EMT and back again: does cellular plasticity fuel neoplastic progression?

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Abstract
Epithelial-mesenchymal transition (EMT) is a cellular transdifferentiation program that facilitates organ morphogenesis and tissue remodeling in physiological processes such as embryonic development and wound healing. However, a similar phenotypic conversion is also detected in fibrotic diseases and neoplasia, in which it is associated with disease progression. EMT in cancer epithelial cells often appears to be an incomplete and bi-directional process. Here we discuss the phenomenon of EMT as it pertains to tumor development, focusing on exceptions to the commonly held rule that EMT promotes invasion and metastasis. We also highlight the role of the Ras-controlled signaling mediators, ERK1, ERK2 and PI3-kinase, as microenvironmental responsive regulators of EMT.

Introduction

Simple epithelia are composed of cohesive sheets of cells connected by tight junctions and polarized in an apical-basal orientation relative to an underlying basement membrane (BM) (Fig. 1). The surrounding mesenchymal cells are embedded within the interstitial extracellular matrix (ECM); lacking intercellular junctions, they manifest primarily anterior-posterior polarity[1, 2]. These structural differences are reflected in the characteristic genes each cell type expresses: epithelial cells express distinct junctional proteins like E-cadherin, and epithelial-specific cytoskeletal proteins like cytokeratins, while mesenchymal cells express N-cadherin and mesenchymal-specific vimentin[3] (Table 1).

Desmoplasia, the appearance of fibrous, mesenchymal-like tissue in the peritumor stroma is associated with poor clinical outcome[4]. Recent gene profiling experiments suggest that the presence of a mesenchymal gene signature in tumors is predictive of poor clinical outcome in colorectal, breast and ovarian cancers[5-10]. The principal cell types that contribute to the desmoplastic stromal reaction and to the mesenchymal gene signature are fibroblasts, which reside in the stroma and produce interstitial ECM molecules, and myofibroblasts, which produce growth factors, cytokines, and ECM, and which also act to contract the ECM. Myofibroblasts have long been thought to derive from fibroblasts, but recent studies show that a substantial proportion of these cells can be derived from EMT[8].

EMT involves fundamental changes in gene expression that disrupt epithelial polarity and that establish a mesenchymal phenotype with concomitant alterations in cytoskeletal organization, cell adhesion, and ECM production (Fig. 2) [1, 8, 11]. This process of phenotype transdifferentiation is well-conserved throughout the vertebrata, having emerged more than 500 million years ago [11, 12]. More recent observations have led to suggestions that EMT contributes to the phenotypic

conversions observed in tissue fibrosis [3, 13 14 15-17], chronic inflammation like that which occurs in rheumatic diseases [18], and cancer progression [1, 8, 19-23]. Several recent reviews have summarized key signaling pathways involved in EMT and have probed the link between the tumor microenvironment, fibrosis, EMT, and cancer progression[8, 24, 25]. Here, we expand upon these reviews by analyzing the mechanistic processes involved in this transdifferentiation as part of the broader function of epithelial plasticity in tumor progression. We focus on the role of Ras signaling in epithelial tissue plasticity because EMT in cancer is a dynamic and often incomplete process regulated by the microenvironment and Ras, a small GTPase, and its effector pathways, most notably ERK1, ERK2 and PI3-kinase/Akt. Surprisingly, these pathways are responsive to the microenvironment even when Ras is mutated into an activated form.

EMT in culture is part of an intrinsic epithelial tumor cell plasticity

Stoker and colleagues[26] first described EMT in cultured cancer cells as a morphological change from epithelial-like tumor cell sheets to scattered, fibroblast-like cells capable of invading the basement membrane (Fig. 3). Since these initial observations, EMT in cultured cancer cells has been characterized on the molecular level; altered expression profiles, subcellular localizations, and activity levels are now commonly used to identify EMT in culture (Table 1) [3]. EMT in culture can be either stable, i.e., the mesenchymal phenotype is sustained after the stimulus provoking the conversion is removed, or reversible, i.e., the cells revert, or undergo a mesenchymal-epithelial transition (MET), when the stimulus is removed. Experiments that quantitatively define the transient and incomplete phenotypic changes often observed in cultured tumor cells provide insight into the dynamic role EMT may play in neoplastic processes.

Insight into the complexity of EMT has been provided by studies that use gene transcription and proteomic microarrays to assess EMT and MET[27]. Additional studies suggest that the gene signatures for EMT and more generally, tumor epithelial cell plasticity are controlled by tissue and microenvironmental factors[8]. A comparison of transcriptional analyses of TGF-β1-induced EMT in EpH4 mouse mammary epithelial cells (transient EMT associated with a scattering phenotype) and EMT induced in EpH4 derivatives such as c-Fos-ER-EpH4 (stable EMT without induction of malignancy) and Ras-EpH4 (stable EMT with induction of malignancy) revealed a common EMT gene signature distinct from that associated with scattering, metastasis or oncogene expression (Fig. 4A). Furthermore, a number of the genes in this signature have been linked to poor outcome in breast cancer[27]. Intriguingly, some changes in gene expression profiles also overlap with those documented to occur during EMT conversion of the medial edge epithelial seam in the embryonic palate[28]. Figure 4 B,C shows the extent of this overlap and compares expression of genes that are upregulated by at least two-fold during EMT of EpH4 mammary cells with those altered (again by at least 2-fold) during EMT in the embryonic palate. Clearly there are similar but not identical alterations in the expression of gene sets. Overlap is also observed when the genes that are down-regulated during EMT of EpH4 cells are compared to the gene profile altered during palatogenesis: 70% of down regulated EpH4 cell EMT-specific genes are also altered by more than two-fold during palatogenesis (Fig. 4D). The similarity between these two EMT signatures may result from the important role of TGF-β1 in driving mesenchymal conversion of both EpH4 cells[27] and the embryonic palate medial ridge epithelium[28]. Even though an EMT involves acquisition of at least some mesenchymal properties, the EMT-specific gene signature of EpH4 cells shares surprisingly few gene expression changes in common with stromal signatures including the fibroblast serum response which captures genes that are commonly upregulated in fibroblasts from different tissues

following serum stimulation *in culture*, and which predicts both poor outcome in breast and other cancers and enhances the prognostic value of an "invasiveness" gene signature that predicts poor outcome in breast cancer[6, 17]. A similarly limited overlap in altered gene expression is observed between the EMT-specific EpH4 cell gene signature and tumor stromal signatures such as head and neck squamous cell carcinoma (HNSCC)[29] and prostate cancer[30] (Fig. 5). Furthermore, the EpH4 mammary cell EMT gene signature bears little resemblance to an EMT signature of HNSCC[38] (Fig. 5). Although these comparisons are limited, gene signature analysis to date suggests that EMT is a process that is distinct from metastasis or tumorigenesis per se and may be tissue and microenvironment-specific, but bears some resemblance to the embryonic process, at least when driven by a common factor such as TGF-β1.

As these and other studies illustrate, EMT in cancer is a complex process that appears to be a subset of an extensive transdifferentiation program[1, 31-35]. The dynamic nature of tumor phenotype inter-conversion is more difficult to capture *in vivo* and has rarely been directly documented. However, a conversion of breast tumor ductal epithelial cells into myoepithelial cells and myofibroblasts is suggested by both the residual expression of epithelial keratin markers in myoepithelial and myofibroblast cells and the simultaneous expression of myoepithelial (e.g. K14, K17 and vimentin) and myofibroblast (vimentin and alpha smooth muscle actin) markers[36]. Retention of some epithelial and myoepithelial markers in "transdifferentiated" myofibroblasts, as well as evidence of non-random X-chromosome inactivation patterns[36] also demonstrate epithelial plasticity towards the fibroblast phenotype. These results suggest that adult epithelial cells have a capacity to acquire aspects of a mesenchymal phenotype and vice versa in culture and in breast cancer.

Clinical significance of EMT

Although EMT has been clearly documented in cultured human cancer cell lines and in some human tumors, its prevalence in aggressive tumors and its role in clinical progression are still controversial[31, 37]. A clear demonstration of EMT in most human neoplastic disease has been compromised by the cellular heterogeneity of most human tumors and by the lack of clear markers for defining mesenchymal and epithelial tumor cells in solid tumor biopsies. Evidence that EMT may be highly localized and transient or limited to specific steps in metastatic colonization [38, 39] further complicates clinical analysis of this process. Certainly, skepticism of a clinical role for EMT in tumor progression is fueled by the rarity of morphological changes observed by pathologists in primary tumors.

Nevertheless, increased expression of accepted EMT markers has been detected at the invasive fronts of aggressive tumors[1]. There is also a large body of data that shows an association between known regulators of EMT (e.g. Snail, Twist, Slug) and both aggressive tumor behavior in animal models and poor clinical outcome in cancer patients, suggesting a role for EMT in tumor progression[1, 23, 40]. However, the pleiotropic nature of EMT regulators such as Snail, makes it difficult to determine the extent to which they are causative of EMT in human cancer[41]. One approach used to identify more diagnostic, tissue-specific EMT markers is to identify gene expression alterations associated with transdifferentiation in human tumors, animal cancer models, or cultured cells and then assess whether or not these gene signatures are correlated with clinical outcome. Such transcriptional profiling experiments resulted in the identification of a "wound" gene signature[7] that predicts poor outcome in breast cancer patients and that increases the predictive

value of other gene signatures for poor outcome in this disease. Similarly, identification of key transcriptional alterations associated with the response of human breast epithelial cells to organotypic 3D culture conditions was also predictive of outcome in breast cancer patients[42]. While the prognostic value of an EMT to breast cancer progression per se has to our knowledge not been reported, a number of genes in the EpH4 mammary cell EMT/metastatic gene signatures/mammary tumor cell signature are associated with poor prognosis in breast cancer[27]. However, comparison of the EMT-specific EpH4 mammary cell gene signature alone has limited overlap with two metastasis/invasion gene signatures that predict poor clinical outcome in breast cancer[10, 43] (Fig. 5). More in depth studies of this type may help to clarify any clinical significance of an EMT to tumor progression.

Possible functions of EMT during tumor progression

Whereas numerous studies have shown that blocking the expression or impairing the function of EMT-regulating factors blocks migration and invasion in cultured epithelial cells, invasion and metastasis of epithelial tumors can occur in the absence of any detectible EMT, and even in the presence of EMT, invasion and metastasis may not occur[1]. For example, metastasis of some bladder cancer cell lines is associated with conversion to an epithelial phenotype (MET) rather than with retention of the mesenchymal phenotype[44], while desmoid tumors are mesenchymal and locally invasive but do not metastasize[45]. EMT can be functionally uncoupled from the processes of invasion and metastasis. For example, conditional expression of TGF-β1 in mouse keratinocytes in the presence of a functional TGF-β1 receptor promoted EMT and metastasis in chemically-induced papilloma; however, expression of a dominant negative TGF-β1 receptor blocked the induction of EMT but did not influence the ability of TGF-β1 to promote metastasis[46]. Such observations suggest that the conceptualization of EMT as responsible only for increased migratory and invasive capacities may be an oversimplification.

A broader perspective of the role of EMT in cancer may be gleaned from the study of EMT in normal development. Growing evidence suggests that EMT is integral to normal tissue repair and renewal processes[47-50] and may contribute to fibrosis when these processes are sustained or otherwise aberrant [1, 8, 15, 18, 51]. As predicted by analysis of EMT gene signatures, EMT has been documented in keratinocyte migration at wound sites[52] and in response to UV irradiation[49]. EMT also occurs transiently at the tips of growing mouse mammary gland branches as they invade the fat pad during branching morphogenesis[53], a process that resembles tumor invasion into adjacent tissues. In this case, EMT-related processes are used for coordinated epithelial cell movement rather than for dissemination.

Transient EMT in cancer may provide fibroblast-like properties to tumor cells even in the absence of complete morphological alteration. Several reports suggest that conversion of non-small cell lung carcinoma (NSCLC) to a mesenchymal phenotype affects their sensitivity to mitogens and to anti-proliferative drugs. EMT in non-small cell lung cancer (NSCLC), detected by a mesenchymal gene signature, predicted loss of response to epidermal growth factor receptor (EGFR) activation and insensitivity to Erlotinib, an EGFR inhibitor[54, 55]. These studies demonstrate that EMT can affect signaling pathways that control cell viability and prompt the question of how the transient alterations associated with EMT can affect these pathways. Fibroblasts possess distinct immunomodulatory activities; by providing stromal guidance cues that permit leukocyte infiltration and retention within tissues at wound sites, by presenting antigens to the immune system, and by modifying T-cell responses, fibroblasts could act to mask tumor

antigens and to protect tumors from immune surveillance[56]. Fibroblasts produce and respond to a different set of cytokines and growth factors than epithelial cells and are more responsive to the mitogenic and motogenic effects of platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) than epithelial cells. These growth factors are abundant in the microenvironment of tissues undergoing extensive remodeling as well as in tumors, and transiently or reversibly, EMT may thus facilitate growth of epithelial tumor cells[57]. Transient EMT may also permit epithelial cells to temporarily evade the effects of growth-inhibitory factors. Using a tissue micropatterning approach, Nelson et al. showed that a transient EMT, detected by expression of green fluorescent protein (GFP) under the control of the vimentin promoter, occurred in regions of lowest concentration of the branching inhibitor TGF-β1[53]. Here, transient EMT may provide the ductal cells with a temporary release from the inhibitory growth effects of TGF-β1, and allow response to other mitogens in the microenvironment [58].

Molecular pathways that regulate EMT

Identifying the molecular pathways that regulate EMT in cancer cells has been the subject of intense investigation [1, 19, 59-64]. While the processes involved in EMT have distinct characteristics in different tissues, Ras-regulated ERK1/ERK2 and PI3-kinase signaling pathways are increasingly recognized as key mediators of some tumor cell plasticity. These pathways are commonly activated through mutations or increased expression of Ras in human tumors.

Ras proteins act as switches controlling many downstream signaling pathways and are triggered by micronenvironmental factors such as growth factors and ECM molecules[65-67]. Increased expression and/or mutation of Ras is a common early event in human tumors[61, 66]. In breast cancer, mutations that result in increased expression of Ras are more common than mutations that result in constitutive activation[68], and increased activity of the Ras-regulated downstream mediators, PI3-kinase and ERK1/ERK2, is a poor prognostic indicator[69-74]. Ras-regulated pathways can induce autonomous, stable EMT in mammary epithelial cells. For example, exposing EpH4 cells containing activating Ras mutants to TGF-β1 stimulates autocrine production of mesenchymal factors such as PDGF-A, PDGF-B, and the PDGF receptors alpha and beta, which collectively maintain EMT even when exogenous TGF-β1 is withheld[75, 76] In the absence of constitutively activated Ras signaling pathways in parental EpH4 mammary cells, TGF-β1 induces an incomplete and transient mesenchymal conversion that is reversible when TGF-β1 is removed [77, 78].

ERK1/ERK2 and PI3-kinase-regulated pathways play central roles in tumor cell EMT. PI3-kinase stimulates proliferation, blocks apoptosis, and promotes cadherin isotype switching upon exposure to interstitial collagens [60, 79]; ERK1/ERK2 promotes disassembly of adherens junctions and induces expression of mesenchymal ECM components such as tenascin-C as well as ECM-remodeling MMPs [63, 80]. Both pathways regulate Slug and Snail transcription factors, which in turn promote EMT by suppressing expression of E-cadherin, genes encoding epithelial tight junction components, and epithelial-specific cytokeratins; it is relevant to this that loss of E-cadherin induced by extracellular MMPs can induce EMT as well[81-83]. Activation of ERK1/ERK2 and PI3-kinase pathways can co-opt normally tumor suppressive effects of environmental factors such as TGF-β1, promoting growth and stabilization of EMT [84], an effect that is achieved by linking TGF-β1 receptor activity to PI3-kinase and ERK1/ERK2 signaling pathways[27, 46, 77]. Microenvironmental control of Ras, PI3-kinase, and ERK1/ERK2 mediators is controlled through alteration of integrin-responsive signaling pathways: β1-integrins modulate the activity of growth factor receptors such as EGFR and PDGFR, and these are required for maximal activation of ERK1/ERK2 and PI3-

kinase [85, 86]. Additionally, the nuclear localization of activated ERK1/ERK2, which is required for its effects on gene transcription, is regulated by hyaluronan[87]. Thus, ECM molecules in combination with growth factors present in the tumor microenvironment control the localization and activation status of these Ras-effectors thereby determining the precise effect of these pathways on tumor cell behavior and differentiation/plasticity (Fig. 6).

Conclusions

A more complete definition of how EMT contributes to cancer progression requires analysis of EMT during normal tissue renewal and development of mechanistic assays for *in situ* detection of EMT as well as continued identification of effectors of EMT that are prognostic for tumor outcome. Ras-regulated ERK1/ERK2 and PI3 kinase signaling pathways are modulated by elements of the tumor microenvironment suggesting functions beyond their well-studied roles in motility and proliferation. Another active field involves identification of EMT regulatory pathways in the context of epithelial plasticity to identify potential targets for therapy. In parallel, technical advances in accurate sampling and visualization of individual cells will enable isolation and analysis of the key regulators of tumor EMT.

Figure legends

Figure 1. Common morphological characteristics of epithelial and mesenchymal cells. Epithelial morphology is characterized by an apical-basal polarity, contact with a basal basement membrane and formation of extensive cell-cell contacts including tight junctions. Mesenchymal morphology is characterized by an anterior-posterior polarity, loose if any cell-cell junctions and residency within a more unstructured interstitial matrix.

Figure 2. EMT of mammary epithelial cells. Treatment of mouse mammary epithelial cells with MMP-3 stimulates breakdown of epithelial structure and acquisition of a mesenchymal morphology. Red, f-actin; blue, DAPI.

Figure 3. **EMT in normal morphogenesis and cancer.** EMT is part of normal branching morphogenesis in mature mammary glands. When it occurs in tumor cells, it can contribute to cancer progression**.**

Figure 4. Overlap between the EMT gene signature of EpH4 mammary cells and embryonic palate overlap. (A) A Venn diagram illustrates the overlap between the Eph4 metastasis and EMT gene signatures. These results show that EMT can be distinguished from metastasis as a molecular process. (B) A Venn diagram shows the number of EMT-specific genes that are commonly upregulated by at least two fold in both EpH4 mammary cells and embryonic palate. Both EMT processes are in response to TGFβ1. Approximately 50% of EMT-specific genes upregulated in EpH4 cells are also upregulated during EMT associated with embryonic palate morphogenesis. (C) The table shows which of the upregulated EpH4 EMT genes are also altered during EMT of the embryonic palate. Of the 21 upregulated EpH4 cell EMT genes, 10 are increased (+) in embryonic palate, 8 are not altered (-) and 3 are down regulated. (D) The table compares the EMT-specific genes in EpH4 mammary cells that are down regulated with expression changes in embryonic palate undergoing EMT.

Figure 5. Comparison of EpH4 mammary cell EMT gene signature with cancer-related gene signatures. Both upregulated and down regulated EMT-specific genes from EpH4 mammary cells were compared to gene expression profile changes during EMT of head and neck squamous cell carcinoma (HNSCC), stromal gene signatures that have prognostic value in breast and other cancers and metastasis invasion gene signatures that predict poor outcome in breast cancer. Limited overlap is seen between the EpH4 EMT-specific gene signature and these cancer-related gene signatures.

Figure 6. Microenvironmental and Spatial Regulation of Signaling Pathways Controlling EMT: The Ras-ERK1/ERK2 pathway provides an example of the key roles for microenvironment and subcellular localization in determining tumor phenotype. When activated integrins are coupled to growth factor receptors (1), farnesylated Ras (2) is transported to the cell membrane where it selectively activates MAP kinase (e.g. ERK1/ERK2) (3) and other pathways (e.g.PI3 kinase), blocks tumor suppression by TGF-β (2) and links the TGF-β pathway to ERK1/ERK2 signaling pathways (4). Activated ERK1/ERK2 must be translocated to specific subcellular sites to have access to target proteins and thereby promote EMT/invasion/progression. Steps 1-4 are reversible and have profound effects on tumor phenotype even when other pathways are mutated.

References

1. **Lee, J. M., Dedhar, S., Kalluri, R. & Thompson, E. W. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol 172, 973-81 (2006).**
2. **Valles, A. M. et al. Acidic fibroblast growth factor is a modulator of epithelial plasticity in a rat bladder carcinoma cell line. Proc Natl Acad Sci U S A 87, 1124-8 (1990).**
3. **Kalluri, R. & Neilson, E. G. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 112, 1776-84 (2003).**
4. **Desmouliere, A., Guyot, C. & Gabbiani, G. The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. Int J Dev Biol 48, 509-17 (2004).**
5. **Nuyten, D. S. et al. Predicting a local recurrence after breast-conserving therapy by gene expression profiling. Breast Cancer Res 8, R62 (2006).**
6. **Chang, H. Y. et al. Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds. PLoS Biol 2, E7 (2004).**
7. **Chang, H. Y. et al. Robustness, scalability, and integration of a wound-response gene expression signature in predicting breast cancer survival. Proc Natl Acad Sci U S A 102, 3738-43 (2005).**
8. **Radisky, D. C., Kenny, P. A. & Bissell, M. J. Fibrosis and cancer: Do myofibroblasts come also from epithelial cells via EMT? J Cell Biochem (2007).**
9. **Adler, A. S. & Chang, H. Y. From description to causality: mechanisms of gene expression signatures in cancer. Cell Cycle 5, 1148-51 (2006).**
10. **Liu, E. T., Kuznetsov, V. A. & Miller, L. D. In the pursuit of complexity: systems medicine in cancer biology. Cancer Cell 9, 245-7 (2006).**
11. **Thiery, J. P. Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol 15, 740-6 (2003).**
12. **Hay, E. D. An overview of epithelio-mesenchymal transformation. Acta Anat (Basel) 154, 8-20 (1995).**
13. **McAnulty, R. J. Fibroblasts and myofibroblasts: Their source, function and role in disease. Int J Biochem Cell Biol (2006).**
14. **Willis, B. C., duBois, R. M. & Borok, Z. Epithelial origin of myofibroblasts during fibrosis in the lung. Proc Am Thorac Soc 3, 377-82 (2006).**
15. **Neilson, E. G. Mechanisms of disease: Fibroblasts--a new look at an old problem. Nat Clin Pract Nephrol 2, 101-8 (2006).**
16. **Faulkner, J. L., Szcykalski, L. M., Springer, F. & Barnes, J. L. Origin of interstitial fibroblasts in an accelerated model of angiotensin II-induced renal fibrosis. Am J Pathol 167, 1193-205 (2005).**
17. **Liu, Y. Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention. J Am Soc Nephrol 15, 1-12 (2004).**
18. **Zvaifler, N. J. Relevance of the stroma and epithelial-mesenchymal transition (EMT) for the rheumatic diseases. Arthritis Res Ther 8, 210 (2006).**
19. **Vincent-Salomon, A. & Thiery, J. P. Host microenvironment in breast cancer development: epithelial-mesenchymal transition in breast cancer development. Breast Cancer Res 5, 101-6 (2003).**
20. **Zhang, Z., Yuan, X. M., Li, L. H. & Xie, F. P. Transdifferentiation of neoplastic cells. Med Hypotheses 57, 655-66 (2001).**

21. Katoh, M. Epithelial-mesenchymal transition in gastric cancer (Review). Int J Oncol 27, 1677-83 (2005).
22. Bates, R. C. & Mercurio, A. M. The epithelial-mesenchymal transition (EMT) and colorectal cancer progression. Cancer Biol Ther 4, 365-70 (2005).
23. Yang, J., Mani, S. A. & Weinberg, R. A. Exploring a new twist on tumor metastasis. Cancer Res 66, 4549-52 (2006).
24. Kalluri, R. & Zeisberg, M. Fibroblasts in cancer. Nat Rev Cancer 6, 392-401 (2006).
25. Thiery, J. P. & Sleeman, J. P. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 7, 131-42 (2006).
26. Stoker, M. & Perryman, M. An epithelial scatter factor released by embryo fibroblasts. J Cell Sci 77, 209-23 (1985).
27. Jechlinger, M. et al. Expression profiling of epithelial plasticity in tumor progression. Oncogene 22, 7155-69 (2003).
28. LaGamba, D., Nawshad, A. & Hay, E. D. Microarray analysis of gene expression during epithelial-mesenchymal transformation. Dev Dyn 234, 132-42 (2005).
29. Roepman, P. et al. Maintenance of head and neck tumor gene expression profiles upon lymph node metastasis. Cancer Res 66, 11110-4 (2006).
30. Bacac, M. et al. A mouse stromal response to tumor invasion predicts prostate and breast cancer patient survival. PLoS ONE 1, e32 (2006).
31. Christiansen, J. J. & Rajasekaran, A. K. Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. Cancer Res 66, 8319-26 (2006).
32. Fridriksdottir, A. J., Villadsen, R., Gudjonsson, T. & Petersen, O. W. Maintenance of cell type diversification in the human breast. J Mammary Gland Biol Neoplasia 10, 61-74 (2005).
33. Petersen, O. W. et al. The plasticity of human breast carcinoma cells is more than epithelial to mesenchymal conversion. Breast Cancer Res 3, 213-7 (2001).
34. Gudjonsson, T., Adriance, M. C., Sternlicht, M. D., Petersen, O. W. & Bissell, M. J. Myoepithelial cells: their origin and function in breast morphogenesis and neoplasia. J Mammary Gland Biol Neoplasia 10, 261-72 (2005).
35. Adriance, M. C., Inman, J. L., Petersen, O. W. & Bissell, M. J. Myoepithelial cells: good fences make good neighbors. Breast Cancer Res 7, 190-7 (2005).
36. Petersen, O. W. et al. Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. Am J Pathol 162, 391-402 (2003).
37. Tarin, D., Thompson, E. W. & Newgreen, D. F. The fallacy of epithelial mesenchymal transition in neoplasia. Cancer Res 65, 5996-6000; discussion 6000-1 (2005).
38. Brabletz, T. et al. Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin. Cells Tissues Organs 179, 56-65 (2005).
39. Spaderna, S. et al. A transient, EMT-linked loss of basement membranes indicates metastasis and poor survival in colorectal cancer. Gastroenterology 131, 830-40 (2006).
40. Chung, C. H. et al. Gene Expression Profiles Identify Epithelial-to-Mesenchymal Transition and Activation of Nuclear Factor-{kappa}B Signaling as Characteristics of a High-risk Head and Neck Squamous Cell Carcinoma. Cancer Res 66, 8210-8218 (2006).
41. Barrallo-Gimeno, A. & Nieto, M. A. The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development 132, 3151-61 (2005).

42. Fournier, M. V. et al. Gene expression signature in organized and growth-arrested mammary acini predicts good outcome in breast cancer. Cancer Res 66, 7095-102 (2006).
43. van 't Veer, L. J. et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 415, 530-6 (2002).
44. Chaffer, C. L. et al. Mesenchymal-to-epithelial transition facilitates bladder cancer metastasis: role of fibroblast growth factor receptor-2. Cancer Res 66, 11271-8 (2006).
45. Nieuwenhuis, M. H. & Vasen, H. F. Correlations between mutation site in APC and phenotype of familial adenomatous polyposis (FAP): a review of the literature. Crit Rev Oncol Hematol 61, 153-61 (2007).
46. Han, G. et al. Distinct mechanisms of TGF-beta1-mediated epithelial-to-mesenchymal transition and metastasis during skin carcinogenesis. J Clin Invest 115, 1714-23 (2005).
47. Zavadil, J. et al. Genetic programs of epithelial cell plasticity directed by transforming growth factor-beta. Proc Natl Acad Sci U S A 98, 6686-91 (2001).
48. Flanders, K. C. Smad3 as a mediator of the fibrotic response. Int J Exp Pathol 85, 47-64 (2004).
49. Hudson, L. G. et al. Ultraviolet radiation stimulates expression of Snail family transcription factors in keratinocytes. Mol Carcinog (2007).
50. Prindull, G. & Zipori, D. Environmental guidance of normal and tumor cell plasticity: epithelial mesenchymal transitions as a paradigm. Blood 103, 2892-9 (2004).
51. Rastaldi, M. P. Epithelial-mesenchymal transition and its implications for the development of renal tubulointerstitial fibrosis. J Nephrol 19, 407-12 (2006).
52. Savagner, P. et al. Developmental transcription factor slug is required for effective re-epithelialization by adult keratinocytes. J Cell Physiol 202, 858-66 (2005).
53. Nelson, C. M., Vanduijn, M. M., Inman, J. L., Fletcher, D. A. & Bissell, M. J. Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures. Science 314, 298-300 (2006).
54. Yauch, R. L. et al. Epithelial versus mesenchymal phenotype determines in vitro sensitivity and predicts clinical activity of erlotinib in lung cancer patients. Clin Cancer Res 11, 8686-98 (2005).
55. Thomson, S. et al. Epithelial to mesenchymal transition is a determinant of sensitivity of non-small-cell lung carcinoma cell lines and xenografts to epidermal growth factor receptor inhibition. Cancer Res 65, 9455-62 (2005).
56. Parsonage, G. et al. A stromal address code defined by fibroblasts. Trends Immunol 26, 150-6 (2005).
57. Hogaboam, C. M., Steinhauser, M. L., Chensue, S. W. & Kunkel, S. L. Novel roles for chemokines and fibroblasts in interstitial fibrosis. Kidney Int 54, 2152-9 (1998).
58. Strutz, F. et al. TGF-beta 1 induces proliferation in human renal fibroblasts via induction of basic fibroblast growth factor (FGF-2). Kidney Int 59, 579-92 (2001).
59. Wu, W. S. The signaling mechanism of ROS in tumor progression. Cancer Metastasis Rev 25, 695-705 (2006).
60. Larue, L. & Bellacosa, A. Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways. Oncogene 24, 7443-54 (2005).
61. Malaney, S. & Daly, R. J. The ras signaling pathway in mammary tumorigenesis and metastasis. J Mammary Gland Biol Neoplasia 6, 101-13 (2001).

62. Huber, M. A., Kraut, N. & Beug, H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol 17, 548-58 (2005).
63. Nawshad, A., Lagamba, D., Polad, A. & Hay, E. D. Transforming growth factor-beta signaling during epithelial-mesenchymal transformation: implications for embryogenesis and tumor metastasis. Cells Tissues Organs 179, 11-23 (2005).
64. Grego-Bessa, J., Diez, J., Timmerman, L. & de la Pompa, J. L. Notch and epithelial-mesenchyme transition in development and tumor progression: another turn of the screw. Cell Cycle 3, 718-21 (2004).
65. Chambard, J. C., Lefloch, R., Pouyssegur, J. & Lenormand, P. ERK implication in cell cycle regulation. Biochim Biophys Acta (2006).
66. Giehl, K. Oncogenic Ras in tumour progression and metastasis. Biol Chem 386, 193-205 (2005).
67. Nottage, M. & Siu, L. L. Rationale for Ras and raf-kinase as a target for cancer therapeutics. Curr Pharm Des 8, 2231-42 (2002).
68. Guerra, E., Vacca, G., Palombo, B. & Alberti, S. Prognostic value of mutations in TP53 and RAS genes in breast cancer. Int J Biol Markers 18, 49-53 (2003).
69. Kim, D., Cheng, G. Z., Lindsley, C. W., Yang, H. & Cheng, J. Q. Targeting the phosphatidylinositol-3 kinase/Akt pathway for the treatment of cancer. Curr Opin Investig Drugs 6, 1250-8 (2005).
70. Milde-Langosch, K. et al. Expression and prognostic relevance of activated extracellular-regulated kinases (ERK1/2) in breast cancer. Br J Cancer 92, 2206-15 (2005).
71. Gee, J. M., Robertson, J. F., Ellis, I. O. & Nicholson, R. I. Phosphorylation of ERK1/2 mitogen-activated protein kinase is associated with poor response to anti-hormonal therapy and decreased patient survival in clinical breast cancer. Int J Cancer 95, 247-54 (2001).
72. Gee, J. M., Barroso, A. F., Ellis, I. O., Robertson, J. F. & Nicholson, R. I. Biological and clinical associations of c-jun activation in human breast cancer. Int J Cancer 89, 177-86 (2000).
73. Janes, P. W., Daly, R. J., deFazio, A. & Sutherland, R. L. Activation of the Ras signalling pathway in human breast cancer cells overexpressing erbB-2. Oncogene 9, 3601-8 (1994).
74. Nakopoulou, L. et al. Effect of different ERK2 protein localizations on prognosis of patients with invasive breast carcinoma. Apmis 113, 693-701 (2005).
75. Jechlinger, M. et al. Autocrine PDGFR signaling promotes mammary cancer metastasis. J Clin Invest 116, 1561-70 (2006).
76. Gotzmann, J. et al. A crucial function of PDGF in TGF-beta-mediated cancer progression of hepatocytes. Oncogene 25, 3170-85 (2006).
77. Janda, E. et al. Ras and TGF[beta] cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. J Cell Biol 156, 299-313 (2002).
78. Grunert, S., Jechlinger, M. & Beug, H. Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis. Nat Rev Mol Cell Biol 4, 657-65 (2003).
79. Shintani, Y., Wheelock, M. J. & Johnson, K. R. Phosphoinositide-3 kinase-Rac1-c-Jun NH2-terminal kinase signaling mediates collagen I-induced cell scattering and up-regulation of N-cadherin expression in mouse mammary epithelial cells. Mol Biol Cell 17, 2963-75 (2006).

80. Zavadil, J. & Bottinger, E. P. TGF-beta and epithelial-to-mesenchymal transitions. Oncogene 24, 5764-74 (2005).
81. Lochter, A. et al. Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. J Cell Biol 139, 1861-72 (1997).
82. Lochter, A. et al. Misregulation of stromelysin-1 expression in mouse mammary tumor cells accompanies acquisition of stromelysin-1-dependent invasive properties. J Biol Chem 272, 5007-15 (1997).
83. Radisky, D. C. et al. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436, 123-7 (2005).
84. Savagner, P. Leaving the neighborhood: molecular mechanisms involved during epithelial-mesenchymal transition. Bioessays 23, 912-23 (2001).
85. Tibbles, L. A. & Woodgett, J. R. The stress-activated protein kinase pathways. Cell Mol Life Sci 55, 1230-54 (1999).
86. Scaltriti, M. & Baselga, J. The epidermal growth factor receptor pathway: a model for targeted therapy. Clin Cancer Res 12, 5268-72 (2006).
87. Tolg, C. et al. Rhamm-/- fibroblasts are defective in CD44-mediated ERK1,2 motogenic signaling, leading to defective skin wound repair. J Cell Biol 175, 1017-28 (2006).

Table 1. Commonly used markers of EMT *in culture*

Increased Expression ***Mesenchymal Markers***	**Decreased Expression** ***Epithelial Markers***	**Altered Location/Activity** ***Signaling factors***
Alpha smooth muscle actin	epithelial cytokeratins (e.g. CK-8, CK-18, CK-19)	β- Catenin
Vimentin	E-cadherin	Starch
Thrombospondin	Occludin	Snail
Fibronectin	Desmoplakin	Slug
N-cadherin	Mucin-1	Twist
Tenascin C	Zo-1	Goosecoid
STRO-1		FOXC2
FSP-1		Sox 10
Vitronectin		NFKB
Collagen I		Smad-2,3
Collagen III		ERK 1,2
PAI-1		PI-3 Kinase/AKT
TGF-β-1		
FGF-1,2,8		
MMP-2		
MMP-9		

Figure 1. Common morphological characteristics of epithelial and mesenchymal cells

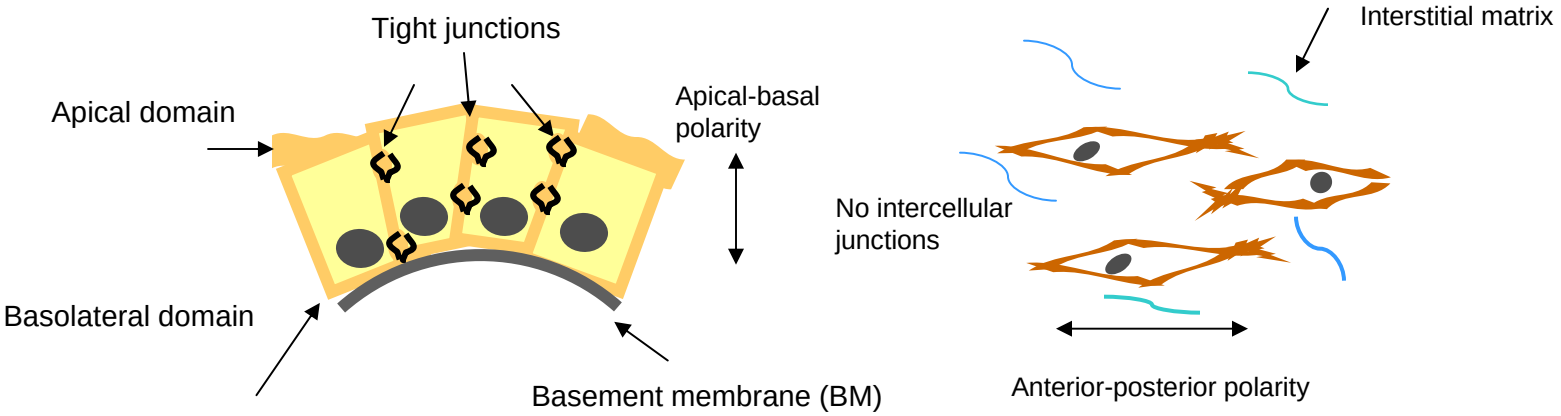


Figure 2. EMT of mammary epithelial cells.

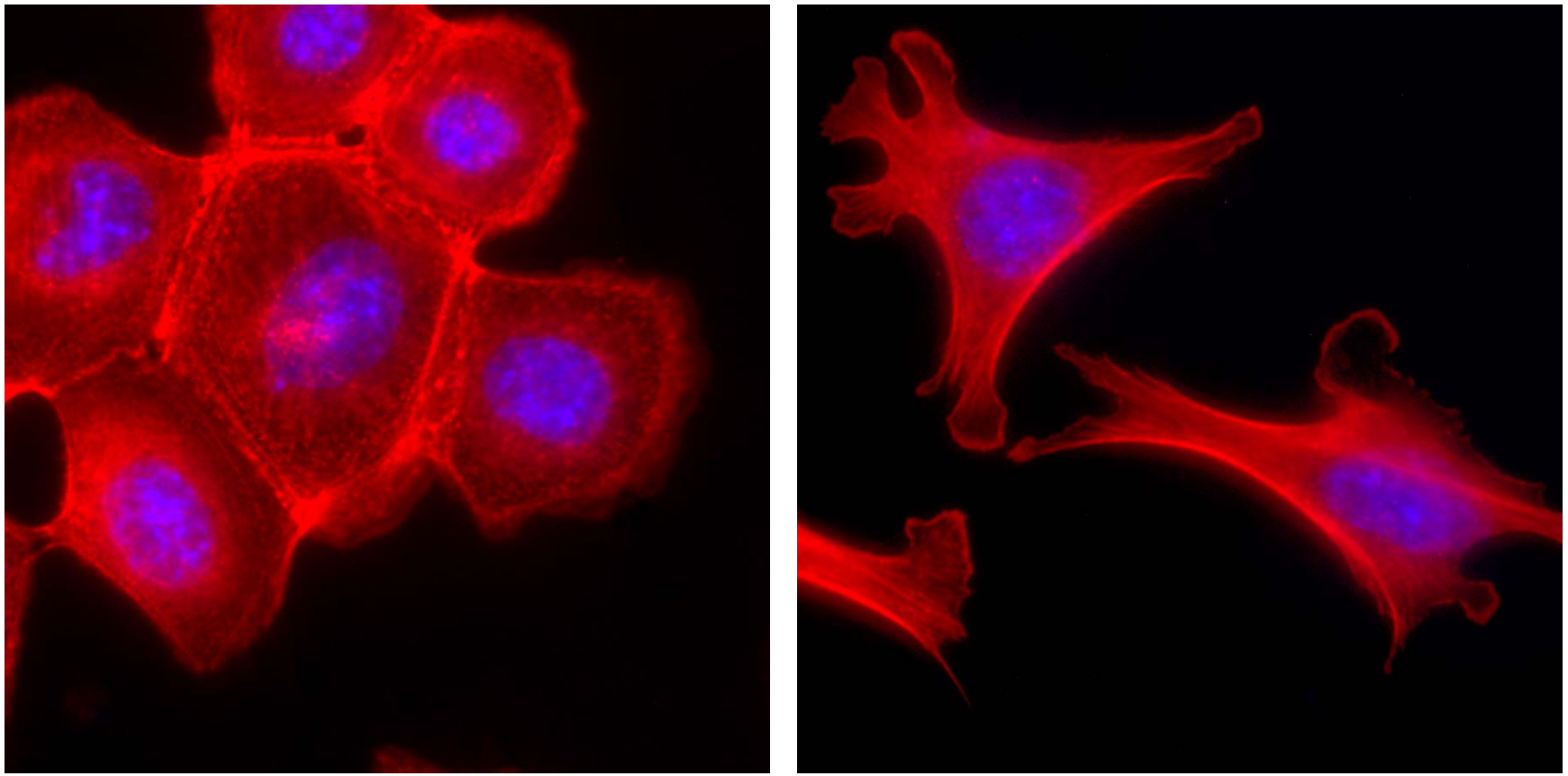


Figure 3. EMT in normal morphogenesis and cancer.

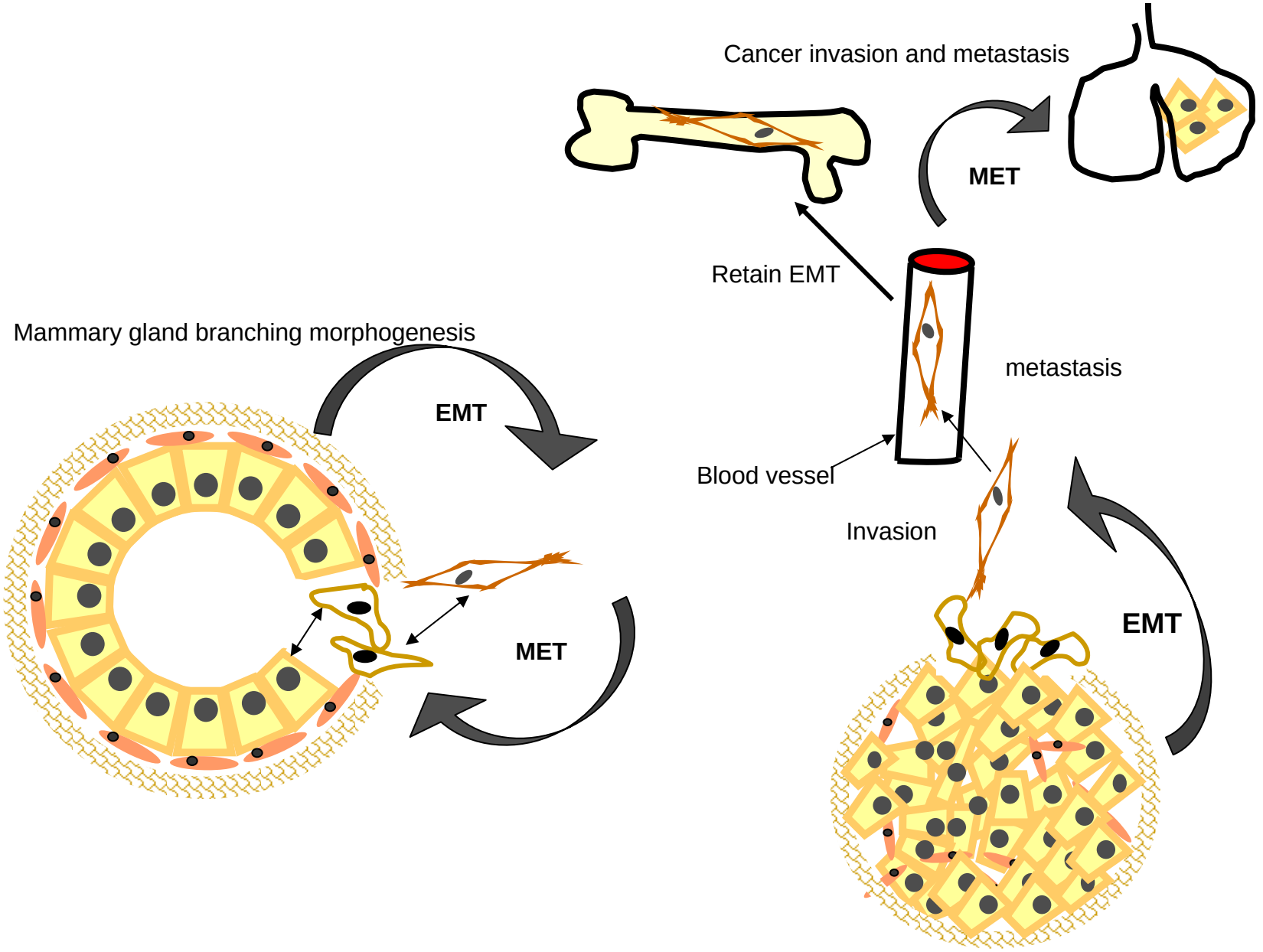
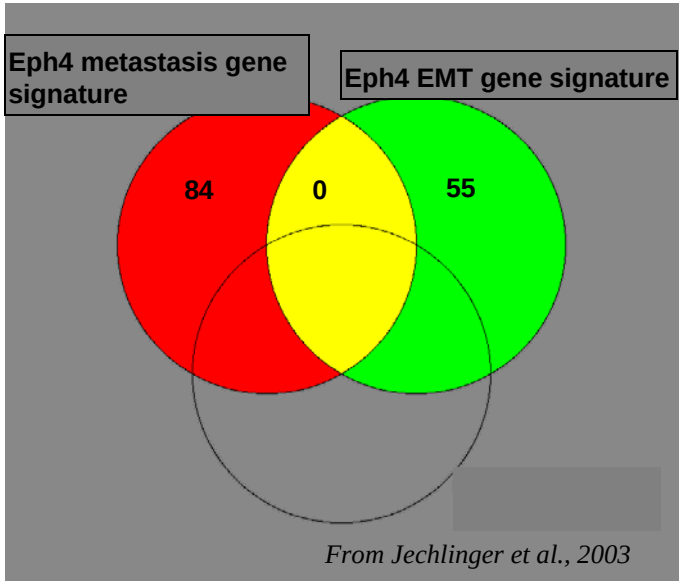
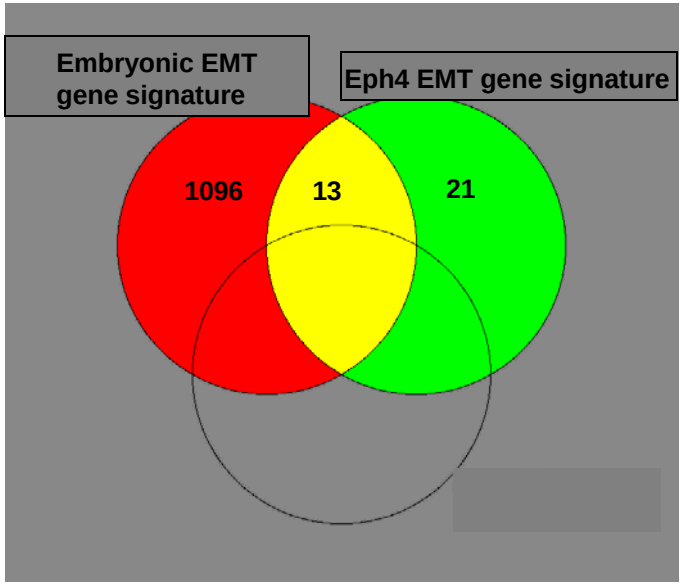


Figure 4. Overlap between the EMT gene signature of EpH4 mammary cells and embryonic palate

A.



B.



C.

EMT gene unicode	Eph4 cells	Embryonic palate
Atf1	+	+
Creg	+	+
F2r	+	↓
Dpys13	+	-
Dab2ip	+	↓
Eng	+	+
Gas1	+	+
Hmox1	+	-
Rpl7a	+	+
Hexb	+	+
Mt1	+	-
Mt2	+	+
Ppic	+	+
Pxmp3	+	+
Pcolce	+	-
Col6a1	+	+
Col6a2	+	↓
Raew07	+	-
Mcp1	+	-
Vdlr	+	-
Zfhxla	+	-

Data in B,C are from Jenchlinger et al., 2003 and LaGamba et al., 2005

Figure 4 cont **D.**

Unicode of downregulated EMT genes in EpH4 cells	embryonic palate
Ap1b1	↓
Actn4	↓
Abcf2	↓
Chka	↓
Cldn4	↓
Ddb1	+
Fln	+
Flii	-
Ier5	+
Gspt1	↓
Hsp110	-
Hnrpdl	+
Irf3	↓
Junb	↓
Jup	↓
Klf5	-
Lamb3	+
Lisch7	↓
Mkrn3	+
Msh2	+
Atp1a3	-
Nasp	-
Pctp	+
Pkp1	+
Pou2fl	-
Arhgef1	↓
Spint2	+
Slc9a9	-
Supt6h	↓
Top2a	-
Sgtb	-
Tgm2	+
Usp5	↓
Hiplr	-

Figure 5. Comparison of EpH4 mammary cell EMT gene signature with cancer-related gene signatures

Gene signature	Eph4 cell EMT gene unicode
<u>EMT signatures</u> HNSCC *Chung et al., 2006*	0
<u>Stromal signatures</u>	
fibroblast serum response (human) *Chang et al, 2004*	Fl n, Mt1, Top2a
Stromal signature of HNSCC (human) *Roepman et al., 2006*	Pcolce
Stromal response of prostate Cancer (mouse) *Bacac et al, 2006*	Mt2
<u>Metastasis/Invasion signatures for breast cancer</u>	
70 gene prognostic signature *Van 't Veer et al., 2002*	Mcp1, Mt1,2
"invasiveness" gene sigature *Liu et al., 2007*	Ier5, Fln

Figure 6. Microenvironmental and spatial regulation of signaling pathways controlling EMT