Environmental guidance of normal and tumor cell plasticity: epithelial mesenchymal transitions as a paradigm

Gregor Prindull and Dov Zipori

Epithelial mesenchymal transitions are a remarkable example of cellular plasticity. These transitions are the hallmark of embryonic development, are pivotal in cancer progression, and seem to occur infrequently in adult organisms. The reduced incidence of transitions in the adult could result from restrictive functions of the microenvironment that stabilizes adult cell phenotypes and prevents plastic behavior. Multipotential progenitor cells exhibiting a mesenchymal phenotype have been derived from various adult tissues. The ability of these cells to differentiate into all germ layer cell types, raises the question as to whether mesenchymal epithelial transitions occur in the adult organism more frequently than presently appreciated. A series of cytokines are known to promote the transitions between epithelium and mesenchyme. Moreover, several transcription factors and other intracellular regulator molecules have been conclusively shown to mediate these transitions. However, the exact molecular basis of these transitions is yet to be resolved. The identification of the restrictive mechanisms that prevent cellular transitions in adult organisms, which seem to be unleashed in cancerous tissues, may lead to the development of tools for therapeutic tissue repair and effective tumor suppression. (Blood. 2004;103:2892-2899)

© 2004 by The American Society of Hematology

Introduction

Cell fate is determined by a variety of factors that control gene transcription/silencing during embryonic development and in normal adult physiology. Traditionally, cell commitment to differentiation has been viewed as consisting of a series of irreversible steps. A descending hierarchy of diminishing capacities of differentiation is begun with the totipotent embryonic stem cell, capable of giving rise to all cells of the embryo. Embryonic stem (ES) cells that originate from the inner cell mass (ICM) were considered pluripotent. In the first step of differentiation, at compaction, embryonic cells lose their ability to differentiate into cells of the placenta but maintain their capacity to differentiate into any cell of the embryonic organism plus a few extra-embryonic cell types. During subsequent stages of organogenesis, the potency of differentiation is further reduced, coupled with a diminished proliferative capacity. Recent experiments have shown that this traditional one-way street of progressive commitment to differentiation may not be irreversible. A recent speculative hypothesis for hematopoiesis suggests that stem cell systems may not be hierarchical but rather show considerable degrees of plasticity. Changes in functional stages may depend on cell cycle transit with gene expression to vary widely, depending on shifting chromatin constellations. Stem cells appear to continuously change surface receptor expression and, thus, respond differently to external stimuli at different points of the cell cycle. Nuclear transfer experiments have shown that fully differentiated nuclei of mature adult cells are converted into ES-like nuclei, with corresponding pluripotency, by cytoplasmic factors of an oocyte. Furthermore, as suggested by a multitude of publications, adult stem cells are far more plastic than previously thought (reviewed in Blau et al) and may undergo transdifferentiation.

To indicate just a few examples, neural stem cells generate blood and skeletal muscle and contribute to several embryonic tissues on implantation into blastocysts. Hematopoietic stem cells (HSCs) give rise to epithelium and mesangial kidney cells, whereas multipotent adult stem cells from the dermis give rise to neurons, glia, smooth muscle, and adipocytes, and bone marrow–derived cells turn into hepatocytes (Figure 1). Some of these reports lack a final proof that transition among lineages has, in fact, occurred at a single cell level. It has not been excluded, beyond reasonable doubt, that stem cells of various lineages are present within the same tissue. Indeed, the muscle seems to contain a variety of cell types, including a population of muscle-specific stem cells, and other stem cells that have hematopoietic potency that seem to be of bone marrow origin and do not have significant myogenic activity. However, an additional recent study suggests that muscle-derived stem cells (MDSCs) transdifferentiate into hematopoietic stem cells but nonetheless retain their myogenic potential. The study of Krause et al demonstrated the transition of rare bone marrow seeking HSCs into epithelium, but other investigators were unable to repeat these results and suggested that HSCs are not capable of significant nonhematopoietic transdifferentiation. In addition, recent studies show that the ability of bone marrow–derived cells to turn into hepatocytes, that was interpreted as representing a process of transdifferentiation, is in fact due to cell fusion. These discrepancies should be resolved by further experiments. Yet, stem cells, other than HSCs, are found in the bone marrow. One type is the mesenchymal stem cell (MSC), known for its ability to differentiate into mesoderm-derived tissues such as muscle, cardiomyocytes, bone, cartilage, and fat. Recent studies indicated that a population of such stem cells, termed multilineage adult progenitor cells (MAPCs), may be derived from...
and accumulation, or block differentiation of cells, which otherwise could disrupt tissue integrity. This review is not intended to resolve the heated debate over the extent of plasticity of stem cells. Rather, we aim to discuss, in detail, one aspect of plastic cell behavior, namely, the process of epithelial-mesenchymal transition (EMT), which is a hallmark of development. EMT, and the reciprocal phenomenon of mesenchymal-epithelial transition (MET), both entail drastic phenotypic and functional changes. Thus, an immobile epithelial cell, bound by cadherin bridges to adjacent cells, becomes a mobile, fibroblast-like mesenchymal cell that loses its cell-cell contacts, in EMT, whereas the reverse occurs during MET (Figure 2). In both EMT and MET, plasticity is indisputable in cases wherein these processes are observed at a single cell level. We shall highlight the dominant role of the microenvironment in the regulation of these processes and argue that in the adult organism there are tighter restrictions on such plasticity than during embryonic development, making it a less frequent phenomenon. Nevertheless, phenotypic plasticity, in regard to the capacity to switch lineage and direction of differentiation, is apparently an inherent property of cells, which they never totally lose. It is the task of future research to discover the molecular basis of the restrictive mechanisms that attenuate the plastic behavior of cells, so that plasticity could be harnessed effectively for medical uses.

Epithelial-mesenchymal transitions at the cellular level

EMT profoundly affects major cell properties. This includes transcriptional down-regulation of epithelial cell markers and up-regulation of mesenchymal markers, including cell motility and mesenchymal molecules such as fibronectin, stromelysin-1 (a matrix metalloproteinase), collagen I, vimentin, and tenascin. Vimentin is a marker of highly motile stromal fibroblasts and, of late, stage metastatic progression. Additional mesenchymal criteria of embryonic cell plasticity include reorganization of the cytoskeleton and degradation of basal lamina. Conversely, in MET, epithelial cell markers, including E-cadherin, a homophilic cell-cell adhesion molecule, are up-regulated, and the cytoskeleton with fibroblast-like actin fibers reorganize. EMT occurs in diverse steps of normal embryonic development, as well as in carcinogenesis (described in "Epithelial-mesenchymal transitions in tumorogenesis"). During embryonic development, epithelial-mesenchymal interactions are intimate. EMT is part of the formation of the extra-embryonic parietal endoderm, the ICM, branching morphogenesis of lungs, kidneys, and mammary gland epithelium. Because, initially, the ICM consists exclusively of epithelial cells, generation of the primary ICM mesenchyme must be initiated, in a subpopulation of these cells, by alterations of gene expression. These changes consist in changes in adhesion bindings of epithelial...
cells to the extracellular matrix (ECM) and to neighboring cells, and in the interruption of direct intercellular information by an exchange of trafficking molecules through gap junctions36-38 (reviewed in Mercer39 and Kidder and Winterhager40). Embryonic cells in transition to mesenchymal cells develop cytoplasmic actin-based machinery for invasion of, and migration through, the ECM. During migration, they receive specific, variable information from the ECM that, in turn, modify by secreting factors.

The primary mesenchyme constitutes the earliest, motile form of the ICM stroma.31 It has been suggested that in the course of further embryonic development, “oscillations” occur between EMT and MET, namely, between the “stable” epithelia and an “unstable” mesenchyme with plastic and exploratory capacities32 (Figure 2). In fact, EMT and MET appear to be reversible processes,33 even in neoplastic cell growth.34 Primary mesenchyme appears to be converted into secondary epithelia which, in turn, give rise to secondary mesenchyme and tertiary epithelial structures, eg, in the urogenital tract. The ability to go through cycles of epithelial and mesenchymal states makes the mesoderm highly plastic and versatile, properties that are major contributors of development of complex organisms32 (Figure 2). An embryonic carcinoma model of EMT indicates that this process may be steered by extracellular factors, including parathyroid hormone-related peptides.35 All vertebrates use MET in embryonic somitogenesis for the generation of segmental plates. The best known example is the conversion of metanephrogenic tissue into excretory tubular epithelium in kidney development, but it also takes place in the ontogeny of the gastrointestinal tract, the lungs, and skin.36 In the placenta, the extravillous cytotrophoblast undergoes EMT, losing some of its epithelial features (E-cadherin, integrin α6β4) but retaining others (eg, cytokeratins) (reviewed in Vicovac and Aplin40). Mesenchymal cells that do not regain an epithelial state through MET differentiate and give rise to muscle, bone, nerve, or connective tissue (reviewed in Perez-Pomares and Munoz-Chapuli41) and form the stromal microenvironment of the hematopoietic organs, and other tissues.

Plastic transitions between cell types, which are fundamental in development, may also occur in the adult organism. It has been suggested that EMT is a feature of pathologic disease states. In biopsy material, progressive stages of EMT have been described in different renal diseases.37 In chronic renal interstitial fibrosis, transforming growth factor (TGF) β1 induced EMT with increased matrix mobility and invasive capacities.38 In adult organisms, processes of angiogenesis entail many traits of cellular transitions and are similarly regulated by common cytokines (reviewed in Perez-Pomares and Munoz-Chapuli41). A recent study, aimed at determining whether tissue repair involves lineage switches, shows that single spinal cord cells turned into muscle or cartilage during amphibian tail regeneration.39 In view of the proposed plasticity of adult mesenchymal stem cells, one should consider the possibility that tissue damage in the adult, such as following wounding, is followed by differentiation and transdifferentiation of mesenchymal stem cells. We propose to revisit wound healing and other regenerative processes, bearing in mind the possible contribution of the mesenchyme to epithelial organization by MET.

The dramatic changes in cell phenotypes, which occur during embryogenesis, and possibly also in the adult organism, in the course of EMT and MET, are induced by environmental factors, the nature of which is discussed in the next section.

### Molecular basis of epithelial-mesenchymal transitions

Communication among neighboring cells, and between cells and their microenvironment, are fundamental requirements for a synchronized development and correct differentiation. The microenvironment is the major driving force in embryogenesis, already in the ICM. Functional coordination, timing and synchronization, and spatial segregation of embryonic cells are mediated by the ECM, cytokines,40,41 adhesion molecules, membrane receptors, and cellular junctions (reviewed in Kidder and Winterhager40 and Fleming et al42). Rearrangement of the ECM occurs by necessity in EMT and MET. The role of the microenvironment in determining gene transcription has been dramatically demonstrated by injection of differentiated somatic epidermal cells43,44 and human adult multipotent stem cells45 into blastocysts. The injected cells acquired lineage differentiation characteristics in accordance with the respective organs in the recipient embryo.

As mentioned earlier, E-cadherin contacts between epithelial cells are lost during EMT. E-cadherin junctional adhesion complexes form multicellular aggregates in normal morphogenesis and tumor invasion. E-cadherin particularly localizes at cell-cell boundary regions and is regulated by integrins.45,46 It increases greatly in concentration during development from the 2 cell embryo to the blastocyst. In murine E-cadherin−/− embryos, maternal E-cadherins seem to suffice quantitatively for initial compaction of the morula. However, when additional, embryo-synthesized cadherin is required for further development, morphologic polarization of adhering cells disintegrates.47 Because E-cadherin stabilizes cell adhesions and inhibits cell migration, down-regulation and transcriptional loss of this adhesion molecule is a major mechanism in EMT for both embryonic mesoderm formation and tumor progression. As an early step in EMT, E-cadherin–mediated adhesions of epithelial cells are loosened to allow for mesenchymal motility. The component of cell-cell junctions, p120 catenin, may participate in regulation of cell motility by being a link between the formation and disruption of cadherin–mediated contacts.48 E-cadherin also has growth-suppressive properties. In tissues lacking E-cadherin, other members of the cadherin family may assume a similar role. The cell surface receptor tyrosine kinases, ephrins, reversibly signal cell boundaries in developmental cell recognition, synergize with E-cadherin, and, thus, may likewise be involved in EMT.49 Integrins50 have an important role in EMT because they mediate intercellular communication by binding to the ECM and signaling information from the ECM, through an extensive network of cytoskeletal molecules.

Wnt glycoproteins are a family of secreted signaling molecules that may promote stem cell renewal, act as hematopoietic growth factors, when abnormally activated they contribute to carcinogenesis51 (reviewed in Behrens52), and have a major instructive role in development. Kidney organogenesis initiates with the condensation of mesenchymal cells and aggregation into a pretubular adenoid stage.53,54 Fibroblast growth factor (FGF) signaling participates in patterning of mesoderm at gastulation, through activation of fibroblast growth factor receptor 1 (FGFR1), leading to EMT and morphogenesis of mesoderm at the primitive
streak. This is mediated through β-catenin, thus forming a link between FGF and Wnt signaling.56 In addition, Wnt signaling also occurs independently of β-catenin action.57

TGFβ1 is a potent inducer of EMT.33,58 Various members of this superfamily are involved in the regulation of development and in EMT. Additional growth factors are major regulators of embryonic development (reviewed in Diaz-Cueto and Gerton59 and Hardy and Spanos60): FGF and hepatocyte growth factor (HGF) are ligands for tyrosine-kinase receptors. FGFR1 orchestrates EMT and the corresponding mesodermal morphogenesis by controlling Snail and E-cadherin expression and, probably, also Brachury and Tbx6. In FGFR−/− embryos, Wnt3a signaling is attenuated but can be rescued by lowering E-cadherin levels.56 HGF is a potent modulator of EMT. It activates the Rho guanosine triphosphatases (GTPases) Cdc42 and Rac, leading to activation of p21-activated kinase (PAK).61 Cell motility and branching morphogenesis in EMT are also promoted by members of the EGF-CFC gene family, in human and mouse, and are associated with a decrease in β-catenin and an increased vimentin expression. EGF-CFC activates the ras/raf/mitogen-activated protein kinase (MAPK) signaling pathways in mammary epithelial cells. Cripto-1, a member of the EGF-CFC family, is active, e.g., in the embryogenesis of ICM. It also enhances migration and branching morphogenesis of mouse mammary epithelial cells to undergo EMT.62 Abrogation of the Cripto gene leads to abnormal mesenchymal development, including failure of gastrulation.63

An extensively studied example of MET is normal nephron development during conversion of the metanephric mesenchyme to an epithelial phenotype (reviewed in Davies64). One of the activator molecules of MET may be the high mobility group 17 (HMG-17) protein that is strongly expressed in the uretic bud where kidney cells start to undergo MET during organogenesis.65 Cells preparing for MET in the ectoderm of the segmental plate express EphA4, a member of the ephrin family, which seems to regulate the process of reorganization of the cytoskeleton. Loss of EphA4 leads to failure of somite formation and irregular kidney morphology.66 In kidney tubulogenesis, MET is critically dependent on the expression of the developmental control gene Pax-2 which, in turn, is regulated by TGFβ.67 In metanephric conversion, alterations of gene transcription have been identified in a multitude of mRNA molecules. Some examples are cell adhesion molecules syndecan-4 and integrins α6, α3, β1; the cytokine TGFβ, epidermal growth factor (EGF), FGF, bone morphogenetic protein 2 (BMP2), BMP4, and Neuregulin (Neu)-differentiation factor (NDF); and among transcription factors, β-catenin.68,69,70

The various molecules discussed earlier, including ECM components, cell surface cytokines, and soluble cytokines that mediate EMT and MET, are available both in the embryo and in adult organisms. Nevertheless, in the adult, the frequency of cellular transitions is very low, suggesting that adult cells either lose their plasticity or that this property stays intact but is suppressed. We argue that the experimental data available to date imply this latter possibility. Indeed, adult tissues contain plastic stem cells, which were identified only following in vitro culture, namely, as a result of destruction of tissue architecture, or following radiation or chemical induced tissue damage in vivo. Furthermore, the plastic nature of cells may be unmasked in the situation of tissue damage as a result of disease70 or wounding.71

Expression of E-cadherin is essential for the maintenance of the epithelial phenotype. Silencing of E-cadherin expression, which is the basis for EMT, involves transcriptional repression. Several factors have been shown to transcriptionally repress E-cadhrin by binding to proximal E-boxes in the E-cadherin promoter. These include Snail and Slug, members of a zinc finger transcription factor family, as well as E47, ZEB-1, and ZEB-2.72,73 Snail and Slug are highly homologous. However, they differ in the intermediate P-S rich protein region that contains a 29-amino-acid sequence termed the Slug domain. Whereas the role of Snail in EMT is indisputable, some conflicting information is available, as for the role of Slug. The latter triggers EMT during chick and Xenopus development, whereas in mammalian cells Slug expression is not always associated with EMT. Nevertheless, recent studies show that Snail and Slug are functionally equivalent, when examined in the epithelial cell line Madin-Darby canine kidney (MDCK), in contributing to EMT and in maintaining the mesenchymal phenotype.74 Studies in Xenopus further show that Slug lies upstream of Slug in the cascade leading to neural crest formation; thus, these proteins may have distinct functions.75 This view is supported by the distinct expression patterns of these proteins in development and during evolution.76 There are also differences in the consequences of knockout of these genes; Snail−/− embryos form abnormal mesoderm germ layer, are defective in gastrulation, and die early in gestation.77 Mice, mutant for Slug, are viable but show growth retardation.82

Human breast cancer is associated with elevated expression of the Erb/Neu receptor tyrosine kinase, which is, in fact, a prognostic factor indicative of increased progression into invasive and highly malignant phenotype. Overexpression of constitutively activated Erb/Neu, in MDCK epithelial cells, causes dissociation of cell-cell contacts and increased motility. In a three-dimensional collagen matrix system activated Erb/Neu endowed the cells with a capacity to form tubules.83

HGF, mentioned earlier as a potent inducer of EMT, operates through binding to a tyrosine kinase receptor, Met. c-Cbl is a downstream target to the Met receptor. Overexpression of c-Cbl in MDCK cells causes flattening of colonies formed in vitro, but no overt EMT occurs. However, cells overexpressing a naturally occurring mutant of Cbl, termed 70z-Cbl, undergo EMT.84 c-Cbl contains multiple protein interaction motifs and serves as a docking site for many proteins, including Crk. Indeed, HGF stimulation causes recruitment of Crk adaptor proteins into Met-dependent signaling complexes. In the absence of HGF stimulation, overexpression of Crk is sufficient to induce EMT.85

Members of the Myb family of transcription factors appear to oppose EMT. They promote E-cadherin and integrin-mediated cell adhesion to extracellular fiber molecules. Myb proteins also contribute to maintenance of the state of differentiation of progenitor cells and are involved in apoptosis. Not surprisingly, ICM of B-Myb−/− blastocysts have severely impaired ES cell proliferation.86

The traditional concept of cell differentiation would predict that in epithelial cells, genes encoding for mesenchymal properties are silenced and become reactivated during EMT. At the same time, genes encoding for epithelial features should be silenced. Although this view is supported by studies of major epithelial markers, such as E-cadherin, as discussed earlier, this may not be the case for many cellular proteins, neither in EMT and MET, nor in the case of plastic behavior of stem cells, in general. Indeed, mesenchymal cells have been shown to express, at a low level, T-cell receptor (TCR) complex genes,57 and mesenchymal stem cells express a whole range of markers of lineages into which they potentially may differentiate.58 Similar promiscuity in gene expression has been demonstrated for HSCs.89 Furthermore, muscle-derived stem cells are able to differentiate into hematopoietic progeny while retaining their myogenic potential.90 It should be considered, therefore, that a
various lineages, within the normal cell population, are in a “standby” state, in which they express low levels of transcripts of different lineages and would readily differentiate on triggering, without the requirement of reversal of gene silencing. This promiscuous gene expression pattern may be the molecular basis for cell plasticity and transdifferentiation and may explain the relative ease of cell phenotype reprogramming. Indeed, it has been shown that it is sufficient to overexpress one single transcription factor, in order to shift completely the direction of differentiation of HSCs.

Epithelial-mesenchymal transitions in tumorigenesis

EMT is believed to be activated during the later stages of tumor progression but does not seem to play a major role in the initiation of neoplasms. Phenotypic EMT may represent an early indication that a cell no longer recognizes or respects its neighbors, is no longer subject to contact inhibition and coordinating synergism, and may serve as a vehicle for tumor dissemination (Figure 3). Some of the factors promoting EMT are briefly mentioned later. By contrast to the role of E-cadherin, in maintaining the epithelial phenotype, forced expression of N-cadherin leads to EMT. The extracellular repeat 4 portion of N-cadherin was shown to be necessary and sufficient to promote EMT and cell motility. The transcription factor HMG-Y, which belongs to the HMGI(Y) family of nonhistone chromatin proteins, seems to be associated with EMT in metastatic tumor progression of human breast epithelial cells.

Snail triggers EMT-associated tumor progression by virtue of its strong suppressor effects on E-cadherin gene expression. Epithelial cells, ectopically expressing snail, adopt a fibroblast phenotype and acquire tumorigenic and invasive properties. E-cadherin-negative oral squamous cell carcinomas strongly express Snail. Clinically, Snail is expressed in all ductal carcinomas with lymph node metastases, including breast tumors. The intensity of Snail expression correlates inversely with the degree of differentiation. Because β-catenin is an E-cadherin–associated protein and a key component of the Wnt signaling pathway, it is not surprising that mutated β-catenin is a pathogenic factor in the transition of premalignant to neoplastic cancer and in invasion and metastasis in several types of cancers (reviewed in Reya et al). Target genes of β-catenin include matrix metalloproteinase 7 (MMP-7), urokinase-type plasminogen activator (uPA), as well as the cell adhesion molecule NrCAM. During promotion of EMT in several premalignant and malignant human carcinomas, members of the EGF/CFC gene family, such as Cripto, suppress β-catenin function. Expression of Cripto is increased several-fold in EMT, not only in human tumors such as colon, gastric, pancreas, lung, and breast carcinoma, but also is detectable already in premalignant lesions of some of these neoplasms (reviewed in Saloman et al). This last observation raises doubts as to whether EMT is indeed only a late event in the course of carcinogenesis. In fact, it has now been shown that cells may leave the local tumor and disseminate to distant sites were they eventually form metastasis, early on, before tumor progression in the primary site has been completed.

Although TGFβ is a major tumor suppressor cytokine during early stages of tumor development, blocking cell cycle progression and initial cell growth, many dedifferentiated late-stage tumors are resistant to this function of TGFβ. They may secrete TGFβ and retain their susceptibility to TGFβ–induced EMT. TGFβ indeed promotes EMT in late stage tumors. Of possible clinical significance is a dominant-negative type II receptor of TGFβ (TGFβ–RIIΔn) that prevents EMT in EpRas cells. This receptor mutant abolishes completely the production of metastases in dedifferentiated, highly metastatic mouse colon carcinoma cells (CT26). In vitro treatment of several human carcinoma cell lines with TGFβ-neutralizing antibody resulted in loss of invasiveness. The capacity of TGFβ to mediate EMT is dependent on cooperation with Ras signaling; Ha-Ras and TGFβ cause sustained EMT and metastatic phenotype. This entails activation of Raf/MAPK. However, phosphatidylinositol 3-kinase (PI3K) activation has a complementary function in promoting, eg, proliferation. Elevated H-ras expression induces nuclear accumulation of Smad-2 which promotes EMT and subsequent invasiveness and metastatic dissemination.

EMT may be a reversible phenomenon, even in aggressive tumor cell lines such as small cell lung cancer, if the cells are allowed to re-establish E-cadherin–mediated adhesion contacts. Transfection with E-cadherin cDNA has a stabilizing effect, impairing the capacity of forming metastases in breast cancer cells. Exposure of small cell lung carcinoma to 5-bromodeoxyuridine leads to reversal, back to a normal cell phenotype, with re-establishment of cell-cell and cell-to-matrix adhesions, down-regulation of vimentin, up-regulation of cytokeratin, and redistribution of the actin cytoskeleton. The elevated degrees of cell plasticity, common to both embryonal development and tumor progression, suggest that a principle of tumor biology could be recapitulation of stages of embryonal development. A comparison between Snail-induced E-cadherin repression in undifferentiated mesoderm and in tissues undergoing EMT, in early mouse development versus tumor cell lines, lead to the conclusion that “the same molecules are used to trigger epithelial-mesenchymal transition during embryonic development and in tumor progression.” Wilms tumor is a good example for comparable developments in embryonal organogenesis and tumorigenesis. These tumors overexpress genes corresponding to the earliest stages of metanephric development and underexpress genes of the later stages, thus mimicking MET. In fact, it has been suggested that dysregulation of MET could be a major pathogenic factor in the pathogenesis of Wilms tumor.

It is possible that the tumor stroma contains mesenchymally transformed epithelial cancer cells, generated by EMT, that participate with the physiologic stroma cells in creating a new tumor’s microenvironment. This issue should be examined along with the possibility that the tumor mesenchyme might be modified such that it loses some of its “normal” features or, alternatively, that it acquires new, “transformation” related properties. Although EMT seems to be closely associated with, and probably is a basic mechanism in, cancer progression, more detailed information is...
needed on the properties of EMT-transformed epithelial cells and on the sequence of steps that take place during cancer progression.

**Summary and future perspectives**

During development, cells respond to microenvironmental factors by specific phenotypic alterations that underlay EMT and MET. These dramatic changes, and the subsequent lineage commitments and formation of the variety of cell types found in metazoa, are the ultimate form of cell “plasticity.” Recent studies seem to propose that such plastic capabilities may not be restricted to the embryo but rather are shared by adult stem cells. Clearly, this issue must be examined with caution, bearing in mind the limitations of the experimental systems used to demonstrate stem cell plasticity and the fact that regeneration of the liver, claimed to result from transdifferentiation of HSCs, has now been shown to result mainly from cell fusion. However, even in this case, the fusion between HSC and hepatocytes results in the reprogramming of the hemopoietic genome, re-emphasizing the plasticity of HSC phenotype. We reviewed the above-mentioned data that indicate that manifestations of cell plasticity are inherent to developmental embryonic processes. In the adult organism, plasticity may be less frequent and, thus, may be subject to tighter restrictions (Figure 4).

However, is it biologically conceivable that this property is completely disposed of by the adult organism? Amphibians have kept remarkable regenerative capacity that entails plasticity. On amputation of a limb or tail in urodele amphibians, a cohort of genes are coexpressed leading to a process of dedifferentiation, changes in cell fates and eventually to the formation of a new organ which is a replica of the lost one. We propose that similar basic rules may apply to any type of regenerative process in the adult organism, namely, phenotypic plasticity is an inherent property of cells which they never lose completely. Indeed, transdifferentiation of pancreas to liver and vice versa has been observed in vivo, in animal models, and in certain human diseases and was reproduced in vitro (reviewed in Shen et al). The recent identification of MAPCs point to a possible mechanism for tissue repair through transdifferentiation; resident MAPCs may be engaged in tissue maintenance by repairing micro-defects, because of cell death, that sporadically occur in healthy tissues. The discovery of MAPCs raises further intriguing possibilities: Is the capacity of MAPCs to give rise to epithelial cells mediated through molecular mechanisms of MET? And conversely, do these cells initially exist in the organism as epithelial cells and convert to MAPCs through EMT?

The gradual reduction in plastic behavior of cells after completion of development suggests, to our minds, the existence of restraining mechanisms that prevent the expression of this inherent cellular property, to ensure the stability of already formed tissues and organs. Such restrictive mode of cell organization has been proposed to account for events within the hemopoietic microenvironment. The blood-generating tissue remains in a highly dynamic form, throughout lifespan, a state that has similarities to embryonic development, and is accordingly carefully controlled. The “restrictive model of cell organization” suggests that the microenvironment imposes restrictions of cell growth and differentiation. The exact localization of cells relative to their environment has a critical role in the determination of cell fate. One outstanding example is the importance of orientation in Drosophila male germ line stem cell positioning. These stem cells are arranged oriented to the Hub, somatic cells that form the “stromal” niche and elaborate renewal signals. Dividing stem cells orient the mitotic spindles perpendicular to the hub. As a result, one of the daughter cells stays in touch with the hub and continues to self renew, while the other is pushed away from the niche and subsequently differentiates. Similarly, the mammalian skin shows a clear organization wherein stem cells are localized adjacent to mesenchyme, separated by basement membrane. Their differentiation commences as they depart to distal layers. A similar effect of physical distance of the stem cells from its stromal niche has been suggested for bone marrow stem cells.

Apparently, the adult state, in which the restrictions on epithelial mesenchymal transitions are overtly lifted, is cancer. EMT is a frequent phenomenon in cancer progression and dissemination. No qualitative differences in signaling from the microenvironment have been identified with certainty to date, as a cause of cancer progression. However, it has been suggested that the stroma may transform adjacent epithelial cells, in the absence of pre-existing tumor cells (reviewed in Tlsty and Hein). Also, some data do suggest, in particular tumors, that the microenvironment may be altered. We suggest that such microenvironmental changes may exist and should be experimentally tested. Better understanding of restrictive mechanisms that control tissue organization will provide molecular tools to relieve tissue restrictions in the adult, to allow for regeneration when needed. Conversely, in tumor states one would wish to block further cancerous development by re-establishing the lost tissue restrictions.

**Acknowledgments**

D. Z. is an incumbent of the Joe and Celia Weinstein professorial chair at the Weizmann Institute of Science. G. P. is a Varon Visiting Professor from the University of Gottingen, Germany. We thank Prof Alexander Bershadsky, from the Department of Molecular Cell Biology, Weizmann Institute of Science, for critically reviewing the manuscript.
References


Tanaka EM. Regeneration: if they can do it, why can't we? Cell. 2003;113:559-562.


Environmental guidance of normal and tumor cell plasticity: epithelial mesenchymal transitions as a paradigm

Gregor Prindull and Dov Zipori