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Definitions and Guidelines for Research on Epithelial-Mesenchymal Transition

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On behalf of The EMT International Association (TEMTIA)

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Abstract:

Epithelial-Mesenchymal Transition (EMT) encompasses dynamic changes in cellular organization between epithelial and mesenchymal phenotypes, which leads to functional changes in cell migration and invasion. A conserved set of inducing signals, transcriptional regulators, and downstream effectors of EMT has been implicated in a diverse range of physiological and pathological EMT events. Rapid expansion in EMT-oriented research is highlighted by over five thousand publications indexed by Web of Science in 2018 alone, warranting consensus-building among researchers for clarity in EMT nomenclature and guidelines for EMT research. Mediated by "The EMT International Association" (TEMTIA), this white paper is the outcome of a two year-long discussion among EMT researchers and aims to provide definitions and guidelines for EMT research in future publications. We trust that this white paper will help to reduce misunderstanding and misinterpretation of research data generated in various experimental models and promote fruitful cross-disciplinary conversation and collaboration through the identification of key questions arising in this field. While recognizing the importance of maintaining diversity in experimental approaches and conceptual frameworks, we emphasize that lasting contributions of EMT research to life sciences, especially in understanding developmental processes and in combatting cancer and other diseases, rests on adoption of a unified terminology to describe EMT, which this white paper strives to achieve.

1. Introduction

Epithelial-Mesenchymal Transition (EMT) is a cellular process during which epithelial cells downregulate their epithelial features and acquire mesenchymal phenotypes and behavior. EMT is triggered in response to signals that cells receive from their microenvironment. The epithelial state of the cells in which EMT is initiated is characterized by epithelial cell-cell junctions, apical-basal

polarity, and interactions with basement membrane. During EMT, these epithelial characteristics become repressed, and cells acquire mesenchymal characteristics due to changes in gene expression and post-translational regulation, displaying a fibroblast-like morphology and cytoarchitecture as well as increased migratory capacity. Furthermore, these now migratory cells often acquire invasive properties.

EMT was first defined as a coherent process via research on early embryogenesis [1,2]. It is now recognized to occur normally during early embryonic development to enable a variety of morphogenetic events as well as later in development and in the adult during wound healing. Moreover, EMT is activated during cancer pathogenesis and tissue fibrosis. The reverse process, known as Mesenchymal-Epithelial Transition (MET), also occurs frequently during development. A salient characteristic of EMT is that during the course of both developmental and pathological EMTs occurring *in vivo*, the transition from an epithelial to a mesenchymal state is often incomplete, resulting in cells that reside in intermediate states that retain both epithelial and mesenchymal characteristics. Of note, these intermediate states can be diverse depending on the biological context [3].

The EMT research field has grown explosively over the last 20 years. More than half of all publications on EMT have appeared in the last five years (Fig. 1), and half of those address EMT in the context of cancer biology. The growing complexity and diversity of the EMT literature has resulted in the currently diffuse definitions of EMT and associated nomenclature. Thus, cell biologists have traditionally focused on shifts in the microscopically visible, profound changes in cell-cell interactions, cell motility, cytoskeletal organization, cell proliferation, and resistance to various stressors. Molecular biologists have focused on EMT-associated transcription factors (EMT-TFs) and how they act, often involving various chromatin modifications to orchestrate changes in EMT-associated gene expression.

Cancer biologists often emphasize the acquisition of various malignancy-associated cell phenotypes, notably invasiveness, dissemination, and the effects of cell responsiveness to various therapeutic modalities. These diverse portrayals of EMT do not reflect current experimental shortcomings. Rather, as we learn more about EMT-associated changes, the recognition of diversity in EMT phenotypic manifestations requires a more encompassing view of EMT, rather than a single narrow definition of this complex cell biological program.

2. Purpose of this Consensus Statement

The use of the term EMT in diverse areas covering developmental biology, cell biology, tissue homeostasis, and disease (notably cancer and fibrosis) has created discrepancies in data interpretation and persistent disagreement [4-9], largely because the plasticity and heterogeneity of EMT programs have been insufficiently considered. EMT was originally described as an important process in embryonic development during which epithelial cells underwent a phenotypic transformation to mesenchymal cells. Early analyses and validations in cell culture focused on the phenotypic changes using a limited set of molecular markers. However, the identification of EMT as a crucial program in cancer initiation and progression indicated that EMT involves more than the original developmental EMT programs and that different variations of the EMT-program clearly exist.

As the complexity of EMTs and EMT regulators in cancer becomes increasingly appreciated, there is a need for the community of EMT researchers to agree on a number of key issues. These include proper description of EMT-related phenomena, definitions of major EMT-related terms, diverse versions of EMT and their context-dependent regulation by EMT regulators and the relationship between core and non-core EMT functions of EMT-TFs; in the context of cancer pathogenesis, the contributions of

genetic alterations, the complex input of the changing tumor environment, and EMT-like changes in non-epithelial cancers, such as melanoma, sarcoma and leukemia must also be considered. Provoked by a passionate town hall discussion in its 2017 meeting, the EMT International Association (TEMTIA; https://temtia.org) proposes the following guidelines to define the EMT program, its phenotypic plasticity, and the resulting multiple intermediate epithelial/mesenchymal states. By building such a consensus on EMT-related concepts, we aim to eliminate semantic problems in the EMT debate and facilitate genuine cross-disciplinary discussion on the roles of EMT in normal developmental and pathological conditions.

3. A brief history of EMT

Modern EMT studies began with research aimed at understanding tissue morphogenesis during development, cell behavior in culture, and carcinoma invasiveness in cancer progression. Elizabeth Hay recognized the importance of EMT in embryogenesis [1] and began to discuss the concept of "epithelial-mesenchymal transformation" in the late 1970s. The EMT concept was examined subsequently in the context of neural crest formation [10,11], heart valve formation [12] and Müllerian duct regression [13], and in epithelial tissue explants in vitro [14]. This "epithelial-mesenchymal transformation" process was alternately referred to as "epithelial-mesenchymal transition" to distinguish it from the process of neoplastic transformation used by the cancer community. "Epithelial-mesenchymal transition" became the consensus usage after the 1st TEMTIA meeting that brought the field together in 2003.

A variety of observations originating more than a quarter century ago described EMT programs induced by a diverse array of contextual signals. For example, cultured amnion cells were "transformed" from an epithelial phenotype into fibroblast-like cells in response to leukocyte medium

[15]. Endocardial cells underwent EMT in response to signals from adjacent cardiac muscle [16]. Hepatocyte Growth Factor (HGF) was found to induce transformation of epithelial cells into migratory fibroblasts [17]. Fibroblast growth factor 1 (FGF1) induced an "epithelial plasticity" response in bladder carcinoma [18], connecting EMT to cancer. TGF-β, overexpressed in cancers and required for cardiac EMT [19], was shown to act as a potent EMT inducer in cultured cells [20]. These observations provided the first indications that diverse extracellular signals, including soluble factors and, quite possibly, components of the extracellular matrix (ECM) could act together to evoke EMT programs in responding cells.

These early descriptive studies shed little light on the mechanisms operating within individual cells that enable induction of EMT programs. Discoveries in the field of *Drosophila* developmental genetics revealed the identities of master regulators of these programs, such as the transcription factors Snail and Twist, which act pleiotropically to orchestrate mesoderm formation during gastrulation [21]. The involvement of related transcription factors in chordates testified to the high degree of conservation of these factors during the course of metazoan evolution, and thus the utility of using various developmental models to study EMT regulation in human cells.

Research aimed at identifying molecular regulators of EMT began on a large scale in the 1990s. For example, the identification of the Snail-related transcription factor Slug (Snai2) as an inducer of EMT during chick gastrulation and neural crest cell formation illustrated that specific transcription factors (EMT-TFs) can act as key upstream regulators of EMT [22]. The finding that Slug expression can convert epithelial carcinoma cells into mesenchymal derivatives strengthened the connection between embryonic EMT and cancer progression [23]. This notion was reinforced by the observation that the Snail family transcription factors are capable of inducing EMT and invasiveness in normal epithelial

cells, in part through transcriptional repression of the gene encoding E-cadherin [24-27]. Additional EMT-TFs, notably E47, Twist1, Zeb1 and Zeb2, were identified through their ability to evoke morphological and molecular changes associated with EMT [28-31]. It is important to note that these EMT-TFs usually co-operate with one another to orchestrate EMT programs. Extensive studies also reveal that various upstream EMT -inducing signals could impinge on these EMT-TFs, doing so via transcriptional and post-transcriptional regulation.

As a better understanding of the inductive signals and transcriptional control of EMT evolved, **it** became apparent that activation and execution of EMT programs do not require changes in DNA sequence and can be reversible. This made it clear, in turn, that EMTs represent complex epigenetic regulatory programs, much like those operative during many steps of development. During development, some cell populations may undergo multiple rounds of EMT and MET, indicating substantial phenotypic plasticity. For example, during renal morphogenesis, the epithelial cells lining renal vesicles are derived from renal mesenchymal cells via MET, while these mesenchymal cells are descendants of epithelial cells in the epiblast via EMT [32]. During somite formation, paraxial mesenchyme cells undergo MET to form epithelial somites, which in turn undergo EMT to give rise to the sclerotome [33]. Likewise, during pathogenesis of cancers and fibrosis, EMT programs are activated to various extents and are often reversible, revealing a plasticity that can yield cells residing in a spectrum of phenotypic states between the fully epithelial and fully mesenchymal endpoints of this program. This issue presents a major challenge to the EMT research community: How to best capture the diversity and plasticity of EMT programs operating in various biological contexts.

4. EMT in development, cancer, and fibrosis

A driving paradigm for the growth of this research field is that EMT operates in normal tissues during development and wound healing, but is also a driver in the pathogenesis of cancer and fibrosis. The common starting point of diverse EMTs is a downregulation of certain epithelial phenotypes. Importantly, however, the EMT programs do not operate as binary switches that shunt cells from fully epithelial to fully mesenchymal extremes. Instead, cells activate EMT programs to differing extents, yielding cells that land at various stopping points along the E-to-M phenotypic spectrum. At present, it remains unclear whether there are discrete phenotypic states arrayed along this spectrum or, alternatively, a continuum of such states lacking distinct, definable boundaries. The extent to which such intermediates represent stable states in specific biological contexts is also unclear. A continuum of EMT intermediate states may enable rapid interconversion between cells possessing various combinations of these traits, a process viewed as having high phenotypic plasticity. Additionally, it is likely that the phenotypic states between the fully epithelial and fully mesenchymal endpoints cannot be arrayed along a linear spectrum, and that multiple alternative paths can operate to enable an epithelial cell to advance toward a mesenchymal state. Finally, cells activating EMT programs in adult tissues under pathological conditions commonly express combinations of epithelial and mesenchymal markers and rarely complete the entire EMT program, suggesting that "partial EMTs" represent the norm rather than the exception.

Development: During animal development, cells of epithelial origin often migrate long distances from their points of origin to their final destinations. These migrations depend on cells transitioning to a more mesenchymal state through EMT in order to enable migration, and in many cases the resulting cells then undergo permanent or temporary reversal to an epithelial state through MET (e.g**.,** endoderm cells) or switch to another state (e.g**.,** neural crest). A great degree of morphological variability is associated with epithelial cells that participate in developmental EMTs, ranging from cells possessing fully formed epithelial cell-cell junctions and an underlying basement membrane, such as the

pluripotent epiblast cells of amniotes[34], to the primitive epithelial cells giving rise to mesendoderm in *Xenopus* and fish, which exhibit only apical-basal polarity and incompletely assembled junctions[35]. In many cases, the quasi-mesenchymal state is not reached through a complete loss of cell-cell junctions, but instead by changes in the nature and dynamics of junction formation and dissolution, which may explain how cells with mesenchymal characteristics can exhibit collective cell migration[36], i.e. the migration of cohorts of cells that appear to be held together by various types of cell-cell junctions. Such plastic, quasi-mesenchymal phenotypes are observed in cells that migrate collectively and form cadherin-based cell-cell contacts, as seen in *Drosophila*, zebrafish, *Xenopus* and in mouse endoderm and mesoderm cells[37-42], neural crest cells of zebrafish, chick, and *Xenopus*[43,44], and cells that migrate while forming only transient cell-cell contacts, such as germ cells of zebrafish[45]. It is important to note that not every migratory process employed by epithelial cells involves EMT, such as chicken epiblast morphogenesis before the formation of the primitive streak [46].

Cancer: During the multi-step progression of carcinomas, initially benign, epithelial cells acquire certain distinctly mesenchymal traits and thereby develop the ability to invade locally and disseminate to distant tissues. Much of this phenotypic progression depends on the activation of EMT programs [3,47-51]. Carcinoma cells may be able to perform collective migration locally without activating EMT programs, possibly using collective migration mechanisms similar to those used during development. However, it is unclear whether primary carcinoma cells can complete the entire process of metastatic dissemination without activating, at least transiently, components of the EMT program. The residence of individual carcinoma cells in intermediate E/M states (i.e., partial EMTs) echoes the behavior of epithelial cells during normal development. In this way, cancer cells proceed through a gradation of phenotypic states, each associated with combinations of epithelial and mesenchymal markers [3,51,52].

Activation of alternative EMT programs and progression of individual cells to different states along the E-to-M spectrum can generate extensive phenotypic heterogeneity within tumors. Supporting this notion, multiple E/M subpopulations with distinct chromatin landscapes and gene expression signatures have been reported in skin and mammary primary tumors, and these subpopulations are often spatially localized within a tumor [53]. Moreover, hybrid E/M states are enriched in the circulating tumors cells (CTCs) released by primary and disseminated breast and lung cancers [54,55], ostensibly reflecting the cellular heterogeneity seen within corresponding primary tumors. Such phenotypic plasticity and heterogeneity may provide cancer cells with substantial adaptability, enabling them to respond to a variety of external cues and physiologic stresses [3,49,51,52,56,57]. Thus, since tumor cells encounter diverse microenvironments as they navigate the multiple steps of the metastatic cascade, various intermediate/hybrid E/M phenotypes may be favored and better adapted for survival in these distinct environments, such as blood and lymphatic vessels and primary and secondary tumor sites. The tissue-of-origin of the tumor cell, specific combinations of expressed EMT-TFs, and modifications of chromatin may also determine the phenotypic heterogeneity arising from residence in various hybrid E/M states.

The diversity of EMT-associated cancer cell phenotypes is reflected in discrepancies in experimental and histopathological observations of human tumors, fueling a long-standing debate regarding the role(s) of EMT in cancer progression [4,5,58-60]. Such differences are often due to the use of different EMT-markers and the analysis of particular EMT-TFs as markers of this program; in addition, the EMTs operating in different tissues may differ from one another. For instance, Snai1 and Twist1 were both shown to be important for metastasis in the PyMT-driven breast cancer model [61,62], but dispensable for metastasis in a pancreatic cancer model [7], which instead depends on Zeb1 [63]. Further complexity comes from the observation that carcinoma cells undergoing a partial EMT can shed their

epithelial phenotype through post-translational mechanisms [64], making it challenging to interpret studies that rely solely on the perturbation of transcriptomes by EMT-TFs. These and other examples indicate that the version of EMT programs and the functions of involved EMT-TFs are tissue context-dependent. Moreover, as often seen during the course of embryonic development, cancer-associated EMT is likewise only activated partially and transiently, making end-stage markers of a fully mesenchymal state uninformative in cancer studies. Further complicating the situation is the fact that EMT programs have been linked to additional traits, possibly yielding traits that are not associated with canonical EMT regulation, such as the stemness, survival, metabolic changes, and, in the case of cancer, resistance to anti-cancer therapeutic drugs [65,66].

Fibrosis: EMT has also been observed to occur and play a role in diverse types of fibrosis (including in the lung, liver and kidney) with EMT-TF expression shown to be a pre-requisite for fibrosis development/progression in mouse models [3]. As in cancer progression, the role of EMT in organ fibrosis has been the subject of active debate. A central issue in this debate has been the origin of the myofibroblasts that accumulate in fibrotic tissues. These cells represent a specialized fibroblast population involved in collagen secretion and thus the development and progression of interstitial fibrosis that lies at the heart of this disease. Early lineage tracing studies supported the EMT-driven conversion [67], but subsequent tracing analyses do not provide compelling evidence of epithelial cells as precursors of fibrosis-associated myofibroblasts [68]. More recent studies show that renal epithelial cells undergo a partial EMT that is crucial for disease progression but do not directly contribute to the formation of the myofibroblast population [68,69]. Instead, they lose their normal tubular function, and these damaged cells relay paracrine signals to the interstitium, reshaping the microenvironment through the release of TGF-β that converts existing fibroblasts into myofibroblasts,and the secretion of additional cytokines and chemokines that are likely to recruit macrophages to the stroma. Hence, damaged renal epithelial cells promote both fibrogenesis and inflammation, these representing

hallmarks of renal fibrosis [69,70]. While the debate concerning the contribution of EMT to different types of fibrosis will likely continue, the demonstrated requirement of EMT-TF expression strongly suggests that activation of EMT is indeed required for the development of several types of fibrosis.

5. Definitions of EMT and its associated terms

To facilitate investigation of multifaceted EMT processes and discussion among diverse groups of EMT researchers, we propose the following definitions of EMT and its associated terms to stand as a reference.

a) EMT (epithelial-mesenchymal transition)

A multifaceted and often reversible change in cellular phenotypes during which epithelial cells lose their apical-basal polarity, modulate their cytoskeleton and exhibit reduced cell-cell adhesive properties. Cells may individually or collectively acquire mesenchymal features and increase motility and invasive ability. Typically, a switch in intermediate filament usage from cytokeratins to vimentin is observed after a complete EMT. Cortical actin filament in epithelial cells also undergoes marked rearrangement during EMT. While characteristics of fully epithelial cells are relatively clearly defined, our current knowledge does not allow us to define the mesenchymal state with specific cellular characteristic or molecular markers that are universal end-products of all EMT programs.

b) MET (mesenchymal-epithelial transition)

Reciprocal changes in cellular phenotype that reverse EMT-induced phenotypes, during which mesenchymal-like cells may acquire apical-basal polarity, re-organize their cytoskeleton, and exhibit

increased cell-cell adhesion, resulting in an organized epithelium. MET occurs during embryonic development (e.g., cardiac development, kidney morphogenesis, somite formation) and cancer.

c) EndoMT (endothelial-mesenchymal transition)

Similarly to epithelial cells, endothelial integrity depends on cell-cell junctions, apical-basal polarity and interactions with an underlying basement membrane. Endothelial-mesenchymal transition (EndoMT) more accurately indicates the affected cell population, and resembles EMT in most aspects except for the replacement of E-cadherin by VE-cadherin. EndoMTthereby enables endothelial cells to attenuate or deconstruct their functional integrity and apical-basal polarity, to acquire motile and invasive behavior, and to activate changes in gene expression that are driven by certain EMT-TFs. Similar to EMT in epithelial cells, endothelial cells that have activated EndoMT programs exhibit a variety of intermediate or partial phenotypes, as discussed above for EMT. EndoMT was initially described during embryonic heart development [71] and subsequently in the context of cardiac fibrosis [72].

d) EMP (epithelial-mesenchymal plasticity)

We favor the use of EMP to describe the ability of cells to adopt mixed E/M features and to interconvert between intermediate E/M phenotypic states arrayed along the epithelial-mesenchymal spectrum that cannot be easily distinguished based on our current understanding. This plasticity has been variably referred to as partial EMT, hybrid E/M status, metastable EMT state, EMT continuum, and EMT spectrum, in all of which cells express a mixture of epithelial (e.g., cytokeratins) and mesenchymal (e.g., migration) features and markers. EMP indicates an ability to move readily between these various states, while the stability of these various states varies in different biological contexts. EMP is widely observed in development, wound healing, and cancer. In addition to a mesenchymal-type of migration, as observed during mesoderm formation, EMP can also participate in collective

migration, e.g., during tubulogenesis and wound healing. EMP also accounts for the reversibility of the EMT program. Epithelial cells going through EMT give rise to cell populations that may enter reversibly into states with various proportions of epithelial and mesenchymal features. EMP is thought to provide cells the fitness and flexibility to fulfill the diverse requirements during the course of both developmental and pathological processes.

e) Definition of EMT transcription factors

In many if not most settings, both in cell culture and *in vivo*, EMP involves some degree of transcriptional regulation. Several transcription factors (TFs) belonging to the Snail, Twist and Zeb families have been found to control cell-cell adhesion, migration and ECM degradation, and to play evolutionally conserved central roles in the operations of EMTs observed in various biological settings in various organisms (Table 1). In fact, all of the developmental EMT processes described to date involve at least one member of these families of core EMT-TFs.

Yet other TFs have been shown to affect EMT in certain contexts (Supplemental Table 1). However, these transcription factors are also involved in other cellular processes (e.g., proliferation, apoptosis, stemness). Typically, these responses involve regulation of cellular fate, survival and dynamics. In addition, many of the EMT-TFs are also expressed in non-epithelial cells, ranging from fibroblasts to hematopoietic precursors**,** and in cancer types involving non-epithelial derivatives (melanoma, glioblastoma, leukemia), where they play important roles during tumor progression, often beyond classic EMT phases. While we use EMT-TFs to describe all transcription factors associated with EMT, it is important to keep in mind that their expression alone does not indicate an EMT process.

6. Recommendations on the features that define the core EMT program

6.1: EMT status cannot be assessed based on one or a small number of molecular markers.

EMT represents changes of cell behavior involving loss of certain epithelial characteristics and gain of certain mesenchymal traits. The complex series of cell-biological changes occurring during EMTs require the cooperation of a large number of molecular factors. Based on their involvement in the process, these factors can be divided into three groups: EMT-inducing signals, EMT-associated transcription factors (EMT-TFs), and EMT markers that define and create various epithelial and mesenchymal cell characteristics. In the literature, diverse cellular and molecular descriptors have been used to define EMT in various biological systems, representing a major source of confusion. For example, some studies define partial loss of E-cadherin as an indication of EMT, while others argue that the maintenance of certain levels of expression of epithelial markers, such as cytokeratins, is indicative of cells not having undergone EMT. Given the complex manifestations of the EMT program, it has become clear that conclusions concerning the actions of EMT cannot rely solely on a few salient molecular markers, such as E-cadherin and vimentin [73].

More importantly, the use of various EMT molecular markers to characterize the phenotypic state of individual tumor cells has revealed that such cells, as described earlier, can simultaneously express both epithelial and mesenchymal genes. The core EMT-TFs are often co-expressed in various combinations in order to orchestrate complex EMT programs and involve various members of EMT-TF families, such as Snai1 vs. Snai2, Zeb1 vs. Zeb2, depending on specific biological context [74]. Importantly also, post-transcriptional regulation of EMT regulators at both the mRNA and protein levels is critical in controlling the EMT outputs. Such regulation is often neglected in studies that use RNA expression exclusively to survey EMT molecular markers. A focus on defining EMT programs based exclusively on the expression of specific molecular markers such as these underrepresents the

enormous complexity and plasticity of the EMT programs in diverse developmental and pathological settings.

6.2: Primary criteria for defining the EMT status should be changes in cellular properties together with a set of molecular markers, rather than relying solely on molecular markers.

One major feature that unites all variant EMT programs is the initial attenuation or deconstruction, to various degrees and with diverse manifestations, of the epithelial phenotype. Epithelial cells harbor complexes mediating cell-cell interactions, most notably adherens junctions, tight junctions and desmosomes. Apical-basal polarity guides the proper organization of tight junctions, adherens junctions and desmosomes in epithelial cells. The polarity complexes, including the Par, Crumbs, and Scribble complexes[75-77], define the apical vs. basal-lateral domains of an epithelial cell. During the early phase of EMT, loss of apical-basal polarity is often the first event to be observed and can lead to the destabilization of adhesion complexes, such as the tight junctions and adherens junctions at the lateral membrane [78,79] , as well as activation of EMT-TFs [80]. The decrease or loss of epithelial adherens junctions and desmosomes occurs via transcriptional repression by the core EMT-TFs of genes encoding junction proteins. . The cytoplasmic relocalization of adherens junction proteins, such as E-cadherin, via post-transcriptional regulation is also an early feature of EMT initiation in various EMT models [81].

Another key function of the EMT programs is to provide stationary epithelial cells with the ability to migrate by invading through extracellular matrices secreted by both epithelial and mesenchymal cells. Thus, during EMT, epithelial cells often need to breach the basement membrane in order to migrate away from their epithelium of origin [34,82]. Migration of cells that have undergone EMT does not

necessarily require cells to lose all epithelial features, and a switch of intermediate filaments from cytokeratin to vimentin can facilitate cell migration. Depending on the extent of cell-cell adhesion loss, epithelial cells can migrate as single cells in a mesenchymal manner or collectively while remaining attached with one another via weakened but still operative cell-cell interactions. This indicates that the breakdown of these tightly regulated epithelial structures, the gain of motility and the ability to degrade ECM during EMT cannot simply be represented by the absence or presence of the expression of selected markers. Furthermore, complex post-translational modifications of key proteins play critical roles in governing these complex processes. For these reasons, EMT or MET events should be described as functional changes of the cell-biological properties rather than focusing largely on changes of a few readily monitored molecular markers. It is with this perspective that our understanding of EMT could faithfully reflect the functional purpose of EMT during developmental and pathological events (Fig. 2). Therefore, whenever it is experimentally feasible, EMT should be assessed by cellular properties together with multiple molecular markers.

6.3: EMT-TFs and other molecular markers are valuable indicators of EMT, but should be assessed in conjunction with changes in cellular characteristics to define EMT.

The morphological and functional changes during EMT are often the result from changes in gene expression. Many, but not all, changes in EMT gene expression result directly or indirectly from actions of EMT-TFs, which play key roles in driving EMT programs. Indeed, most EMT programs are associated with activation of expression of one or several core EMT-TFs. While core EMT-TFs often initiate EMT-associated changes in gene expression (Table 1), a large number of other EMT-TFs and numerous microRNAs and lncRNAs have also been shown to contribute to or play critical roles in diverse EMTs (Supplementary Table 1). Decreased association between β-catenin and p120-catenin to

E-cadherin, achieved by post-translational modifications,can also greatly weaken the adhesive functions of adherens junctions. Reduced expression of junctional and polarity proteins is often visible during EMT. Depending on the cell type and extent to which cells advance through an EMT program, cells undergoing an EMT may begin to express vimentin, suppress cytokeratin, shift expression of key integrins etc. These changes in gene expression are often seen as indicative of EMT or as markers of EMT, although, considering the extensive variations in EMT, their overall value in the diagnosis of EMT needs to be considered with caution. Beyond this small set of commonalities, it is difficult to define other changes as contributing universally to all the diverse manifestations of EMT programs that have been described in the rapidly expanding literature. Furthermore, our current knowledge does not allow us to know whether there is a linear succession of cell-biological changes as cells advance progressively through an EMT program, or whether a diverse series of routes radiates in multiple directions from the starting point of attenuation or loss of epithelial junctions.

6.4: Finding reliable EMT markers requires a combinatorial approach, together withdistinguishing between EMT-associated and non-EMT-associated functions.

An analysis of EMT status often requires the use of markers specific to a specific biological context. It may also be critical to assess the position of cells along the EMT spectrum using a standardized set of criteria in a specific biological context. Obtaining quantitative EMT marker measurements should always be coupled with cellular and functional analyses of the EMT status as described above. Importantly, recent studies have linked EMT to various other cellular programs and functions, including cancer cell stemness, resistance to apoptosis, genome instability, cancer drug resistance, and metabolic adaptation. Many components of EMT regulatory pathways, including EMT-TFs, also affect other important cellular phenotypes and are themselves regulated through diverse signals that may or may not involve canonical EMT. For example, EMT-TFs, such as Snai1/2 and Twist1, also regulate

cell survival and cancer drug resistance [66,83], which are currently unknown to be due to their EMT functions or not. Likewise, many extracellular matrix remodeling proteins that are important in breaching basement membrane can be regulated in both EMT-dependent and -independent manners. Furthermore, while cells may switch to a different cell fate upon EMT, EMT represents changes of epithelial cell characteristics to more mesenchymal cones, which is independent of cell differentiation or dedifferentiation. Therefore, it is important to note that the cell biological definition of EMT strictly refers to the core EMT program, while we should keep these associated phenotypes in mind when examining the impact of EMT in various biological settings.

7. Conclusions and implications for the future of EMT research

Fifty years and over 36,000 publications after Betty Hay's pioneering observations, the concept of EMT has now been widely applied in biomedical research. It provides a unifying framework for developmental and cancer studies, which is evidenced by the exponentially growing number of EMT-related publications. Such a framework holds the promise of far-reaching breakthroughs in cancer diagnosis and treatment for cancer biologists, and for bridging the gap in understanding normal and pathological epithelial organization and morphogenesis for developmental and cell biologists. To realize this promise, it is desirable for the EMT community to reach a consensus on the definition of EMT-related terms as outlined in the previous sections and on the conceptual framework of approaching EMT research as a cell biological process with quantifiable molecular descriptors and cellular readouts. All co-authors of this white paper agree to use the terms that are defined in this white paper in future research publications and recommend other researchers in the EMT and the larger biological research community also follow these guidelines. Only by minimizing semantic

misinterpretation and data miscommunication can we begin to appreciate the diversity of individual EMTs and uncover conserved themes in EMT regulation in development and disease.

Studies using cell lines, developmental systems and cancer models have revealed a diversity of EMT-induced phenotypes and highlighted the remarkable complexity in the execution and regulation of EMT. Looking forward, to decipher the complexity and plasticity of the EMT program, we propose that EMT research, while anchored in traditional developmental, cell and cancer biology, should be explored within a broader conceptual context. The EMT field has in recent years attracted the interest of a diverse group of researchers with their expertise in systems biology, biophysics, stem cell biology, pathology and mathematical modeling. This represents remarkable strength and will surely enable genuine cross-disciplinary collaborations. We expect that future EMT studies will apply multi-disciplinary approaches to gain increased mechanistic understanding of various EMT events. One open question in the cancer EMT field is the extent to which stabilization of specific hybrid E/M states or switches between E/M states in a dynamic fashion in response to distinct microenvironmental cues favors the metastatic process. With many important factors to EMT remaining insufficiently explored, advancing EMT research requires innovation in investigative tools for single-cell level studies of developmental and cancer EMTs. This includes single-cell live imaging, lineage tracing, gene expression analyses, and studies of genetic and epigenetic modifications. Finally, a combination of mathematical modeling with carefully constructed experimental analyses will be fruitful to gain mechanistic understanding of EMT plasticity.

Another major challenge will involve the translation of the existing concepts of EMT heterogeneity and plasticity into clinical practice. While we are far from understanding the full consequences of EMT heterogeneity, several clinical trials already incorporate the notion of dynamic EMT plasticity,

thereby opening the way for novel therapies exploiting EMT heterogeneity. Promising advances will come from single-cell sequencing of normal tissues, primary tumors, circulating tumor cells, and disseminated metastatic lesions, together with cellular analyses and functional validations, in order to better capture the diversity and plasticity of EMT and to reveal the underlying molecular alterations following tumor progression and therapy response. An increasing understanding of the manifestations and underlying mechanisms of EMTs offers the potential for targeted therapy to prevent cancer metastasis. For example, while inhibition of EMT-associated changes may reduce cancer cell dissemination in early-stage carcinoma, preventing MET in disseminated tumor cells might inhibit metastatic outgrowth in distant organs. Experimental and clinical studies show that resistance arising to various therapies, including chemotherapies and immunotherapies, is tightly associated with EMT phenotypes [83]. These studies suggest that targeting EMT, or the cells capable of expressing it, hold promise in overcoming therapy resistance, a major challenge to cancer treatment.

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References

1 Hay, E. D. in *Epithelial-Mesenchymal Interactions: 18th Hahnemann Symposium.* (ed R. Fleischmajer, Billingham, R.E.) 31-35 (Williams and Wilkins).

2 Hay, E. D. An overview of epithelio-mesenchymal transformation. *Acta Anat (Basel)* **154**, 8-20 (1995).

3 Nieto, M. A., Huang, R. Y., Jackson, R. A. & Thiery, J. P. Emt: 2016. *Cell* **166**, 21-45, doi:10.1016/j.cell.2016.06.028 (2016).

4 Tarin, D., Thompson, E. W. & Newgreen, D. F. The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res* **65**, 5996-6000; discussion 6000-5991 (2005).

5 Thompson, E. W., Newgreen, D. F. & Tarin, D. Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? *Cancer Res* **65**, 5991-5995; discussion 5995 (2005).

6 Fischer, K. R. *et al.* Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. *Nature* **527**, 472-476, doi:10.1038/nature15748 (2015).

7 Zheng, X. *et al.* Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature* **527**, 525-530, doi:10.1038/nature16064 (2015).

8 Ye, X. *et al.* Upholding a role for EMT in breast cancer metastasis. *Nature* **547**, E1-E3, doi:10.1038/nature22816 (2017).

9 Aiello, N. M. *et al.* Upholding a role for EMT in pancreatic cancer metastasis. *Nature* **547**, E7-E8, doi:10.1038/nature22963 (2017).

10 Newgreen, D. F., Ritterman, M. & Peters, E. A. Morphology and behaviour of neural crest cells of chick embryo in vitro. *Cell Tissue Res* **203**, 115-140, doi:10.1007/bf00234333 (1979).

11 Thiery, J. P., Duband, J. L., Rutishauser, U. & Edelman, G. M. Cell adhesion molecules in early chicken embryogenesis. *Proc Natl Acad Sci U S A* **79**, 6737-6741, doi:10.1073/pnas.79.21.6737 (1982).

12 Markwald, R. R., Fitzharris, T. P. & Manasek, F. J. Structural development of endocardial cushions. *Am J Anat* **148**, 85-119 (1977).

13 Trelstad, R. L., Hayashi, A., Hayashi, K. & Donahoe, P. K. The epithelial-mesenchymal interface of the male rate Mullerian duct: loss of basement membrane integrity and ductal regression. *Dev Biol* **92**, 27-40, doi:10.1016/0012-1606(82)90147-6 (1982).

14 Greenburg, G. & Hay, E. D. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. *J Cell Biol* **95**, 333-339 (1982).

15 Nahmias, A. J., Cooperband, S. R., Green, J. A. & Kibrick, S. Transformation of epithelial cells in vitro. *Nature* **216**, 1349-1350, doi:10.1038/2161349a0 (1967).

16 Runyan, R. B. & Markwald, R. R. Invasion of mesenchyme into three-dimensional collagen gels: a regional and temporal analysis of interaction in embryonic heart tissue. *Dev Biol* **95**, 108-114 (1983).

17 Stoker, M. & Perryman, M. An epithelial scatter factor released by embryo fibroblasts. *J Cell Sci* **77**, 209-223 (1985).

18 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**, 1124-1128, doi:10.1073/pnas.87.3.1124 (1990).

19 Potts, J. D. & Runyan, R. B. Epithelial-mesenchymal cell transformation in the embryonic heart can be mediated, in part, by transforming growth factor beta. *Dev Biol* **134**, 392-401 (1989).

20 Miettinen, P. J., Ebner, R., Lopez, A. R. & Derynck, R. TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. *The Journal of cell biology* **127**, 2021-2036 (1994).

21 Leptin, M. twist and snail as positive and negative regulators during Drosophila mesoderm development. *Genes Dev* **5**, 1568-1576. (1991).

22 Nieto, M. A., Sargent, M. G., Wilkinson, D. G. & Cooke, J. Control of cell behavior during vertebrate development by Slug, a zinc finger gene. *Science* **264**, 835-839 (1994).

23 Savagner, P., Yamada, K. M. & Thiery, J. P. The zinc-finger protein slug causes desmosome dissociation, an initial and necessary step for growth factor-induced epithelial-mesenchymal transition. *J Cell Biol* **137**, 1403-1419, doi:10.1083/jcb.137.6.1403 (1997).

24 Batlle, E. *et al.* The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol* **2**, 84-89. (2000).

25 Cano, A. *et al.* The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* **2**, 76-83. (2000).

26 Hajra, K. M., Chen, D. Y. & Fearon, E. R. The SLUG zinc-finger protein represses E-cadherin in breast cancer. *Cancer Res* **62**, 1613-1618. (2002).

27 Bolos, V. *et al.* The transcription factor Slug represses E-cadherin expression and induces epithelial to mesenchymal transitions: a comparison with Snail and E47 repressors. *J Cell Sci* **116**, 499-511, doi:10.1242/jcs.00224 (2003).

28 Perez-Moreno, M. A. *et al.* A new role for E12/E47 in the repression of E-cadherin expression and epithelial-mesenchymal transitions. *J Biol Chem* **276**, 27424-27431 (2001).

29 Yang, J. *et al.* Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* **117**, 927-939 (2004).

30 Comijn, J. *et al.* The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. *Mol Cell* **7**, 1267-1278. (2001).

31 Eger, A. *et al.* DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells. *Oncogene* **24**, 2375-2385 (2005).

32 Stark, K., Vainio, S., Vassileva, G. & McMahon, A. P. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature* **372**, 679-683, doi:10.1038/372679a0 (1994).

33 Christ, B. & Ordahl, C. P. Early stages of chick somite development. *Anat Embryol (Berl)* **191**, 381-396, doi:10.1007/bf00304424 (1995).

34 Nakaya, Y., Sukowati, E. W., Wu, Y. & Sheng, G. RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation. *Nat Cell Biol* **10**, 765-775, doi:10.1038/ncb1739 (2008).

35 Shook, D. & Keller, R. Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development. *Mech Dev* **120**, 1351-1383 (2003).

36 Mayor, R. & Etienne-Manneville, S. The front and rear of collective cell migration. *Nat Rev Mol Cell Biol* **17**, 97-109, doi:10.1038/nrm.2015.14 (2016).

37 Campbell, K. & Casanova, J. A role for E-cadherin in ensuring cohesive migration of a heterogeneous population of non-epithelial cells. *Nat Commun* **6**, 7998, doi:10.1038/ncomms8998 (2015).

38 Montero, J. A. *et al.* Shield formation at the onset of zebrafish gastrulation. *Development* **132**, 1187-1198, doi:10.1242/dev.01667 (2005).

39 Clark, I. B., Muha, V., Klingseisen, A., Leptin, M. & Muller, H. A. Fibroblast growth factor signalling controls successive cell behaviours during mesoderm layer formation in Drosophila. *Development* **138**, 2705-2715, doi:10.1242/dev.060277 (2011).

40 Dumortier, J. G., Martin, S., Meyer, D., Rosa, F. M. & David, N. B. Collective mesendoderm migration relies on an intrinsic directionality signal transmitted through cell contacts. *Proc Natl Acad Sci U S A* **109**, 16945-16950, doi:10.1073/pnas.1205870109 (2012).

41 Weber, G. F., Bjerke, M. A. & DeSimone, D. W. A mechanoresponsive cadherin-keratin complex directs polarized protrusive behavior and collective cell migration. *Dev Cell* **22**, 104-115, doi:10.1016/j.devcel.2011.10.013 (2012).

42 Viotti, M., Nowotschin, S. & Hadjantonakis, A. K. SOX17 links gut endoderm morphogenesis and germ layer segregation. *Nat Cell Biol* **16**, 1146-1156, doi:10.1038/ncb3070 (2014).

43 Theveneau, E. & Mayor, R. Neural crest delamination and migration: from epithelium-to-mesenchyme transition to collective cell migration. *Dev Biol* **366**, 34-54, doi:10.1016/j.ydbio.2011.12.041 (2012).

44 Scarpa, E. *et al.* Cadherin Switch during EMT in Neural Crest Cells Leads to Contact Inhibition of Locomotion via Repolarization of Forces. *Dev Cell* **34**, 421-434, doi:10.1016/j.devcel.2015.06.012 (2015).

45 Raz, E. Primordial germ-cell development: the zebrafish perspective. *Nat Rev Genet* **4**, 690-700, doi:10.1038/nrg1154 (2003).

46 Rozbicki, E. *et al.* Myosin-II-mediated cell shape changes and cell intercalation contribute to primitive streak formation. *Nat Cell Biol* **17**, 397-408, doi:10.1038/ncb3138 (2015).

47 Ocana, O. H. *et al.* Metastatic colonization requires the repression of the epithelial-mesenchymal transition inducer Prrx1. *Cancer Cell* **22**, 709-724, doi:10.1016/j.ccr.2012.10.012 (2012).

48 Tsai, J. H., Donaher, J. L., Murphy, D. A., Chau, S. & Yang, J. Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. *Cancer Cell* **22**, 725-736, doi:10.1016/j.ccr.2012.09.022 (2012).

49 Tsai, J. H. & Yang, J. Epithelial-mesenchymal plasticity in carcinoma metastasis. *Genes & development* **27**, 2192-2206, doi:10.1101/gad.225334.113 (2013).

50 Brabletz, T. To differentiate or not — routes towards metastasis. *Nat Rev Cancer* **12**, 425-436 (2012).

51 Chaffer, C. L., San Juan, B. P., Lim, E. & Weinberg, R. A. EMT, cell plasticity and metastasis. *Cancer and Metastasis Reviews* **35**, 645-654, doi:10.1007/s10555-016-9648-7 (2016).

52 Lambert, A. W., Pattabiraman, D. R. & Weinberg, R. A. Emerging Biological Principles of Metastasis. *Cell* **168**, 670-691, doi:http://dx.doi.org/10.1016/j.cell.2016.11.037 (2017).

53 Pastushenko, I. *et al.* Identification of the tumour transition states occurring during EMT. *Nature* **556**, 463-468, doi:10.1038/s41586-018-0040-3 (2018).

54 Yu, M. *et al.* Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. *Science* **339**, 580-584, doi:10.1126/science.1228522 (2013).

55 Schliekelman, M. J. *et al.* Molecular portraits of epithelial, mesenchymal and hybrid states in lung adenocarcinoma and their relevance to survival. *Cancer research* **75**, 1789-1800, doi:10.1158/0008-5472.CAN-14-2535 (2015).

56 Diepenbruck, M. & Christofori, G. Epithelial–mesenchymal transition (EMT) and metastasis: yes, no, maybe? *Current Opinion in Cell Biology* **43**, 7-13, doi:http://dx.doi.org/10.1016/j.ceb.2016.06.002 (2016).

57 Santamaria, P. G., Moreno□Bueno, G., Portillo, F. & Cano, A. EMT: Present and future in clinical oncology. *Mol Oncol* **11**, 718-738, doi:10.1002/1878-0261.12091 (2017).

58 Ledford, H. Cancer therory faces doubts. *Nature* **472**, 273 (2011).

59 Maheswaran, S. & Haber, D. A. Transition loses its invasive edge. *Nature* **527**, 452, doi:10.1038/nature16313 (2015).

60 Ruben, B. & Gerhard, C. The relevance of EMT in breast cancer metastasis: Correlation or causality? *FEBS Lett* **589**, 1577-1587, doi:doi:10.1016/j.febslet.2015.05.002 (2015).

61 Tran, H. D. *et al.* Transient SNAIL1 expression is necessary for metastatic competence in breast cancer. *Cancer Res* **74**, 6330-6340, doi:10.1158/0008-5472.CAN-14-0923 (2014).

62 Xu, Y. *et al.* Breast tumor cell-specific knockout of Twist1 inhibits cancer cell plasticity, dissemination, and lung metastasis in mice. *Proc Natl Acad Sci U S A* **114**, 11494-11499, doi:10.1073/pnas.1618091114 (2017).

63 Krebs, A. M. *et al.* The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. *Nat Cell Biol* **19**, 518-529, doi:10.1038/ncb3513

http://www.nature.com/ncb/journal/v19/n5/abs/ncb3513.html - supplementary-information (2017).

64 Aiello, N. M. *et al.* EMT Subtype Influences Epithelial Plasticity and Mode of Cell Migration. *Dev Cell* **45**, 681-695 e684, doi:10.1016/j.devcel.2018.05.027 (2018).

65 Goossens, S., Vandamme, N., Van Vlierberghe, P. & Berx, G. EMT transcription factors in cancer development re-evaluated: Beyond EMT and MET. *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* **1868**, 584-591, doi:https://doi.org/10.1016/j.bbcan.2017.06.006 (2017).

66 Puisieux, A., Brabletz, T. & Caramel, J. Oncogenic roles of EMT-inducing transcription factors. *Nature cell biology* **16**, 488-494, doi:10.1038/ncb2976 (2014).

67 Iwano, M. *et al.* Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* **110**, 341-350, doi:10.1172/JCI15518 (2002).

68 LeBleu, V. S. *et al.* Origin and function of myofibroblasts in kidney fibrosis. *Nat Med* **19**, 1047-1053, doi:10.1038/nm.3218 (2013).

69 Grande, M. T. *et al.* Snail1-induced partial epithelial-to-mesenchymal transition drives renal fibrosis in mice and can be targeted to reverse established disease. *Nat Med* **21**, 989-997, doi:10.1038/nm.3901 (2015).

70 Lovisa, S. *et al.* Epithelial-to-mesenchymal transition induces cell cycle arrest and parenchymal damage in renal fibrosis. *Nat Med* **21**, 998-1009, doi:10.1038/nm.3902 (2015).

71 Boyer, A. S. *et al.* TGFbeta2 and TGFbeta3 have separate and sequential activities during epithelial-mesenchymal cell transformation in the embryonic heart. *Dev Biol* **208**, 530-545 (1999).

72 Zeisberg, E. M. *et al.* Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* **13**, 952-961, doi:10.1038/nm1613 (2007).

73 Savagner, P. Epithelial-mesenchymal transitions: from cell plasticity to concept elasticity. *Curr Top Dev Biol* **112**, 273-300, doi:10.1016/bs.ctdb.2014.11.021 (2015).

74 Stemmler, M. P., Eccles, R. L., Brabletz, S. & Brabletz, T. Non-redundant functions of EMT transcription factors. *Nat Cell Biol* **21**, 102-112, doi:10.1038/s41556-018-0196-y (2019).

75 Assemat, E., Bazellieres, E., Pallesi-Pocachard, E., Le Bivic, A. & Massey-Harroche, D. Polarity complex proteins. *Biochim Biophys Acta* **1778**, 614-630, doi:10.1016/j.bbamem.2007.08.029 (2008).

76 Lee, M. & Vasioukhin, V. Cell polarity and cancer--cell and tissue polarity as a non-canonical tumor suppressor. *J Cell Sci* **121**, 1141-1150, doi:10.1242/jcs.016634 (2008).

77 Ngok, S. P., Lin, W. H. & Anastasiadis, P. Z. Establishment of epithelial polarity--GEF who's minding the GAP? *J Cell Sci* **127**, 3205-3215, doi:10.1242/jcs.153197 (2014).

78 Barrios-Rodiles, M. *et al.* High-throughput mapping of a dynamic signaling network in mammalian cells. *Science* **307**, 1621-1625 (2005).

79 Ozdamar, B. *et al.* Regulation of the polarity protein Par6 by TGFbeta receptors controls epithelial cell plasticity. *Science* **307**, 1603-1609 (2005).

80 Jung, H. Y. *et al.* Apical-basal polarity inhibits epithelial-mesenchymal transition and tumour metastasis by PAR-complex-mediated SNAI1 degradation. *Nat Cell Biol* **21**, 359-371, doi:10.1038/s41556-019-0291-8 (2019).

81 Janda, E. *et al.* Raf plus TGFbeta-dependent EMT is initiated by endocytosis and lysosomal degradation of E-cadherin. *Oncogene* **25**, 7117-7130, doi:10.1038/sj.onc.1209701 (2006).

82 Eckert, M. A. *et al.* Twist1-induced invadopodia formation promotes tumor metastasis. *Cancer Cell* **19**, 372-386, doi:10.1016/j.ccr.2011.01.036 (2011).

83 Dongre, A. & Weinberg, R. A. New insights into the mechanisms of epithelial–mesenchymal transition and implications for cancer. *Nature Reviews Molecular Cell Biology* **20**, 69-84, doi:10.1038/s41580-018-0080-4 (2019).

84 Boulay, J. L., Dennefeld, C. & Alberga, A. The Drosophila developmental gene snail encodes a protein with nucleic acid binding fingers. *Nature* **330**, 395-398, doi:10.1038/330395a0 (1987).

85 Nieto, M. A., Bennett, M. F., Sargent, M. G. & Wilkinson, D. G. Cloning and developmental expression of Sna, a murine homologue of the Drosophila snail gene. *Development* **116**, 227-237 (1992).

86 Boutet, A. *et al.* Snail activation disrupts tissue homeostasis and induces fibrosis in the adult kidney. *The EMBO Journal* **25**, 5603-5613, doi:10.1038/sj.emboj.7601421 (2006).

87 Funahashi, J., Sekido, R., Murai, K., Kamachi, Y. & Kondoh, H. Delta-crystallin enhancer binding protein delta EF1 is a zinc finger-homeodomain protein implicated in postgastrulation embryogenesis. *Development* **119**, 433-446 (1993).

88 Oba, S. *et al.* miR-200b Precursor Can Ameliorate Renal Tubulointerstitial Fibrosis. *PLoS One* **5**, e13614, doi:10.1371/journal.pone.0013614 (2010).

89 Verschueren, K. *et al.* SIP1, a novel zinc finger/homeodomain repressor, interacts with Smad proteins and binds to 5'-CACCT sequences in candidate target genes. *J Biol Chem* **274**, 20489-20498, doi:10.1074/jbc.274.29.20489 (1999).

90 Thisse, B., Stoetzel, C., Gorostiza-Thisse, C. & Perrin-Schmitt, F. Sequence of the twist gene and nuclear localization of its protein in endomesodermal cells of early Drosophila embryos. *EMBO J* **7**, 2175-2183 (1988).

91 Kida, Y., Asahina, K., Teraoka, H., Gitelman, I. & Sato, T. Twist relates to tubular epithelial-mesenchymal transition and interstitial fibrogenesis in the obstructed kidney. *J Histochem Cytochem* **55**, 661-673, doi:10.1369/jhc.6A7157.2007 (2007).

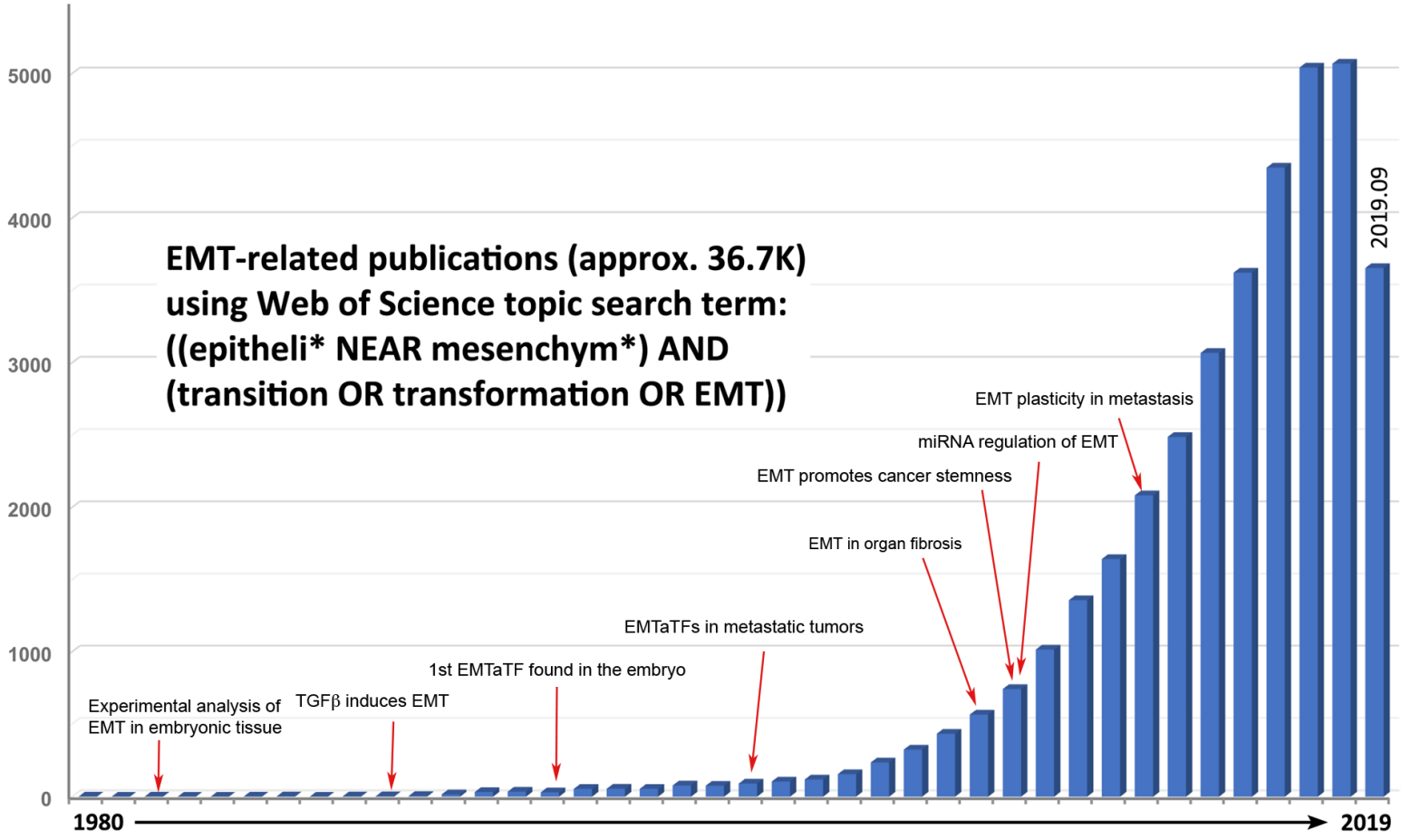
TF Name	Type	Key References reporting the original discoveries		
		Development	Cancer	Fibrosis
Snai1 (Snail)	zinc finger	Boulay et al., 1987 [84]; Nieto et al., 1992 [85]	Batlle et al., 2000 [24]; Cano et al., 2000 [25]	Boutet et al., 2006 [86]
Snai2 (Slug)	zinc finger	Nieto et al., 1994 [22]	Savagner et al., 1997 [23]	
Zeb1	zinc finger	Funahashi et al., 1993 [87]	Grooteclaes and Frisch, 2000	Oba et al., 2010 [88]
Zeb2 (SIP1)	zinc finger	Verschueren et al., 1999 [89]	Comijn et al., 2001 [30]	Oba et al., 2010 [88]
Twist1	bHLH	Thisse et al., 1988 [90]	Yang et al., 2004 [29]	Kida et al., 2007 [91]; Lovisa et al., 2015 [70]

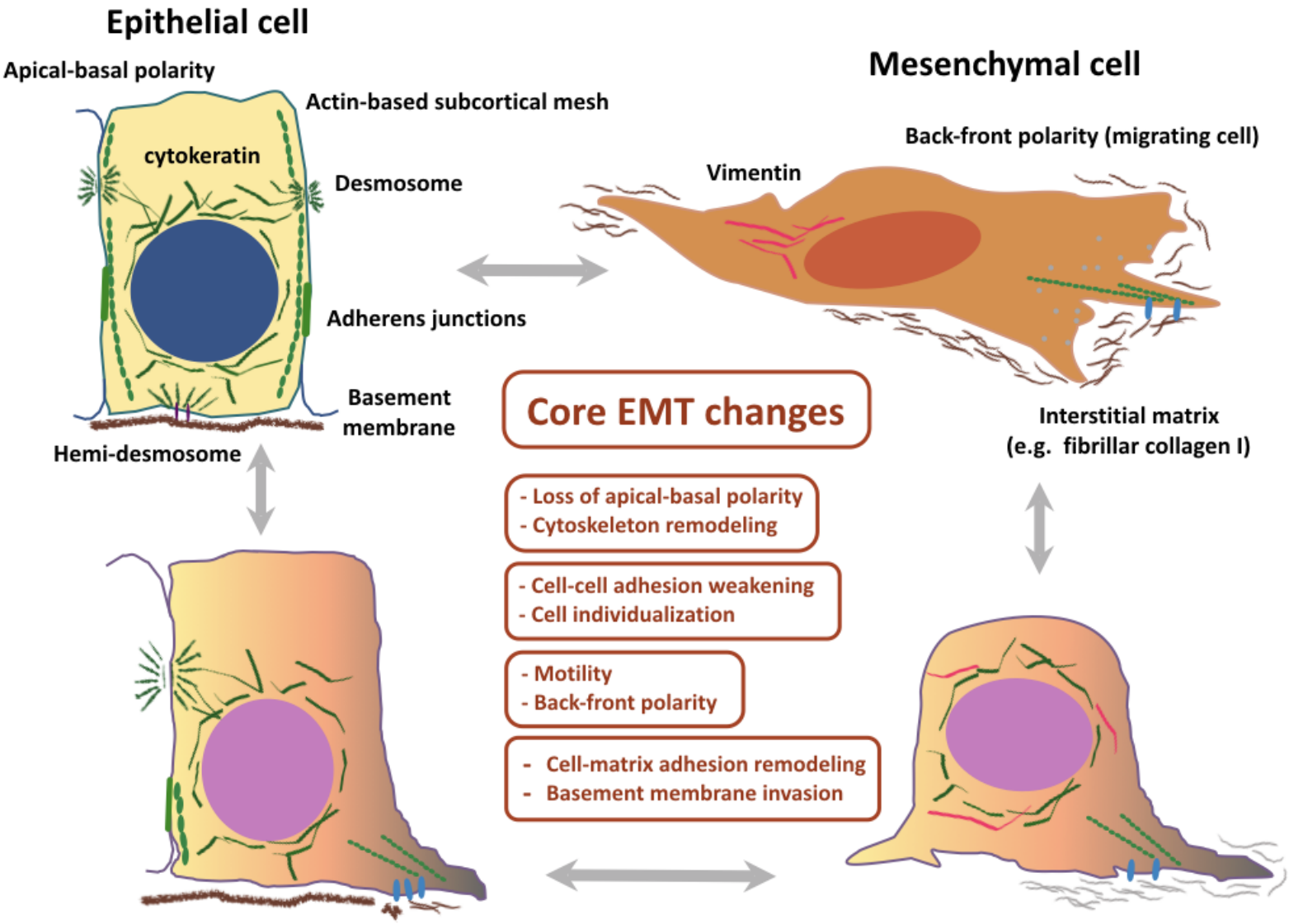
Table I. A list of core EMT transcription factors.

Figure legends:

Figure 1. Growth of the primary literature in EMT. The first experimental analysis of EMT in development was published in 1979. The relationship of EMT to growth factors was found in 1989. Transcriptional regulation of EMT was identified in 1994. Subsequent growth was stimulated by linkage to metastasis, organ fibrosis and stem cells. Growth in the field has been logarithmic since the time of the first TEMTIA meeting in 2003. Graph indicates primary papers published each year by a search in the Web of Science database. Abstracts and reviews were excluded. The total number of publications for 2018 exceeded 5000 papers.

Figure 2. EMT diversity represented by an epithelial-mesenchymal plasticity model. Various cellular features associated with an epithelial or a mesenchymal cell state are found in a range of combinations in cells in different developmental contexts and to different degrees. The accumulated loss or gain of epithelial/mesenchymal characteristics pushes a cell towards various intermediate states (Bottom left and right) in both a fluid and reversible manner between a complete epithelial (Top left) and a complete mesenchymal (Top right) state.


EMT-related publications (approx. 36.7K)
using Web of Science topic search term:
((epitheli* NEAR mesenchym*) AND
(transition OR transformation OR EMT))
5000
4000
3000
2000
1000
0
1980
2019
2019.09
Experimental analysis of
EMT in embryonic tissue
TGFβ induces EMT
1st EMTaTF found in the embryo
EMTaTFs in metastatic tumors
EMT in organ fibrosis
EMT promotes cancer stemness
miRNA regulation of EMT
EMT plasticity in metastasis


Epithelial cell
Apical-basal polarity
Actin-based subcortical mesh
cytokeratin
Desmosome
Adherens junctions
Basement membrane
Hemi-desmosome
Mesenchymal cell
Back-front polarity (migrating cell)
Vimentin
Interstitial matrix
(e.g. fibrillar collagen I)
Core EMT changes
- Loss of apical-basal polarity
- Cytoskeleton remodeling
- Cell-cell adhesion weakening
- Cell individualization
- Motility
- Back-front polarity
- Cell-matrix adhesion remodeling
- Basement membrane invasion

Additional Transcription Factors associated with EMT				
TF Name	Type of factor	References		
		Development	Cancer	Fibrosis
Alx1	homeobox	Saunders & McClay, 2014	Yuan et al., 2013	-
AP1 (Jun/Fos Atf)	Ap1	Reichmann et al., 1992	Bakiri et al. 2015	Alcorn et al., 2009
Brachyury (T)	T-box	Beddington et al., 1992	Fernando et al., 2010	Sun et al., 2014
CBF-A (NF-YA)	CCAT box binding	-	Venkov et al., 2007	Venkov et al., 2007
E2-2 (TCF4) (TCF7L2)	bHLH	Sobrado et al., 2009	Sobrado et al., 2009	Contreras et al., 2016
E47 (TCF3)	bHLH	Perez-Moreno et al., 2001	Perez-Moreno et al., 2001	Slattery et al., 2006
Erg	ETS	Saunders & McClay, 2014	Petrovics et al., 2005	-
Ets1	ETS	Fafeur et al., 1997	Nakayama et al., 1996	Nakamura et al., 2004.
FoxC2 (FoxB)	Forkhead	Saunders & McClay, 2014	Mani et al., 2007	Sipos & Galamb, 2012
GATA4/6 (Srp)	GATA	Campbell et al, 2011	-	-
Goosecoid (GSC)	homeobox	Blum et al., 1992	Hartwell et al., 2006	-
HoxB7	homeobox	-	Wu et al, 2006	-
Id1	HLH	Jen et al., 1996	Tobin et al, 2011	Li et al., 2007
KLF2	Sp/KLF	Chiplunkar et al., 2013	-	-
Klf4	Klf	Liu et al., 2012	Lin et al., 2012	Tiwari et al., 2013
KLF6 (Z9f)	Sp/KLF	-	-	Holian et al., 2008
KLF8	Sp/KLF	-	Wang et al, 2007	-
NF-□b	NF-□B/Rel	DeLaughter et al., 2016	Huber et al, 2004	Sunami et al., 2012
Prrx1	Paired related homeobox	Ocana et al. 2012	Ocana et al., 2012; Takano et al., 2016	Gong et al., 2017
Runx2	Runt-related	Tavares et al. 2017	Pratap et al., 2005	Mummler et al., 2018
Six1	homeobox	Grifone et al., 2005	Micalizzi et al., 2009; McCoy et al., 2009	Smith et al., 2008
Sox4	homeobox	Zhang et al, 2012	Liu et al., 2006	Xiao et al., 2015

Sox9	homeobox	Akiyama et al., 2004	Acevedo et al., 2007	Hanley et al., 2008
Tbx3	T-box	Singh et al., 2012	Rodriguez et al. 2008	Wensing & Campos, 2014
Tead (Yap/Taz)	Tead	Diepenbruck et al., 2014	Overholtzer et al., 2006	Seo et al 2016
Tgif1	homeobox	Saunders & McClay, 2014	Xiang et al., 2015	-
Twist2 (Dermo1)	bHLH	-	Ansieau et al., 2008	Grunz-Borgmann et al., 2017

Supplemental Table 1. A list of additional EMT associated Transcription Factors. Transcription factors with a minimum of 10 citations in the EMT literature. "–" indicates lack of a pertinent reference.

References for supplemental table

Acevedo, V.D., Gangula, R. D., Freeman, K. W., Li, R. L., Zhang, Y. Y., Wang, F., Ayala, G. E., Peterson, L. E., Ittmann, M. & Spencer, D. M. Inducible FGFR-1 activation leads to irreversible prostate adenocarcinoma and an epithelial-to-mesenchymal transition. Cancer Cell 12, 559-571 (2007).

Akiyama, H., Chaboissier, M. C., Behringer, R. R., Rowitch, D. H., Schedl, A., Epstein, J. A. & de Crombrugghe, B. Essential role of Sox9 in the pathway that controls formation of cardiac valves and septa. Proc Natl Acad Sci U S A 101, 6502-6507 (2004).

Alcorn, J.F., van der Velden, J., Brown, A. L., McElhinney, B., Irvin, C. G. & Janssen-Heininger, Y. M. W. c-Jun N-Terminal Kinase 1 Is Required for the Development of Pulmonary Fibrosis. American Journal of Respiratory Cell and Molecular Biology 40, 422-432 (2009).

Ansieau, S., Bastid, J., Doreau, A., Morel, A. P., Bouchet, B. P., Thomas, C., Fauvet, F., Puisieux, I., Doglioni, C., Piccinin, S., Maestro, R., Voeltzel, T., Selmi, A., Valsesia-Wittmann, S., Caron de Fromentel, C. & Puisieux, A. Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. Cancer Cell 14, 79-89 (2008).

Bakiri, L., Macho-Maschler, S., Custic, I., Niemiec, J., Guio-Carrion, A., Hasenfuss, S. C., Eger, A., Muller, M., Beug, H. & Wagner, E. F. Fra-1/AP-1 induces EMT in mammary epithelial cells by modulating Zeb1/2 and TGF beta expression. Cell Death Differ 22, 336-350 (2015).

Beddington, R.S.P., Rashbass, P.R. & Wilson, V. Brachyury--a gene affecting mouse gastrulation and early organogenesis. Development.-.Supplement., 157-165 (1992).

Blum, M., Gaunt, S. J., Cho, K. W., Steinbeisser, H., Blumberg, B., Bittner, D., & De Robertis, E. M. Gastrulation in the mouse: the role of the homeobox gene goosecoid. Cell 69, 1097-1106 (1992).

Campbell, K., Whissell, G., Franch-Marro, X., Batlle, E. & Casanova, J. Specific GATA factors act as conserved inducers of an endodermal-EMT. *Dev Cell* **21**, 1051-1061, doi:10.1016/j.devcel.2011.10.005 (2011)

Chiplunkar, A.R., Lung, T. K., Alhashem, Y., Koppenhaver, B. A., Salloum, F. N., Kukreja, R. C., Haar, J. L. & Lloyd, J. A. Kruppel-like factor 2 is required for normal mouse cardiac development. Plos One 8, e54891 (2013).

Contreras, O., Rebolledo, D.L., Oyarzun, J.E., Olguin, H.C. & Brandan, E. Connective tissue cells expressing fibro/adipogenic progenitor markers increase under chronic damage: relevance in fibroblast-myofibroblast differentiation and skeletal muscle fibrosis. Cell and Tissue Research 364, 647-660 (2016).

DeLaughter, D.M., Clark, C. R., Christodoulou, D. C., Seidman, C. E., Baldwin, H. S., Seidman, J. G. & Barnett, J. V. Transcriptional Profiling of Cultured, Embryonic Epicardial Cells Identifies Novel Genes and Signaling Pathways Regulated by TGFbetaR3 In Vitro. Plos One 11, e0159710 (2016).

Diepenbruck, M., Waldmeier, L., Ivanek, R., Berninger, P., Arnold, P., van Nimwegen, E.& Christofori, G. Tead2 expression levels control the subcellular distribution of Yap and Taz, zyxin expression and epithelial-mesenchymal transition. Journal of Cell Science 127, 1523-1536 (2014).

Fafeur, V., Tulasne, D., Queva, C., Vercamer, C., Dimster, V., Mattot, V., Stehelin, D., Desbiens, X. & Vandenbunder, B. The ETS1 transcription factor is expressed during epithelial-mesenchymal transitions in the chick embryo and is activated in scatter factor-stimulated MDCK epithelial cells. Cell Growth & Differentiation 8, 655-665 (1997).

Fernando, R.I., Litzinger, M., Trono, P., Hamilton, D. H., Schlom, J. & Palena, C. The T-box transcription factor Brachyury promotes epithelial-mesenchymal transition in human tumor cells. J Clin Invest 120, 533-544 (2010).

Gong, J., Han, J., He, J. Y., Liu, J. M., Han, P., Wang, Y. W., Li, M. K., Li, D. X., Ding, X. M., Du, Z. P., Liao, J. Z. & Tian, D. Paired related homeobox protein 1 regulates PDGF-induced chemotaxis of hepatic stellate cells in liver fibrosis. Laboratory Investigation 97, 1020-1032 (2017).

Grifone, R., Demignon, J., Houbron, C., Souil, E., Niro, C.,. Seller, M. J., Hamard, G. & Maire, P. Six1 and Six4 homeoproteins are required for Pax3 and Mrf expression during myogenesis in the mouse embryo. Development 132, 2235-2249 (2005).

Grunz-Borgmann, E.A., Nichols, L.A., Wang, X.H. & Parrish, A.R. Twist2 Is Upregulated in Early Stages of Repair Following Acute Kidney Injury. Int J Mol Sci 18 (2017).

Hanley, K.P., Oakley, F., Sugden, S., Wilson, D. I., Mann, D. A. & Hanley, N. A. Ectopic SOX9 mediates extracellular matrix deposition characteristic of organ fibrosis. Journal of Biological Chemistry 283, 14063-14071 (2008).

Hartwell, K.A., Muir, B., Reinhardt, F., Carpenter, A. E., Sgroi, D. C. & Weinberg, R. A. The Spemann organizer gene, Goosecoid, promotes tumor metastasis. Proc Natl Acad Sci U S A 103, 18969-18974 (2006).

Holian, J., Qi, W., Kelly, D. J., Zhang, Y., nMreich, E., Pollock, C. A. & Chen, X. M. Role of Kruppel-like factor 6 in transforming growth factor-beta1-induced epithelial-mesenchymal transition of proximal tubule cells. Am J Physiol Renal Physiol 295, F1388-1396 (2008).

Huber, M.A., Azoitei, N., Baumann, B., Grunert, S., Sommer, A., Pehamberger, H., Kraut, N., Beug, H. & Wirth, T. NF-kappa B is essential for epithelial-mesenchymal transition and metastasis in a model of breast cancer progression. Journal of Clinical Investigation 114, 569-581 (2004).

Jen, Y., Manova, K. & Benezra, R. Expression patterns of Id1, Id2, and Id3 are highly related but distinct from that of Id4 during mouse embryogenesis. Developmental Dynamics 207, 235-252 (1996).

Li, Y., Yang, J., Luo, J.H., Dedhar, S. & Liu, Y. Tubular epithelial cell dedifferentiation is driven by the helix-loop-helix transcriptional inhibitor Id1. J Am Soc Nephrol 18, 449-460 (2007).

Lin, Z.S., Chu, H.C., Yen, Y.C., Lewis, B.C. & Chen, Y.W. Kruppel-like factor 4, a tumor suppressor in hepatocellular carcinoma cells reverts epithelial mesenchymal transition by suppressing slug expression. Plos One 7, e43593 (2012).

Liu, P.B., Ramachandran, S., Ali Seyed, M., Scharer, C. D., Laycock, N., Dalton, W. B., Williams, H., Karanam, S., Datta, M. W., Jaye, D. L. & Moreno, C. S. Sex-determining region Y box 4 is a transforming oncogene in human prostate cancer cells. Cancer Research 66, 4011-4019 (2006).

Liu, Y.N., Abou-Kheir, W., Yin, J. J., Fang, L., Hynes, P., Casey, O., Hu, D., Wan, Y., Seng, V., Sheppard-Tillman, H., Martin, P. & Kelly, K. Critical and reciprocal regulation of KLF4 and SLUG in transforming growth factor beta-initiated prostate cancer epithelial-mesenchymal transition. Mol Cell Biol 32, 941-953 (2012).

Mani, S.A., Yang, J., Brooks, M., Schwaninger, G., Zhou, A., Miura, N., Kutok, J. L., Hartwell, K., Richardson, A. L. & Weinberg, R. A. Mesenchyme Forkhead 1 (FOXC2) plays a key role in metastasis and is associated with aggressive basal-like breast cancers. Proceedings of the National Academy of Sciences of the United States of America 104, 10069-10074 (2007).

McCoy, E.L., Iwanaga, R., Jedlicka, P., Abbey, N. S., Chodosh, L. A., Heichman, K. A., Welm, A. L. & Ford, H. L.Six1 expands the mouse mammary epithelial stem/progenitor cell pool and induces mammary tumors that undergo epithelial-mesenchymal transition. J Clin Invest 119, 2663-2677 (2009).

Micalizzi, D.S., Christensen, K. L., Jedlicka, P., Coletta, R. D., Baron, A. E., Harrell, J. C., Horwitz, K. B., Billheimer, D., Heichman, K. A., Welm, A. L., Schiemann, W. P.& Ford, H. L. The Six1 homeoprotein induces human mammary carcinoma cells to undergo epithelial-mesenchymal transition and metastasis in mice through increasing TGF-beta signaling. Journal of Clinical Investigation 119, 2678-2690 (2009).

Mummler, C., Burgy, O., Hermann, S., Mutze, K., Gunther, A., & Konigshoff, M. Cell-specific expression of runt-related transcription factor 2 contributes to pulmonary fibrosis. Faseb Journal 32, 703-716 (2018).

Nakamura, Y., Esnault, S., Maeda, T., Kelly, E. A. B., Malter, J. S. & Jarjour, N. N. Ets-1 regulates TNF-alpha-induced matrix metalloproteinase-9 and tenascin expression in primary bronchial fibroblasts. Journal of Immunology 172, 1945-1952 (2004).

Nakayama, T., Ito, M., Ohtsuru, A., Naito, S., Nakashima, M., Fagin, J. A., Yamashita, S. & Sekine, I. Expression of the Ets-1 proto-oncogene in human gastric carcinoma - Correlation with tumor invasion. American Journal of Pathology 149, 1931-1939 (1996).

Ocana, O.H., Corcoles, R., Fabra, A., Moreno-Bueno, G., Acloque, H., Vega, S., Barrallo-Gimeno, A., Cano, A. & Nieto, M. A. Metastatic Colonization Requires the Repression of the Epithelial-Mesenchymal Transition Inducer Prrx1. Cancer Cell 22, 709-724 (2012).

Overholtzer, M., Zhang, J., Smolen, G. A., Muir, B., Li, W., Sgroi, D. C., Deng, C. X., Brugge, J. S. & Haber, D. A. Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proceedings of the National Academy of Sciences of the United States of America 103, 12405-12410 (2006).

Perez-Moreno, M.A., Locascio, A., Rodrigo, I., Dhondt, G., Portillo, F., Nieto, M. A. & Cano, A. A new role for E12/E47 in the repression of E-cadherin expression and epithelial-mesenchymal transitions. Journal of Biological Chemistry 276, 27424-27431 (2001).

Petrovics, G., Liu, A. J., Shaheduzzaman, S., Furasato, B., Sun, C., Chen, Y. M., Nau, M., Ravindranath, L., Chen, Y. D., Dobi, A., Srikantan, V., Sesterhenn, I. A., McLeod, D. G., Vahey, M., Moul, J. W. & Srivastava, S. Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome. Oncogene 24, 3847-3852 (2005).

Pratap, J., Javed, A., Languino, L. R., van Wijnen, A. J., Stein, J. L., Stein, G. S.

& Lian, J. B. The Runx2 Osteogenic Transcription Factor Regulates Matrix Metalloproteinase 9 in Bone Metastatic Cancer Cells and Controls Cell Invasion. Molecular and Cellular Biology 25, 8581-8591 (2005).

Reichmann, E., Schwarz, H., Deiner, E. M., Leitner, I., Eilers, M., Berger, J., Busslinger, M. & Beug, H. Activation of an inducible c-FosER fusion protein causes loss of epithelial polarity and triggers epithelial-fibroblastoid cell conversion. Cell 71, 1103-1116 (1992).

Rodriguez, M., Aladowicz, E., Lanfrancone, L. & Goding, C.R. Tbx3 represses E-cadherin expression and enhances melanoma invasiveness. Cancer Research 68, 7872-7881 (2008).

Saunders, L.R. & McClay, D.R. Sub-circuits of a gene regulatory network control a developmental epithelial-mesenchymal transition. Development 141, 1503-1513 (2014).

Seo, E., Kim, W. Y., Hur, J., Kim, H., Nam, S. A., Choi, A., Kim, Y. M., Park, S. H., Chung, C., Kim, J., Min, S., Myung, S. J., Lim, D. S. & Kim, Y. K. The Hippo-Salvador signaling pathway regulates renal tubulointerstitial fibrosis. Scientific Reports 6 (2016).

Singh, R. et al. Tbx2 and Tbx3 induce atrioventricular myocardial development and endocardial cushion formation. Cell Mol Life Sci 69, 1377-1389 (2012).

Sipos, F. & Galamb, O. Epithelial-to-mesenchymal and mesenchymal-to-epithelial transitions in the colon. World J Gastroentero 18, 601-608 (2012).

Slattery, C., McMorrow, T. & Ryan, M.P. Overexpression of E2A proteins induces epithelial-mesenchymal transition in human renal proximal tubular epithelial cells suggesting a potential role in renal fibrosis. Febs Letters 580, 4021-4030 (2006).

Smith, J.C., Boone, B.E., Opalenik, S.R., Williams, S.M. & Russell, S.B. Gene profiling of keloid fibroblasts shows altered expression in multiple fibrosis-associated pathways. Journal of Investigative Dermatology 128, 1298-1310 (2008).

Sobrado, V.R., Moreno-Bueno, G., Cubillo, E., Holt, L. J., Nieto, M. A., Portillo, F. & Cano, A. The class I bHLH factors E2-2A and E2-2B regulate EMT. Journal of Cell Science 122, 1014-1024 (2009).

Sun, S., Sun, W., Xia, L., Liu, L., Du, R., He, L., Li, R., Wang, H & Huang, C. The T-box transcription factor Brachyury promotes renal interstitial fibrosis by repressing E-cadherin expression. Cell Commun Signal 12, 76 (2014).

Sunami, Y., Leithauser, F., Gul, S., Fiedler, K., Guldiken, N., Espenlaub, S., Holzmann, K. H., Hipp, N., Sindrilaru, A., Luedde, T., Baumann, B., Wissel, S., Kreppel, F., Schneider, M., Scharffetter-Kochanek, K., Kochanek, S., Strnad, P. & Wirth, T. Hepatic activation of IKK/NFkappaB signaling induces liver fibrosis via macrophage-mediated chronic inflammation. Hepatology 56, 1117-1128 (2012).

Tavares, A.L.P., Brown, J.A., Ulrich, E.C., Dvorak, K. & Runyan, R.B. Runx2-I is an Early Regulator of Epithelial-Mesenchymal Cell Transition in the Chick Embryo. Dev Dyn (2017).

Tiwari, N., Meyer-Schaller, N., Arnold, P., Antoniadis, H., Pachkov, M., van Nimwegen, E. & Christofori, G. Klf4 Is a Transcriptional Regulator of Genes Critical for EMT, Including Jnk1 (Mapk8). Plos One 8 (2013).

Tobin, N.P., Sims, A.H., Lundgren, K.L., Lehn, S. & Landberg, G. Cyclin D1, Id1 and EMT in breast cancer. Bmc Cancer 11, 417 (2011).

Venkov, C.D., Link, A. J., Jennings, J. L., Plieth, D., Inoue, T., Nagai, K., Xu, C., Dimitrova, Y. N., Rauscher, F. J. & Neilson, E. G. A proximal activator of transcription in epithelial-mesenchymal transition. Journal of Clinical Investigation 117, 482-491 (2007).

Wang, X., Zheng, M., Liu, G., Xia, W., McKeown-Longo, P. J., Hung, M. C. & Zhao, J. Kruppel-like factor 8 induces epithelial to mesenchymal transition and epithelial cell invasion. Cancer Res 67, 7184-7193 (2007).

Wensing, L.A. & Campos, A.H. TBX3, a downstream target of TGF-beta 1, inhibits mesangial cell apoptosis. Experimental Cell Research 328, 340-350 (2014).

Wu, X., Chen, H., Parker, B., Rubin, E., Zhu, T., Lee, J. S., Argani, P. & Sukumar, S. HOXB7, a homeodomain protein, is overexpressed in breast cancer and confers epithelial-mesenchymal transition. Cancer Res 66, 9527-9534 (2006).

Xiang, G., Yi, Y., Weiwei, H. & Weiming, W. TGIF1 promoted the growth and migration of cancer cells in nonsmall cell lung cancer. Tumour Biol 36, 9303-9310 (2015).

Xiao, L., Zhou, X., Liu, F. Y., Hu, C., Zhu, X. J., Luo, Y., Wang, M., Xu, X. X., Yang, S. K., Kanwar, Y. S. & Sun, L. MicroRNA-129-5p modulates epithelial-to-mesenchymal transition by targeting SIP1 and SOX4 during peritoneal dialysis. Laboratory Investigation 95, 817-832 (2015).

Yuan, H., Kajiyama, H., Ito, S., Yoshikawa, N., Hyodo, T., Asano, E., Hasegawa, H., Maeda, M., Shibata, K., Hamaguchi, M., Kikkawa, F. & Senga, T. ALX1 induces snail expression to

promote epithelial-to-mesenchymal transition and invasion of ovarian cancer cells. Cancer Res 73, 1581-1590 (2013).

Zhang, J., Liang, Q., Lei, Y., Yao, M., Li, L., Gao, X., Feng, J., Zhang, Y., Gao, H., Liu, D. X., Lu, J. & Huang, B. SOX4 induces epithelial-mesenchymal transition and contributes to breast cancer progression. Cancer Res 72, 4597-4608 (2012).