas Notch4 has a crucial function in promoting cell proliferation and in regulating an aggressive phenotype of melanoma cell lines (Lin et al., 2016). Expression of active Notch in human melanocytes promotes their transformation (Pinnix et al., 2009) and in addition, Notch 1 signaling facilitates melanoma development in xenograft model by maintaining cell proliferation and by protecting cells from stress-induced death (Bedogni, 2014).

Master Transcription Factor Networks Regulate Plasticity

Other well-studied mechanisms of plasticity involve master transcription factors (TFs), the Snail, Zeb, and Twist families, that orchestrate transcriptional networks that drive de-differentiation.

These TFs mediate sequence-specific interactions with DNA. The SNAIL family of zinc finger transcriptional repressors, of which Snail/SNAI1, Slug/SNAI2, and Smug/SNAI3 are members, are conserved among vertebrate species and have critical functions in various developmental and cellular processes. SNAIL family member functions include, but are not limited to, mesoderm formation, neural crest migration, determination of left-right asymmetry, cell migration, the regulation of cell motility, apoptosis, and cancer initiation and progression (Hemavathy et al., 2000; Inukai et al., 1999; Isaac et al., 1997; Nieto, 2002; Vega et al., 2004).

Slug and Snail both control epigenetic repression of target genes that harbor the E-box consensus CAGGTG motif recognized by the C-terminal zinc-fingers of Slug and Snail (Barallo-Gimeno and Nieto, 2005; Cobaleda et al., 2007; Nieto, 2002). The evolutionarily conserved SNAG transactivation domain, located in the N termini of Slug and Snail, recruits epigenetic silencing complexes such as polycomb repressive complex 2 (PRC2) and co-repressor Lys-specific demethylase 1 (LSD1). This coupling enables the deposition of repressive histone marks (e.g., H3K4me3) to silence the expression of Snail or Slug target genes (Chiang and Ayyanathan, 2013; Lin et al., 2010; Phillips et al., 2014; Wu et al., 2012; Choi et al., 2015; Barallo-Gimeno and Nieto, 2005; Cobaleda et al., 2007; Nieto, 2002; Nieto et al., 1994).

The ZEB family of zinc finger proteins, of which ZEB1 and ZEB2 are members, contains two widely separated and conserved zinc-finger domain clusters with a centrally located homeodomain. This homeodomain is POU-like and does not bind DNA, so it is likely involved in protein-protein interactions. Much like the SNAIL family, the ZEB family of TFs represses transcription by an epigenetic mechanism at specific DNA sequences. The PXDLS motifs in both ZEB1 and ZEB2 recruit epigenetic silencing complexes, such as the CtBP core complex 2 and co RE1 silencing transcription factor (coREST), and this coupling enables the alteration of repressive histone marks to silence the expression of ZEB target genes.

The Twist family (Twist1 and Twist2) is composed of basic helix-loop-helix (bHLH) domain-containing transcription factors. Twist family bHLH proteins regulate expression of target genes by binding as dimers to canonical E-box responsive elements (Zhu et al., 2016; Ansieau et al., 2010). The Twist family of TFs is composed of key regulators in embryonic development and organogenesis (Zhao et al., 2017). Twist family members can act as transcriptional repressors, by recruiting histone deacetylases or inhibiting acetyl-transferases, or they can function as transcriptional activators. Twist can also regulate transcription by interacting with several TFs (MyoD, RUNX1, RUNX2, p53, NF-kB) and by inhibiting or enhancing Slug gene transcription (Casas et al., 2011; Ansieau et al., 2010). Twist2 is a regulator of embryonic development, but its function in tumor initiation, metastasis, and growth is not well documented (Zhu et al., 2016).

Cellular plasticity in mammary epithelial cells can also originate from epigenetic reprogramming via a coordinated process of *de novo* DNA methylation by DNMT3a and gene silencing by DOT1L-mediated reduction in histone H3K79 methylation. This process causes loss of both cell-cycle regulators and lineage-specific genes Breindel et al., 2017; Hinshelwood et al., 2009). Although the temporal nature of de-differentiation is not entirely

Table 1. Role of Known Genes and Transcription Factors in Cancer-Related Plasticity

Gene/TF	Role in Cancer Cell Plasticity	References
Brca1	cancer-cell-of-origin-related breast cancer heterogeneity	Molyneux et al., 2010
	dysregulation of lineage restriction in preneoplastic BRCA1 mutated breast tissues	Lim et al., 2009
	aberrant increase in Slug protein stability	Proia et al., 2011
Pik3ca	cancer-cell-of-origin-related breast cancer heterogeneity	Van Keymeulen et al., 2015
	activation of multipotent genetic program in normally lineage-restricted mammary gland populations	
Zeb1	stemness, colonization capacity and phenotypic/metabolic plasticity of pancreatic tumors driven by activation of oncogenic Kras and deletion of p53	Krebs et al., 2017
	promotes stem-like and tumorigenic phenotype and resistance to MAPK inhibitors in melanoma cell lines	Bedogni et al., 2008; Bedogni, 2014
	increase tumor propagating cell frequency and cell plasticity through repression of miR-200 family and interaction with YAP in pancreatic and colorectal cancers	Wellner et al., 2009; Lehmann et al., 2015; Preca et al., 2015
Twist1	cell survival, proliferation tumor maintenance, and propagation	Beck et al., 2015; Feng et al., 2014; Del Vecchio et al., 2014; Bedogni, 2014
	repress differentiation by activation of MAPK pathways in melanoma	
	activation of Perk kinase promoting therapy resistance	
Snai1	promotes tumor growth, invasion, migration of cancer cells	Olmeda et al., 2007, 2008
	activation of Perk kinase promoting therapy resistance through Nrf2 activation	Shin et al., 2012; Zheng et al., 2015a; Feng et al., 2014; Del Vecchio et al., 2014; Zhou et al., 2014; Proia et al., 2011;
	decrease E-Cadherin expression, Aldh expression and colony forming capacity in pancreatic cancer cell lines	
Slug/Snai2	prevents cell death and promotes cell survival upon cancer therapy by repressing PUMA	Wu et al., 2015
Smarce1	drives invasion in early stage *in situ* tumors promoting partial EMT	Sokol et al., 2017
Jmjd3	promotes tumor-initiation abilities of hepatocarcinoma cells through deposition of active histone mark on Snai2 promoter	Tang et al., 2016
Imp3, Sirt2	stabilizes Snai2 transcripts in breast cancer	Samanta et al., 2016; Zhou et al., 2016
Taz	alters differentiation, induces plasticity and stemness in mammary epithelial cells	Cordenonsi et al., 2011; Skibinski et al., 2014
	interaction with SWI/SNF complex to mediate cellular plasticity	
Dnmt3a, Dot1l	loss of cell-cycle regulators and lineage-specific genes	Breindel et al., 2017; Hinshelwood et al., 2009

clear, this work sheds light on the epigenetic basis of cellular plasticity, knowledge that could prove useful in understanding similar instances of dedifferentiation in other systems.

Regulatory Networks Controlling Tumor Cell Stemness and Metastasis

By repressing adhesion barriers, these TFs mediate the partial reprograming of epithelial cells to acquire invasive behavior (De Craene and Berx, 2013; Lamouille et al., 2014) and the acquisition of mesenchymal behavior by inducing matrix deposition and secretion. In addition, TF overexpression commonly corre-

lates with tumor progression and predicts poor clinical outcomes in many cancer types (Cobaleda et al., 2007; De Craene and Berx, 2013; de Herreros et al., 2010; Lamouille et al., 2014) thus raising immense therapeutic interest for targeting these TFs in metastatic disease.

Zeb1, TWIST1, SNAIL, Slug, or treatment with transforming growth factor β (TGF-β) promote tumorigenicity and stemness of cancer cells. For instance, Zeb1 is known to act as strong repressor of the miR-200 family, whose members are potent inducers of epithelial differentiation (Wellner et al., 2009; Krebs et al., 2017) thus promoting cellular motility, stemness, and

survival properties. In addition to this known mechanism, Zeb1 has been described to directly interact with Hippo pathway effector YAP, switching its function to a transcriptional co-activator (Lehmann et al., 2015), consequently increasing tumor propagating cell frequency and cell plasticity in pancreatic and colorectal cancer cells. Zeb1 promotes expression of the cancer stem cell surface marker CD44 in pancreatic and breast cancer cells in part by CD44 isoform switching by blocking ESRP1 (Preca et al., 2015). Knockdown of Snail or Slug in breast or pancreatic cancer cells decreased invasion, increased E-cadherin expression, and inhibited ALDH expression, together with decreased sphere and colony forming capacity (Zhou et al., 2014; Proia et al., 2011). Similar observations were made in cell line-derived tumors from tongue SCC, in which overexpression of Snail was associated with EMT features and CSC-like features (Zhu et al., 2012).

In addition, modulation of YAP and TAZ are also capable on its own of inducing plasticity and stemness in mammary epithelial cells (Cordenonsi et al., 2011; Skibinski et al., 2014) and for skin cancer initiation (Bebaugnies et al., 2018). Many data indicate that YAP and TAZ act on similar sets of target genes. However, there are some specific non-redundant features of YAP and TAZ in the mammary gland. While YAP is dispensable for mammary gland development (Chen et al., 2014), TAZ acts as a molecular switch regulating luminal and basal phenotypes, and toggling of the switch is sufficient to alter differentiation state. Overexpression of TAZ causes luminal cells to adopt basal and ME features, and depletion of TAZ induces basal and ME cells to acquire luminal characteristics. The ability of TAZ to induce cellular plasticity depends on chromatin remodeling factors to effect changes in differentiation state. The SWI/SNF complex directly interacts with TAZ and is essential in mediating TAZ function (Skibinski et al., 2014). Although both BRG1 and BRM retain the ability to bind to TAZ by their PPXY motifs, cellular plasticity is achieved only by BRM recruitment of TAZ to target genes and not by TAZ/BRG1 complexes. Therefore, the lack of redundancy between BRM and BRG1 may result from binding to distinct sets of cofactors or other transcription factors that provide specificity for particular promoter sequences to drive transdifferentiation. It is worth noting that, although BRG1 does not seem to be important for TAZ-mediated transcription in mammary epithelial cells, it is possible that BRG1 regulates TAZ target genes and plasticity in other cell types. Whether these findings may also hold true for YAP is not yet known.

Cancer cell plasticity can also originate by epigenetic mechanisms. For instance, the chromatin remodeling factor JMJD3 binds to and deposits the active histone mark H3K27me3 on the *SNAI2* gene promoter, thereby promoting the tumor-initiating abilities of hepatocellular carcinoma cells (Tang et al., 2016). The RNA-binding protein IMP3 directly stabilizes SNAI2 transcripts, as does the deacetylase SIRT2, thereby promoting Slug protein expression and expanding TIC population in breast cancer (Samanta et al., 2016; Zhou et al., 2016). These observations suggest that activation of the EMT program in cancer cells is closely related to CSC state and increased cell plasticity in many cancer types. However, these two phenomena, although closely related, are not synonymous, and some EMT TFs promote tumor stemness independently of their effect on EMT. Supporting this notion, conditional ablation of Twist1 in benign skin tumors causes increased apoptosis, reduced cell proliferation, and defective tumor maintenance and propagation independently of Twist1's EMT function (Beck et al., 2015).

Conclusions

Development is still considered essentially hierarchical, deterministic, and in most cases, unidirectional. Cellular phenotypes are the product of discrete epigenetic configurations, or differentiation states, that have the property of metastability—they resist change except in response to some kind of signal or stimulus (Raj and van Oudenaarden, 2008). The topography of this epigenetic landscape is sculpted by a complex interplay of genetic and microenvironmental factors that conspire to generate distinct differentiation states. In modern times, we understand that differentiation states are epigenetically encoded by chromatin structure and DNA-binding transcription factors. Yet, the discovery of somatic cell plasticity in adults is an unanticipated theme of contemporary biology. The study of plasticity is gradually moving from phenomenology toward a more precise identification of the mechanisms underlying dedifferentiation and transdifferentiation (Varga and Greten, 2017).

Phenotypic plasticity relates directly to the cellular origins of cancer as well as cancer progression and therapy response. The relevant factors that dictate the switch from hierarchy to plasticity is beginning to emerge, however, a deeper understanding about the signatures and mechanisms that drive transdifferentiation or dedifferentiation transitions is needed. In addition, understanding the generation of inter- and intratumor diversity as a result of phenotypic plasticity is far from complete. Finally, it is important to determine whether phenotypic plasticity can be exploited as anticancer therapies since they may give rise to unexpected vulnerabilities that can be used to target cancer cells.

DECLARATION OF INTERESTS

P.B.G. is a founder of Naveris, Inc. and a member its management and scientific board. C.K. is a shareholder of Naveris, Inc. and a member of its scientific board of advisors.

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