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DEVELOPMENT AT A GLANCE

Defining epithelial-mesenchymal transitions in animal development

Guojun Sheng

ABSTRACT

Over 50 years after its discovery in early chick embryos, the concept of epithelial-mesenchymal transition (EMT) is now widely applied to morphogenetic studies in both physiological and pathological contexts. Indeed, the EMT field has witnessed exponential growth in recent years, driven primarily by a rapid expansion of cancer-oriented EMT research. This has led to EMT-based therapeutic interventions that bear the prospect of fighting cancer, and has given developmental biologists new impetus to investigate EMT phenomena more closely and to find suitable models to address emerging EMT-related questions. Here, and in the accompanying poster, I provide a brief summary of the current status of EMT research and give an overview of EMT models that have been used in developmental studies. I also highlight dynamic epithelialization and de-epithelialization events that are involved in many developmental processes and that should be considered to provide a broader perspective of EMT. Finally, I put forward a set of criteria to separate morphogenetic phenomena that are EMT-related from those that are not.

KEY WORDS: EMT, MET, Cancer metastasis, Developmental models, Epithelium, Mesenchyme

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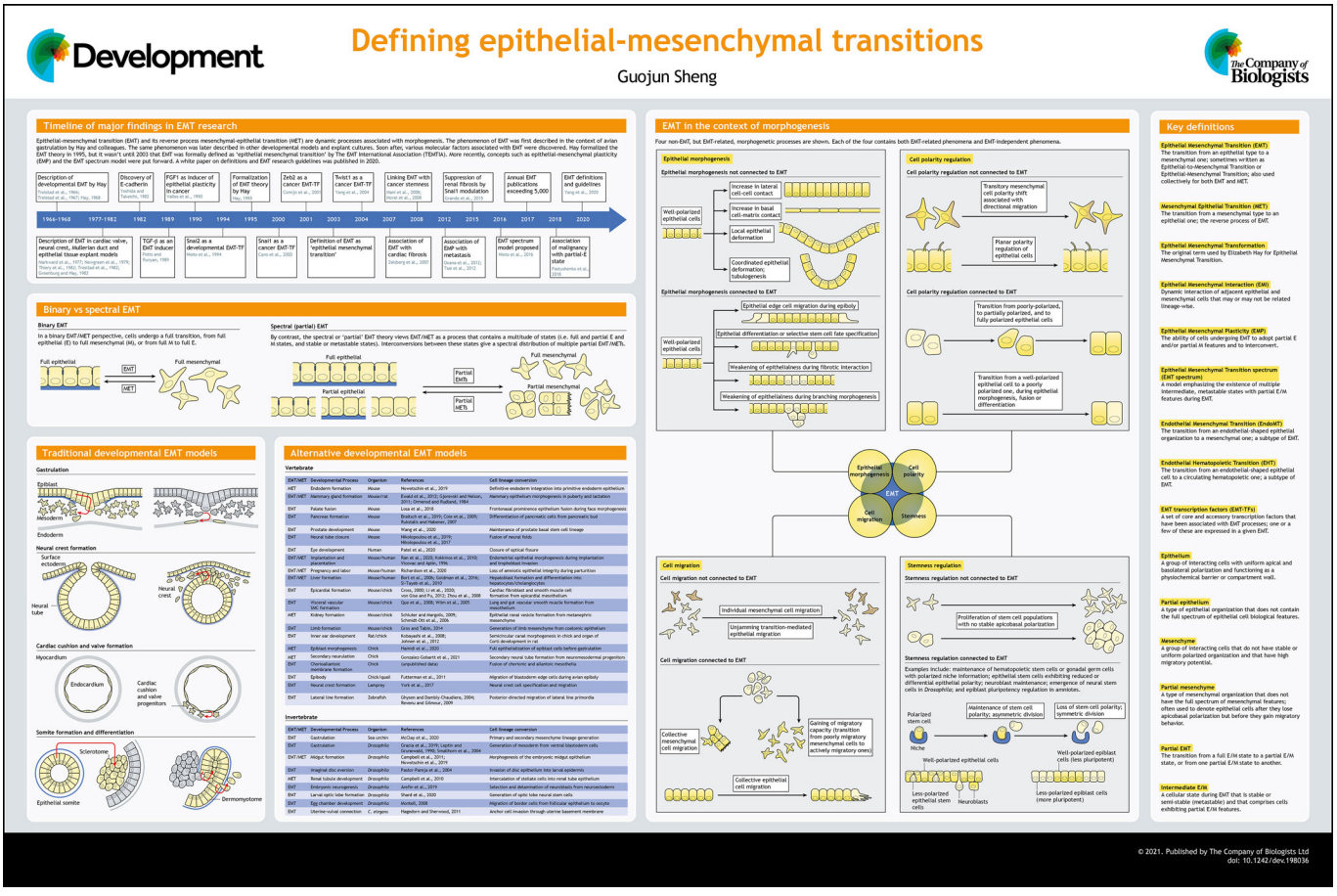
Introduction

Epithelial-mesenchymal transition (EMT) and its reverse process mesenchymal-epithelial transition (MET) are dynamic processes – collectively referred to as EMTs – that are associated with multicellular morphogenesis during animal development (Thiery et al., 2009; Yang and Weinberg, 2008). The concept of EMT originated in the late 1960s in the wake of modern cell biology. At this time, Elizabeth Hay and colleagues, using electron microscopy



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techniques, first discussed EMT phenomena when investigating subcellular organization and intercellular contacts in gastrulation-stage chick embryos (Hay, 1968; Trelstad et al., 1967, 1966). The EMT concept was then extended to include similar phenomena of multicellular morphogenetic transformations *in vitro* (Greenburg and Hay, 1982) and in cancer (Valles et al., 1990), and was formalized in the following decade (Hay, 1995); for a timeline of landmark events in EMT, please see the accompanying poster. Epithelial-mesenchymal transformation, the original term used by Hay, was changed to epithelial-mesenchymal transition at the first meeting of The EMT International Association (TEMTIA) in 2003 to avoid direct association with tumorigenic transformation, although both terms had been in use in the literature since the late eighties. Rooted in developmental biology, EMT is now widely studied in many disease settings, with data from pathological EMTs making up the majority of EMT-related papers published yearly (Yang et al., 2020).

This article provides an overview of EMT models that have been studied in the context of animal development and are potentially of interest to researchers studying EMT in the context of cancer. It highlights the relevance of developmental models to evolving conceptual debates based largely on data obtained from disease EMTs. The concepts of partial EMT and epithelial-mesenchymal plasticity (EMP), emerging themes in EMT studies, are also discussed. Finally, an overview of the molecular markers associated with EMT, together with guidelines on how best to consider these markers, is provided.

EMT: cell biology and terminology

Epithelial cells are viewed as having all or some of the following cell biological characteristics: apicobasal polarity, tight junctions, adherens junctions, gap junctions, desmosomes, hemidesmosomes, keratin-based intermediate filaments, cortical actin fiber bundling and specialized basal and/or apical extracellular matrices (basement membrane and apical ECM, respectively). Most epithelial tissues in animal development, however, do not contain this full spectrum of epithelial characteristics, and molecular components generally associated with these subcellular structures are not uniquely or universally present in epithelial cells (Nakaya and Sheng, 2013). Expression of E-cadherin (CDH1), for example, can be detected in non-epithelial cells (Filimonow et al., 2019), and many epithelial structures such as the vascular endothelium lack CDH1 expression altogether (Giampietro et al., 2012; van Roy, 2014). Even when the same developmental process is being discussed (e.g. gastrulation or neural crest formation), the types and dynamics of cadherin expression may be model organism-dependent. Thus, instead of using molecular markers, an epithelium can be better defined as a group of cells that are collectively polarized with shared apical and basolateral surfaces and functioning as a physiochemical barrier between two compartments (Hay, 1995; Nakaya and Sheng, 2013; Yang et al., 2020).

Mesenchymal cells, on the other hand, are defined as non-epithelial cells. They do not maintain stable cellular polarity or cell-cell and cell-matrix interactions, have vimentin-based intermediate filaments and actin stress fibers, and are more migratory than epithelial cells. In development, cells often have to move long distances from their place of origin to their final destination. According to Hay, such migration is better achieved through mesenchymal-type migration, as opposed to intra-epithelial migration or epithelial tubular morphogenesis. Molecular markers associated with mesenchymal cells are N-cadherin (CDH2), vimentin and EMT-promoting transcription factors (e.g. members of the Twist, Snail and Zeb families) and signaling molecules (e.g. TGF-β). As in the case of epithelial organization, phenotypic diversity in mesenchymal organization is also common. Examples of non-typical mesenchymal structures and cells are mammalian inner cell mass cells and epiblast cells before their epithelialization, which are non-uniformly polarized and non-migratory because of spatial confinement, and neural crest cells, which maintain stable front-back polarization and cell-cell interactions during collective migration.

Variations in epithelial and mesenchymal cellular organizations and interconversions have been described using various terms. These include: fully epithelial (full-E state; exhibiting all or most epithelial characteristics), fully mesenchymal (full-M state; exhibiting all or most mesenchymal characteristics), partially epithelial (partial-E state; exhibiting some epithelial characteristics above the minimal requirement but not meeting the criteria to be classified as an M state), and partially mesenchymal (partial-M state; not meeting the minimal requirement for an epithelium, but also not exhibiting strong full-M features). Likewise, transitions between any two states from the full-E to the full-M can be viewed as either full or partial EMT, depending on the start/end points of a given transition under investigation. The multitude of intermediate states (which can be either stable or partially stable) is referred as the EMT spectrum (Nieto et al., 2016; Yang et al., 2020). The hybrid states (i.e. partial-E or partial-M) that a cell can adopt, and the dynamic, reversible nature of transitions between states, is referred to as EMP (Pinto et al., 2013; Tsai and Yang, 2013; Yang et al., 2020).

Traditional and non-traditional developmental models of EMT

Several developmental processes and models have long been used for studies of EMT. These include gastrulation (Bardot and Hadjantonakis, 2020; Keller et al., 2003; Nakaya and Sheng, 2008; Yamashita et al., 2004), neural crest formation (Ahsan et al., 2019; Piacentino et al., 2020; Shellard and Mayor, 2019), somitogenesis (Christ and Scaal, 2008; Kalcheim and Ben-Yair, 2005; Nakaya et al., 2004; Pourquie, 2018; Takahashi et al., 2005) and cardiac cushion and valve formation (Nath et al., 2008; Person et al., 2005; Tavares et al., 2018; von Gise and Pu, 2012; Wittig and Munsterberg, 2020).

In amniotic vertebrates (i.e. mammals, birds and reptiles), gastrulation generates three germ layers from a pluripotent epiblast sheet that needs to reach a full-E state before the onset of gastrulation. Amniote gastrulation EMT that gives rise to migratory mesoderm cells is a full EMT (Nakaya and Sheng, 2008). By contrast, gastrulation EMT in amniotes that gives rise to definitive endoderm cells may be viewed as a partial EMT because soon after it occurs, definitive endoderm precursor cells undergo an MET before becoming fully migratory, and polarize and intercalate themselves into a pre-existing primitive endoderm-derived epithelium (Bardot and Hadjantonakis, 2020). Gastrulation in anamniotes and invertebrates (e.g. in amphibian, sea urchin and fly models) also involves EMT (Keller et al., 2003; McClay et al., 2020; Smallhorn et al., 2004; Yamashita et al., 2004), but the pre- and post-gastrulation tissue architectures in these species are different to those in amniotes, and caution is needed when associating EMT with gastrulation in general.

The neural crest is an ectoderm-derived, vertebrate-specific cell population that gives rise to a diverse array of cell types. Neural crest precursor cells are specified at the border between neural and epidermal ectoderm territories. They then undergo delamination. The delamination is an EMT process that is linked to, but distinct from, the neurulation process that generates the central nervous

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system (Ahsan et al., 2019; Piacentino et al., 2020; Shellard and Mayor, 2019). Post-delamination neural crest cells undergo extensive migration to reach their lineage-dependent destinations. Neural crest EMT has been studied in the context of the both delamination and the subsequent migration of neural crest cells. Both events are considered as partial EMTs because post-delamination and migrating neural crest cells maintain a collective front-back cellular polarity with dynamic intercellular contacts, although individual neural crest cell-based migration is also observed in chick and mouse models.

Somitogenesis is the process that generates somites, which are paired, metameric structures that give rise to axial bones, dorsal dermis and axial and peripheral skeletal muscles (Christ and Scaal, 2008; Pourquie, 2018). Somites are organized from mesenchymal-shaped presomitic mesoderm (PSM) through periodic budding controlled by cyclic and coordinated cellular polarization/epithelialization events at the tip of the PSM. This is an MET process (Nakaya et al., 2004; Takahashi et al., 2005). Subsequent morphogenesis of the somites, to give rise to the sclerotome, myotome and dermatome, involves de-epithelialization of the epithelized somite and can be viewed as containing at least three separately regulated EMT processes, with sclerotome EMT taking place first, followed by myotome EMT and, finally, by dermatome EMT (Kalcheim and Ben-Yair, 2005; Nakaya and Sheng, 2013).

Cardiac valves form primarily from a tissue known as the endocardium, which is the endothelial inner lining of the heart (Wittig and Munsterberg, 2020). The basal delamination of endocardial cells into the cardiac jelly, a special ECM secreted by the myocardium (heart muscle), generates mesenchymal-shaped endocardial cushion cells and, later, cardiac valve cells (Nath et al., 2008; Person et al., 2005; Tavares et al., 2018; von Gise and Pu, 2012). This process is considered to involve partial EMT, as post-delamination cells are spatially confined and not fully migratory.

In addition to the well-studied EMT processes mentioned above, EMT events can also be found in over 30 developmental processes (in both vertebrate and invertebrate animals) that are not well known for EMT research; see the associated poster for details. Each of these additional examples of EMT/MET has its own unique developmental and tissue context, and involves both generalizable and process-specific molecular regulators. As the EMT concept is applied to more studies, it is expected that novel EMT models will emerge and some of them may be more suitable for experimental investigation than those listed above.

Partial EMT and EMP

Partial EMT is an important notion that has emerged in recent years from both developmental- and disease-based EMT studies (Hamidi et al., 2021; Nieto et al., 2016; Sha et al., 2019). The key assumption of partial EMT is that most morphogenetic transitions that can be considered as EMT are in fact partial ones, from either a full E/M state to a partial E/M state, or from one partial E/M state to another partial E/M state. Partial EMT implies: (1) that there exists one or more intermediate E/M states that are different from the full E and full M states; (2) that these intermediate states are metastable, meaning that they are not transitory and that it is possible to describe them with molecular characteristics and capture them experimentally; and (3) that transition(s) between these states may be more plastic than previously thought, and interconversion between partial E/M states may occur dynamically with appropriate physicochemical cues. By replacing binary E/M transitions with spectral, quantum-like, shifts among intermediate E and M states (Nieto et al., 2016), the partial EMT concept offers a framework under which most EMT processes can be studied and compared with each other.

The widespread observation of partial EMTs has also led to the introduction of EMP as a key emerging concept in EMT research. EMP refers to the hybrid E/M features exhibited by many EMT cells, to the stable or metastable states that those cells can occupy along the EMT spectrum, and to the dynamic nature of transitions between cells occupying those states. The partial EMT and EMP concepts have recently attracted strong interest from scientists working in the field of mathematical and computational biology, empowered with tools that allow better and more accurate descriptions of partial EMT, intermediate E/M states and EMP (Celia-Terrassa et al., 2018; Jolly and Levine, 2017; Tripathi et al., 2020; Xing and Tian, 2019).

Markers and regulators of EMT

TEMTIA recently published a white paper (Yang et al., 2020) stressing the importance of returning EMT to its cell biology roots without downplaying the value of molecular markers in EMT studies. Key to the proper use of molecular markers in EMT research is to understand the cellular context of these markers and regulators. A four-point guideline, emphasizing cell biology and using a combinatorial approach in assessing EMT molecular markers, was the cornerstone of the white paper. First, this guideline states that it is not advisable to use one or a small number of molecular markers to describe or assess EMT status. Most EMTs occur with unique and complex cellular contexts and require cooperation between many molecular factors. For example, levels of the adherens junction marker CDH1 or the intermediate filament maker cytokeratin are not reliable indicators of EMT status (Savagner, 2015). Neither are levels of EMT-associated transcription factors, e.g., members of the Snail, Zeb and Twist families (Stemmler et al., 2019; Yang et al., 2020). Second, the guideline emphasizes that changes in cell biological properties should be used as the primary criteria for defining EMT status. Cellular morphogenesis involved in EMT can and should be described in precise, unambiguous cell biological terms. If possible, cell biological changes should be the primary descriptors of EMT and EMT status [see Yang et al. (2020) for references of cell biological features of importance to EMT regulation]. This may be supported by a combination of molecular markers, without relying solely on one or a few molecular markers. Third, the guideline recommends that, although EMT-associated transcription factors are valuable indicators of EMT, they should be used in assessing EMT status in conjunction with changes in cellular characteristics and with knowledge of the non-EMT associated functions of these transcription factors. To date, all EMT processes appear to involve at least one of several core EMT-associated transcription factors (Snai1, Snai2, Zeb1, Zeb2 and Twist1), strongly suggesting a certain level of conservation in EMT-related transcriptional regulation. However, the absolute level of any single EMT-associated transcription factor is often not a good indicator of EMT status. Furthermore, it remains possible that some EMT processes can be regulated transcriptionally by factors other than the five core transcription factors [see Yang et al. (2020) for a list of core and associated EMT-related transcription factors]. Finally, the guideline highlights that finding reliable EMT status markers will require a combinatorial approach and a clear distinction between EMT-associated and non-EMT-associated functions of many EMT markers. This last point outlines a scenario in which EMT status and EMP regulators can be assessed objectively and compared across diverse biological contexts. The relevance of developmental EMT studies to cancer biology, for instance, would

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rely on the assumption that cellular and molecular insight gained by studying developmental EMT processes can be directly applied to a comparable disease EMT process. It is the long-term goal of the EMT research community to capture EMP and the multitude of partial EMTs with a common set of cellular and molecular descriptors applicable to both physiological and pathological EMTs.

Considering EMT-related morphogenetic phenomena

In multicellular systems either *in vivo* (e.g. metazoans and tumors) or *in vitro* (e.g. organoids), cells can generally be categorized as having either an epithelial or a mesenchymal organization. As discussed above, cells adopting an epithelial organization have a collectively uniform and relatively stable apicobasal polarity and perform barrier functions within a multicellular system. In contrast, cells taking on a mesenchymal organization have non-uniform and/or transitory polarization and dynamically occupy the space created by epithelial barriers. This broad categorization implies that the study of EMT runs the risk of encompassing all morphogenetic processes taking place in a multicellular system. Meaningful discussion of EMT therefore requires disentanglement of EMT processes from other EMT-related morphogenetic phenomena. These phenomena include, but are not limited to: the morphogenesis of epithelial tissues (e.g. changing from a flat epithelium to a curved or tubular one, or from a cuboidal epithelium to a columnar one); the dynamic regulation of cellular polarity (e.g. the direction of mesenchymal cell migration and transitory gain/loss of epithelial polarity); the migration of epithelial or mesenchymal cells (e.g. during wound healing, collective mesenchymal migration or intraepithelial migration controlled by a newly described process called unjamming transition; Mitchel et al., 2020; Mongera et al., 2018); and the regulation of pluripotency and/or other stem cell properties within an epithelial or mesenchymal context [e.g. prostate basal stem cells within the prostate epithelium (Wang et al., 2020); hematopoietic stem cells in a polarized niche environment (Hamidi and Sheng, 2018)]. These non-EMT morphogenetic phenomena have EMT-related features as well as EMT-independent ones. Both types of features may receive molecular inputs similar to those used in EMT regulation and produce interacting and overlapping cellular outputs. Therefore, in order to gain mechanistic insight into EMT-specific regulation and achieve therapeutic intervention of pathological EMTs, it is paramount for developmental, cell and cancer biologists to discuss EMT processes with an awareness of other morphogenetic processes taking place in conjunction with EMT.

Conclusions and perspectives

EMT is an exciting research field, the emerging theme of which is that EMT is context-dependent and phenotypically diverse. No two EMTs are alike, and transitions are spectral and plastic. Developmental EMTs do not come in isolation, but rather take place in conjunction with other, distinct morphogenetic processes. Importantly, it is clear that studies of developmental EMTs have laid the foundation for this field, and their diversity and reproducibility serve as a rich resource for understanding cancer EMTs.

In recent years, the EMT research field has sustained exponential growth in research output and is attracting attention from scientific communities that a decade ago had little shared interest with the traditional three pillars of EMT research (developmental, cell and cancer biologists). TEMTIA biennial meetings provide a forum to bring these diverse disciplines together. A better understanding and appreciation of the dynamic complexity of developmental EMT models (as well as of cancer EMT models) will be an important starting point for achieving mechanistic insight through cross-disciplinary collaborations. For developmental biologists, looking at their favorite developmental problems through the lens of EMT may bring about conceptual clarity in disentangling morphogenesis phenomena surrounding a given developmental process. By doing so, this approach could eventually help find the most appropriate physiological models for cancer EMT studies. For cancer biologists, the characterization of partial EMTs and EMP in normal physiological settings can provide crucial templates in unravelling seemingly chaotic molecular and cellular behaviors during cancer progression. Together, this combinatorial and multi-angled approach will lead to a better understanding of how multicellular systems organize their morphological and functional compartmentalization, the disruption of which appears to underlie many human diseases.

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Competing interests

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