



Mini-review

EMT in developmental morphogenesis



Yukiko Nakaya, Guojun Sheng*

Lab for Early Embryogenesis, RIKEN Center for Developmental Biology, 2-2-3 Minatojima-minamimachi, Chuo-Ku, Kobe, Hyogo 650-0047, Japan

ARTICLE INFO

Keywords:

Developmental EMT
Epithelium
Stratification
Mesenchyme
Amniote
Vertebrate
Mammals
Birds
Cancer

ABSTRACT

Carcinomas, cancers of epithelial origin, constitute the majority of all cancers. Loss of epithelial characteristics is an early step in carcinoma progression. Malignant transformation and metastasis involve additional loss of cell-cycle control and gain of migratory behaviors. Understanding the relationships among epithelial homeostasis, cell proliferation, and cell migration is therefore fundamental in understanding cancer. Interestingly, these cellular events also occur frequently during animal development, but without leading to tumor formation. Can we learn anything about carcinomas from developmental biology? In this review, we focus on one aspect of carcinoma progression, the Epithelial–Mesenchymal Transition (EMT), and provide an overview of how the EMT is involved in normal amniote development. We discuss 12 developmental and morphogenetic processes that clearly involve the EMT. We conclude by emphasizing the diversity of EMT processes both in terms of their developmental context and of their cellular morphogenesis. We propose that there is comparable diversity in cancer microenvironment and molecular regulation of cancer EMTs.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Epithelial–Mesenchymal Transition (EMT) and Mesenchymal–Epithelial Transition (MET) are fundamental processes of cell shape changes during animal development and disease progression (collectively referred to as EMT here, except when a distinction is needed) [1]. Many excellent reviews have covered this subject in recent years [2–6]. Its involvement in cancer underscores the need for better molecular and cellular understanding of EMTs during normal development. Developmental EMTs exhibit a wide range of phenotypic variations. For developmental models to be useful in cancer studies, one needs to appreciate unique morphogenetic parameters associated with each developmental EMT. In this review we survey developmental EMTs with an emphasis on their morphogenetic variables. The examples given are not meant to be an exhaustive list, but to highlight the wide presence of EMT events in development and the variations in their cellular organization. Many of them represent important but transitory morphogenetic events and do not have cancer counterparts. But many occur during organogenesis and have a direct link, in cellular origin and intercellular organization, to organ-specific cancer EMTs in adults. We will also provide a general discussion on epithelial structures in development and propose a conceptual framework to integrate diverse EMT phenomena in cell, developmental and cancer biology.

2. Developmental EMTs with no cancer association

2.1. Blastula formation

The earliest MET during mammalian development takes place when blastomeres differentiate into three primitive cell lineages: the trophoblast, epiblast and hypoblast [7]. This process actually consists of three separate METs. The MET of the trophoblasts generates trophoblast, the first epithelial structure in a mammalian embryo (Fig. 1A, left). The second and third METs epithelialize the hypoblast and epiblast cells, respectively, from the inner cell mass (Fig. 1A, right). Formation of these three cell lineages has often been studied from the perspective of fate specification and pluripotency maintenance, but of equal importance and involving distinct molecular mechanisms is the cell biological regulation of these MET processes [8–16]. Early mammalian development is heavily influenced by maternal environment. The timing of blastomere compaction, trophoblast epithelialization, and epiblast and hypoblast epithelialization, relative to that of fate specification, varies among mammalian species. Differences of these METs in primates and rodents may underlie difficulties in applying the advances in the ES cell field, mostly derived from the mouse model, to the humans [17,18].

2.2. Gastrulation and neural crest cell formation

Gastrulation and neural crest cell formation are two major examples of developmental EMT. They have been reviewed extensively and are described here only briefly. In gastrulation EMT,

* Corresponding author. Tel.: +81 78 306 3132 (Lab); fax: +81 78 306 3146.
E-mail address: sheng@cdb.riken.jp (G. Sheng).

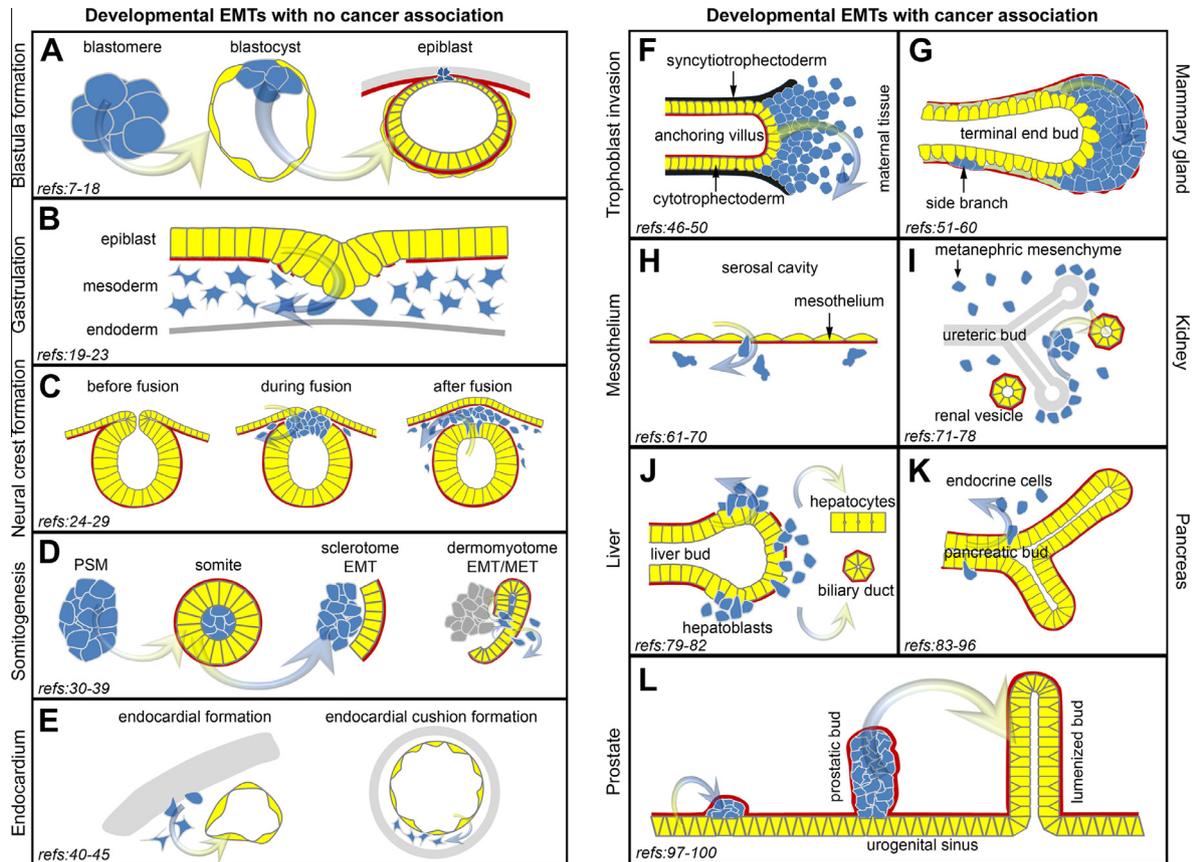


Fig. 1. Developmental EMTs. (A–E) Developmental EMTs with no direct cancer association. (A) Polarization and epithelialization of some blastomeres result in trophoblast formation (1st MET arrow). The inner cell mass is still mesenchymal at this stage. Epithelialization of the inner cell mass generates two additional epithelia (2nd MET arrow): the epiblast and the hypoblast. (B) During gastrulation, epiblast cells undergo EMT to form mesoderm cells. (C) Neural crest cells leave the ectoderm during and after neural tube closure. (D) Somitic mesoderm formation and differentiation involve several rounds of MET/EMT. PSM: presomitic mesoderm. (E) Formation of endocardium (the endothelial lining of the heart) involves MET. Endocardial cushion and cardiac valve formation involves EMT. (F–L): Developmental EMT with cancer association. (A) Anchoring chorionic villus; (B) Terminal end bud of the mammary gland; (C) Mesothelial cells; (D) Renal vesicles; (E) Liver bud; (F) Pancreatic bud; (G) Prostate gland. Epithelial structures: yellow; mesenchymal structures: blue; basement membrane: red. EMT: yellow to blue arrow. MET: blue to yellow arrow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mesenchymal-shaped mesoderm cells are generated from the epithelial-shaped epiblast [19–23] (Fig. 1B). This is the earliest developmental EMT. During the neural crest cell formation, precursor cells delaminate from border regions of neural and non-neural ectoderm territories prior to and during neural tube closure and from dorsal neural tube after the closure [24–29] (Fig. 1C). Both are stereotypic EMT events because mesoderm and neural crest precursor cells are polarized epithelial cells with a basement membrane before EMT and start active migration after EMT. It is worth pointing out that both EMTs exhibit variations in terms of species, regions and developmental time, and should not be generalized. There is no direct cancer association for gastrulation or neural crest cell formation EMT. Postnatally, many cancers are of the mesoderm origin, and neural crest cells are involved in melanoma and neuroblastoma.

2.3. Somitogenesis

Somites are derived from the paraxial mesoderm and begin as mesenchymal-shaped presomitic mesoderm. Segmentation clock regulates periodic budding of somites from its anterior tip [30]. Newly generated somite polarizes to form an epithelial ball, with an internal apical surface and external basement membrane (Fig. 1D, left). This is a MET process [31–33]. Each somite can be broadly divided into two subdomains: the sclerotome and dermomyotome. Cells in the sclerotome subdomain, located ven-

tromedially, lose their epithelial characteristics first [34] (Fig. 1D, middle). Somitocoel cells, located inside the epithelialized somite, remain mesenchymal and contribute to the sclerotome without undergoing MET or EMT. Dermomyotome cells undergo complex EMT and MET processes to generate the myotome (later on giving rise to skeletal muscles) and an EMT process to generate the precursors of dorsal dermis [35–39] (Fig. 1D, right). Formation and dissolution of somites thus involve several rounds of extremely dynamic MET/EMTs.

2.4. Endocardium and endocardial cushion formation

Cardiac morphogenesis is important for embryonic survival. The endocardium is the endothelial lining of the heart [40] and three successive EMT/METs are involved in its formation and differentiation [41]. Cardiac mesodermal precursor cells undergo EMT during gastrulation, when these cells are specified as cardiac progenitors. After subsequent bilateral migration within the lateral plate, mesenchymal-shaped endocardial progenitors separate from myocardial progenitors before epithelialization of the latter. The endocardial progenitors epithelialize to form endothelial plexus and then tubes (Fig. 1E, left). This is a MET process. Early embryonic heart contains inner endocardial layer and outer myocardial layer, separated by cardiac jelly, the extracellular matrix secreted mainly by the myocardium. Endocardial cells delaminate basally in the atrioventricular canal and outflow tract into this extracellu-

lar matrix, contributing to the endocardial cushion in these areas and cardiac valves later in development [41] (Fig. 1E, right). This is an EMT process. In addition, similar to endocardial formation, MET is involved in vacuologenesis in the entire embryo [42–44], and further EMT is involved in hematopoietic stem cell formation from aortic endothelium [45].

3. Developmental EMTs with cancer association

3.1. Trophoblast invasion

The trophoblast is a special type of primitive ectoderm and its origin in development precedes that of the three germ layers. Trophoblast cells (trophoblasts) mark the external boundary of a mammalian embryo and mediate materno-fetal exchanges during placental development. To achieve optimal contact with maternal environment, trophoblasts proliferate, invade and branch out within uterine endometrium. In most areas, the trophoblasts form a cytotrophoblast inner layer and a syncytiotrophoblast outer layer (Fig. 1F). The former is a stereotypic epithelial sheet with a basement membrane. The latter is a fusion product of the former and covers its apical surface. EMT occurs in the anchoring villi and some floating villi where cytotrophoblasts stratify and breach the syncytiotrophoblast layer, forming extra-villous and interstitial cytotrophoblasts with mesenchymal characteristics [46–50] (Fig. 1F). This EMT occurs apically and the inner-most cytotrophoblast layer remains epithelial and has an intact basement membrane. Associated cancer: Chorionic carcinoma.

3.2. Mammary gland

The mammary gland is an ectoderm-derived organ [51–54]. Thickening of mammary placode ectoderm is followed by local epithelial cell movement, leading to formation of mammary bud [55]. Cells within the mammary bud partially lose their epithelial characteristics, but re-epithelialize to form rudimentary mammary gland [56]. This early phase of mammary gland development thus involves partial EMT and MET processes. Onset of puberty initiates rapid ductal growth and morphogenesis, and pregnancy and lactation cycles regulate the formation and involution of milk-producing mammary lobular alveoli. Ductal elongation and bifurcation during puberty occur primarily at the terminal end buds (TEBs), the front of stromal invasion for the ectoderm-derived mammary branches. The TEB is a multi-layered epithelioid structure and is covered at its leading edge by a broken basement membrane [51,57,58] (Fig. 1G). Its luminal layer, and to some extent the basal layer, have partial epithelial characteristics, but its inner layers have mesenchymal features [59]. The TEB also engages in collective cell migration [59,60]. Its formation therefore can be viewed as partial EMT. Side branching also contributes to ductal growth and morphogenesis and may likewise involve similar partial EMT (Fig. 1G). Associated cancer: Breast cancer is the leading cancer in women.

3.3. Mesothelium

The mesothelium is a thin layer of epithelium lining the serosal (peritoneal, pleural and pericardial) cavities [61]. Derived from the lateral plate mesoderm during germ layer formation, the mesothelium is a genuine epithelium with tight junctions, a basement membrane, and an apical surface facing the serosal cavities. Formation of the mesothelium thus involves both EMT and MET processes. After their formation, mesothelial cells can undergo further EMTs (Fig. 1H) to generate coronary vascular smooth muscle cells and cardiomyocytes in the pericardial mesothelium [62–64], pulmonary vascular smooth muscle cells and lung mesen-

chyme in the pleural mesothelium [65] and gut vascular smooth muscle cells in parietal peritoneal mesothelium [66]. EMT is also involved in generating sertoli cells from the mesothelium covering the male gonad [67] and the granulosa cells from the mesothelium of the ovary [68,69]. Furthermore, EMT has been implicated in the repair of ovarian mesothelium after ovulation and in morphological responses of the granulosa cells to follicle activation [68,70]. Associated cancer: Mesothelioma is the leading asbestos-related cancer. Ovarian epithelial carcinoma makes up 90% of all ovarian cancer.

3.4. Kidney

Kidney is a mesoderm-derived organ. Adult kidney development begins when ureteric bud at posterior part of the nephric duct grows into surrounding metanephric mesenchyme [71–73]. The ureteric bud is an epithelial structure (formed at a much earlier stage of development through MET of the intermediate mesoderm) and remains so during its branching morphogenesis. The metanephric mesenchyme cells organize themselves around branching ureteric buds, coalesce into aggregates, and epithelialize to form renal vesicles (Fig. 1I) [74,75]. Renal vesicles are surrounded by a basement membrane and have an apical lumen. This is a typical MET process [72,75–77]. Renal vesicles undergo further tubular morphogenesis to give rise to renal tubules and non-vascular part of renal glomeruli [78]. Associated cancer: Renal cell carcinoma is the most common kidney cancer and is caused by malignant transformation of renal tubule epithelial cells.

3.5. Liver

The liver starts off as liver bud, a ventral diverticulum of the foregut [79]. Hepatoblasts in the liver bud are initially of an epithelial morphology and are surrounded by a basement membrane. The hepatoblasts proliferate and thicken to form a pseudostratified epithelium at the tip of the liver bud. This is followed by breaches in the basement membrane, and hepatoblasts delaminate from the liver bud epithelium [80,81] (Fig. 1J, left). This is a typical EMT process. Mesenchymal hepatoblasts aggregate around the portal vein and differentiate into two types of epithelial cells: the biliary epithelial cells and hepatocytes (Fig. 1J, right). MET is involved in the formation of both. The biliary epithelium is a simple, stereotypic epithelium with an apical lumen and a basal basement membrane [82]. The hepatocytes form a special epithelial structure [79]. It has no basement membrane, but its basal side faces the sinusoid endothelial cells and its apical side faces the canalicular lumen. Tight junctions separate the basolateral membrane from the apical membrane, from which bile acids and salts are secreted. Associated cancer: Hepatocellular carcinoma and cholangiocarcinoma.

3.6. Pancreas

The pancreas is an endoderm-derived organ [83,84]. All of its three main cell lineages, the exocrine, ductal and endocrine cells, originate from the pancreatic bud epithelium. Pancreatic buds are outgrowths of the foregut. Their formation involves local cell proliferation, lumenization and tubulogenesis [85]. During this process, pancreatic bud cells are collectively bound by an intact basement membrane. These cells transit from an initial foregut epithelial state to a stratified epithelioid and then back to an epithelial one [85,86], involving partial EMT and MET. From this secondarily formed epithelium, pancreatic endocrine precursors undergo a typical EMT process by breaking down the basement membrane and leaving the epithelium [87–89] (Fig. 1K). Delaminated cells coalesce to form pancreatic islets that secrete insulin, glucagon and other pancreatic hormones into circulation. Endocrine islet cells are generally considered to be mesenchymal be-

cause they do not have a typical epithelial organization and have no lumen or basement membrane. But this notion may need revision. Each islet is enclosed by a sheet of basement membrane externally, and internally-located islet cells rest on the basement membrane of infiltrated vasculature [90–94]. They have asymmetrical membrane preference for glucose transporter localization [95] and insulin secretion [96]. Formation of pancreatic islets therefore represents a special type of MET. Associated cancer: Pancreatic cancer is the fourth most common cancer and has among the lowest survival rate after diagnosis.

3.7. Prostate

The prostate gland is derived from androgen-regulated endodermal outgrowths of the urogenital sinus [97,98]. These outgrowths (prostate buds) are bound by the basement membrane [99], but cells in the buds form solid epithelioid mass with no lumen (Fig. 1L, left). Lumenization of prostate buds can therefore be viewed as a partial MET process (Fig. 1L, right). Epithelialized buds then undergo branching morphogenesis and cellular differentiation, making up the endoderm portion of the prostate gland. Partial reversion of this MET in adult may underlie prostatic intraepithelial neoplasia, an early stage of prostate cancer [98,100]. Associated cancer: Prostate cancer tops new cancer cases and is the second most deadly cancer in men.

4. What is an epithelium?

A stereotypic epithelium is generally considered to have the following characteristics: (1) It is a sheet of cells with a shared apico-basal polarity (e.g., polarities in membrane lipids and proteins, in intracellular molecular localization, in vesicular transport, and in cytoskeletal and organellar organization); (2) There is no free passage of membrane lipids and proteins between the apical and basolateral compartments (e.g., through lipid rafts); (3) There is no free

passage of extracellular molecules between the apical and basolateral extracellular space (e.g., by forming tight junctions); (4) their lateral membrane adheres to each other (e.g., through adherens junctions); (5) Their basal membrane interacts with a specialized extracellular matrix, the basement membrane (e.g., through integrins and dystroglycan) [2–4,6,20]. Cells organized this way are considered to be fully epithelial (Fig. 2A). However, although these cellular features are interconnected in their molecular regulation, many epithelial-like structures do not have this full set of characteristics (Fig. 2A). This is especially true during embryonic development, when intercellular organizations are modified constantly to accommodate for dynamic growth, morphogenesis and pattern formation. We propose that the minimal criterion of an epithelial structure is for its constituent cells to have a shared apico-basal polarity. A group of cells without this minimal feature should be called mesenchymal. If these mesenchymal cells are not migratory and are constrained spatially, they can be viewed as having an epithelioid mesenchymal structure (Fig. 2A) (e.g., in luminal neoplasia of many cancers and in the terminal end bud of growing mammary gland). Transition from an epithelial structure (either full or partial epithelium) to this epithelioid mesenchymal structure can therefore be considered as a partial EMT process. Molecularly, this basic characteristic of an epithelium, the apico-basal polarity, is achieved through three polarity complexes: the PAR, the Crumbs and the Scribble complexes [4]. The PAR complex contains PAR3, PAR6 and aPKC, and sets the boundary between the apical and basolateral membrane compartments. The Crumbs complex contains transmembrane protein Crumbs and cytoplasmic proteins PLAS1 and PATJ, and controls the apical membrane formation. The Scribble complex contains Scribble, LGL and DLG, and maintains the basolateral membrane compartment. Mutations in many of the apicobasal polarity complex genes have been associated with cancer EMTs [4] and known EMT inducers, such as Snail and ZEB1, can suppress transcription of adherens junction gene *E-cadherin* and cell polarity genes *Crumbs3*, *Pals1* and *Lgl2* [101–103].

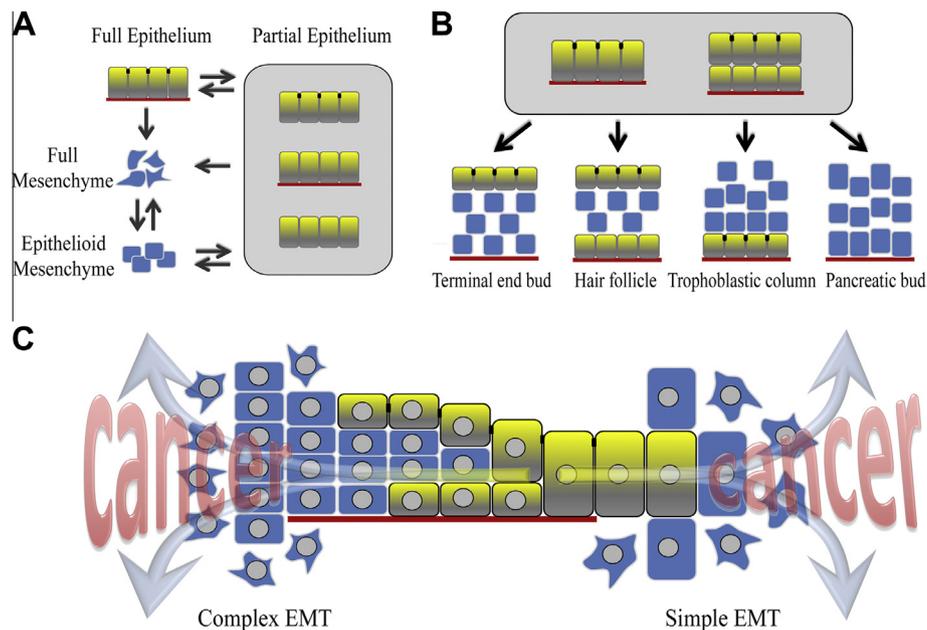


Fig. 2. Partial EMT, epithelial stratification and cancer EMT. (A) Between fully epithelial and fully mesenchymal structures, one can recognize partial epithelial and partial (epithelioid) mesenchymal structure. A partial EMT may mean one of the several stepwise transitions needed to convert a full epithelium to a full mesenchyme, or the modest change from a partial epithelium to epithelioid mesenchyme by losing coordinated apico-basal polarity. (B) Stably-maintained stratified epithelium can be considered as an epithelial unit. Unstable stratification causes temporary loss of epithelial characteristics. This happens both in development and during cancer progression. (C) A model for how graded loss of epithelialness can lead to cancer. The stepwise nature of this transition is emphasized in both simple and complex EMTs. In cancer biology, this implies that multiple independent molecular lesions are required for malignant transformation.

5. Epithelial stratification and EMT

In most cases, epithelial structures exist as a single-cell-layered, two-dimensional sheet. Further morphogenetic modifications often result in forming complex, three-dimensional tissue architecture from such a two-dimensional sheet (e.g., lung, ureter). Occasionally, single-layered epithelial structure can be organized as a three-dimensional cell mass through special types of apico-basal polarization (e.g., among hepatocytes or pancreatic endocrine cells) [79,91]. Epithelial structures can also be found as multi-layered, stratified cell sheets (e.g., mammary duct, skin epidermis). Because all stratified epithelia originate in development as single-cell-layered epithelial structures, a stably-organized stratified epithelium can be viewed as one polarized epithelial unit. Maintenance of a stratified epithelium, however, requires delicate balance of proliferation, mitotic spindle orientation, cell loss, and cross-talk between layers [104], and failure to do so often leads to cancer. In many normal developmental scenarios (Fig. 2B), epithelial stratification also leads to loss of epithelial characteristics among some or all of its constituent cells. This can be viewed as partial EMT. Often times, the non-epithelial cells originating from epithelial stratification are spatially restricted and are not very motile. They can quickly revert to proper epithelial polarity and rarely result in malignant growth. In cancer biology, such epithelial stratification of normally single-cell-layered epithelia and the resulting partial EMT in adult epithelial tissues are often signs of tumor initiation [105–107]. Most of these tumors are benign. Malignant transformation requires additional triggers for unregulated proliferation and the ability to breach the basement membrane (Fig. 2C).

6. Conclusions and outlook

EMT is a fundamental component of animal development, and as discussed in accompanying reviews, a hallmark of cancer progression. In this review, we have shown that there are many types of epithelial organization and EMT in developmental morphogenesis. Not all epithelial structures undergo EMT (e.g., lung, colon), and not all EMTs involve the conversion of fully epithelial cells to fully mesenchymal ones. Recognizing partial epithelial structures and partial EMTs may be the key to understanding both developmental and cancer EMTs. Epithelial stratification in development and cancer is often coupled with partial EMT. Diversity of developmental EMTs in their cellular organization and surrounding tissue architecture strongly suggests that there is a similar level of diversity in the molecular regulation of EMTs. Developmental EMT studies, with an emphasis on each's uniqueness, may help us understand cancer EMTs. Conversely, emerging concepts in cancer and cancer-EMT research [108] offer fresh perspectives for developmental EMT studies, and together these two research fields, development and cancer, can benefit from a win-win partnership.

Conflict of Interest

None

References

- [1] E.D. Hay, An overview of epithelial-mesenchymal transformation, *Acta Anat. (Basel)* 154 (1995) 8–20.
- [2] H. Aclouque, M.S. Adams, K. Fishwick, M. Bronner-Fraser, M.A. Nieto, Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease, *J. Clin. Invest.* 119 (2009) 1438–1449.
- [3] J.P. Thiery, H. Aclouque, R.Y. Huang, M.A. Nieto, Epithelial-mesenchymal transitions in development and disease, *Cell* 139 (2009) 871–890.
- [4] F. Martin-Belmonte, M. Perez-Moreno, Epithelial cell polarity, stem cells and cancer, *Nat. Rev. Cancer* 12 (2012) 23–38.
- [5] G. Moreno-Bueno, F. Portillo, A. Cano, Transcriptional regulation of cell polarity in EMT and cancer, *Oncogene* 27 (2008) 6958–6969.
- [6] R. Kalluri, R.A. Weinberg, The basics of epithelial-mesenchymal transition, *J. Clin. Invest.* 119 (2009) 1420–1428.
- [7] L. Larue, A. Bellacosa, Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways, *Oncogene* 24 (2005) 7443–7454.
- [8] H. Sasaki, Mechanisms of trophectoderm fate specification in preimplantation mouse development, *Dev. Growth Differ.* 52 (2010) 263–273.
- [9] F.C. Thomas, B. Sheth, J.J. Eckert, G. Bazzoni, E. Dejana, T.P. Fleming, Contribution of JAM-1 to epithelial differentiation and tight-junction biogenesis in the mouse preimplantation embryo, *J. Cell Sci.* 117 (2004) 5599–5608.
- [10] K. Takaoka, H. Hamada, Cell fate decisions and axis determination in the early mouse embryo, *Development* 139 (2012) 3–14.
- [11] K. Moriwaki, S. Tsukita, M. Furuse, Tight junctions containing claudin 4 and 6 are essential for blastocyst formation in preimplantation mouse embryos, *Dev. Biol.* 312 (2007) 509–522.
- [12] R.O. Stephenson, Y. Yamanaka, J. Rossant, Disorganized epithelial polarity and excess trophectoderm cell fate in preimplantation embryos lacking E-cadherin, *Development* 137 (2010) 3383–3391.
- [13] G. Wu, L. Gentile, T. Fuchikami, J. Sutter, K. Psathaki, T.C. Esteves, M.J. Arauzo-Bravo, C. Ortmeyer, G. Verberk, K. Abe, H.R. Scholer, Initiation of trophectoderm lineage specification in mouse embryos is independent of Cdx2, *Development* 137 (2010) 4159–4169.
- [14] M. Zernicka-Goetz, S.A. Morris, A.W. Bruce, Making a firm decision: multifaceted regulation of cell fate in the early mouse embryo, *Nat. Rev. Genet.* 10 (2009) 467–477.
- [15] M. Albert, A.H. Peters, Genetic and epigenetic control of early mouse development, *Curr. Opin. Genet. Dev.* 19 (2009) 113–121.
- [16] J. Rossant, P.P. Tam, Blastocyst lineage formation, early embryonic asymmetries and axis patterning in the mouse, *Development* 136 (2009) 701–713.
- [17] A. Trounson, U. Grieshammer, Chimeric primates: embryonic stem cells need not apply, *Cell* 148 (2012) 19–21.
- [18] M. Tachibana, M. Sparman, C. Ramsey, H. Ma, H.S. Lee, M.C. Penedo, S. Mitalipov, Generation of chimeric rhesus monkeys, *Cell* 148 (2012) 285–295.
- [19] Y. Nakaya, E.W. Sukowati, Y. Wu, G. Sheng, RhoA and microtubule dynamics control cell-basement membrane interaction in EMT during gastrulation, *Nat. Cell Biol.* 10 (2008) 765–775.
- [20] Y. Nakaya, G. Sheng, Epithelial to mesenchymal transition during gastrulation: an embryological view, *Dev. Growth Differ.* 50 (2008) 755–766.
- [21] Y. Nakaya, G. Sheng, An amicable separation: chick's way of doing EMT, *Cell Adhes. Migr.* 3 (2009) 160–163.
- [22] K.M. Hardy, T.A. Yatskevich, J. Konieczka, A.S. Bobbs, P.B. Antin, FGF signalling through RAS/MAPK and PI3K pathways regulates cell movement and gene expression in the chicken primitive streak without affecting E-cadherin expression, *BMC Dev. Biol.* 11 (2011) 20.
- [23] A. Ferrer-Vaquer, M. Viotti, A.K. Hadjantonakis, Transitions between epithelial and mesenchymal states and the morphogenesis of the early mouse embryo, *Cell Adhes. Migr.* 4 (2010) 447–457.
- [24] L. Kerosuo, M. Bronner-Fraser, What is bad in cancer is good in the embryo: importance of EMT in neural crest development, *Semin. Cell Dev. Biol.* 23 (2012) 320–332.
- [25] P.H. Strobl-Mazzulla, M.E. Bronner, Epithelial to mesenchymal transition: new and old insights from the classical neural crest model, *Semin. Cancer Biol.* (2012).
- [26] M.R. Clay, M.C. Halloran, Regulation of cell adhesions and motility during initiation of neural crest migration, *Curr. Opin. Neurobiol.* 21 (2011) 17–22.
- [27] J.L. Duband, Diversity in the molecular and cellular strategies of epithelium-to-mesenchyme transitions: insights from the neural crest, *Cell Adhes. Migr.* 4 (2010) 458–482.
- [28] S. Krispin, E. Nitzan, C. Kalcheim, The dorsal neural tube: a dynamic setting for cell fate decisions, *Dev. Neurobiol.* 70 (2010) 796–812.
- [29] E. Theveneau, R. Mayor, Neural crest delamination and migration: from epithelium-to-mesenchyme transition to collective cell migration, *Dev. Biol.* 366 (2012) 34–54.
- [30] O. Pourquie, Vertebrate segmentation: from cyclic gene networks to scoliosis, *Cell* 145 (2011) 650–663.
- [31] Y. Takahashi, Y. Sato, R. Suetsugu, Y. Nakaya, Mesenchymal-to-epithelial transition during somitic segmentation: a novel approach to studying the roles of Rho family GTPases in morphogenesis, *Cells Tissues Organs* 179 (2005) 36–42.
- [32] C. Kalcheim, R. Ben-Yair, Cell rearrangements during development of the somite and its derivatives, *Curr. Opin. Genet. Dev.* 15 (2005) 371–380.
- [33] Y. Nakaya, S. Kuroda, Y.T. Katagiri, K. Kaibuchi, Y. Takahashi, Mesenchymal-epithelial transition during somitic segmentation is regulated by differential roles of Cdc42 and Rac1, *Dev. Cell* 7 (2004) 425–438.
- [34] B. Christ, R. Huang, M. Scaal, Formation and differentiation of the avian sclerotome, *Anat. Embryol. (Berl)* 208 (2004) 333–350.
- [35] J. Gros, M. Scaal, C. Marcelle, A two-step mechanism for myotome formation in chick, *Dev. Cell* 6 (2004) 875–882.
- [36] B. Christ, R. Huang, M. Scaal, Amniote somite derivatives, *Dev. Dyn.* 236 (2007) 2382–2396.

- [37] F. Yusuf, B. Brand-Saberi, The eventful somite: patterning, fate determination and cell division in the somite, *Anat. Embryol. (Berl)* 211 (Suppl 1) (2006) 21–30.
- [38] J. Gros, M. Manceau, V. Thome, C. Marcelle, A common somitic origin for embryonic muscle progenitors and satellite cells, *Nature* 435 (2005) 954–958.
- [39] C. Anderson, S. Thorsteinsdottir, A.G. Borycki, Sonic hedgehog-dependent synthesis of laminin alpha1 controls basement membrane assembly in the myotome, *Development* 136 (2009) 3495–3504.
- [40] Y. Ishii, J. Langberg, K. Rosborough, T. Mikawa, Endothelial cell lineages of the heart, *Cell Tissue Res.* 335 (2009) 67–73.
- [41] A.D. Person, S.E. Klewer, R.B. Runyan, Cell biology of cardiac cushion development, *Int. Rev. Cytol.* 243 (2005) 287–335.
- [42] G. Sheng, Primitive and definitive erythropoiesis in the yolk sac: a bird's eye view, *Int. J. Dev. Biol.* 54 (2010) 1033–1043.
- [43] M. Shin, H. Nagai, G. Sheng, Notch mediates Wnt and BMP signals in the early separation of smooth muscle progenitors and blood/endothelial common progenitors, *Development* 136 (2009) 595–603.
- [44] F. Nakazawa, H. Nagai, M. Shin, G. Sheng, Negative regulation of primitive hematopoiesis by the FGF signaling pathway, *Blood* 108 (2006) 3335–3343.
- [45] J.C. Boisset, W. van Cappellen, C. Andrieu-Soler, N. Galjart, E. Dzierzak, C. Robin, In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium, *Nature* 464 (2010) 116–120.
- [46] A. Bruning, J. Makovitzky, A. Gingelmaier, K. Friese, I. Mylonas, The metastasis-associated genes MTA1 and MTA3 are abundantly expressed in human placenta and chorionic carcinoma cells, *Histochem. Cell Biol.* 132 (2009) 33–38.
- [47] M.I. Kokkinos, P. Murthi, R. Wafai, E.W. Thompson, D.F. Newgreen, Cadherins in the human placenta–Epithelial–Mesenchymal Transition (EMT) and placental development, *Placenta* 31 (2010) 747–755.
- [48] L. Vicovac, J.D. Aplin, Epithelial–mesenchymal transition during trophoblast differentiation, *Acta Anat. (Basel)* 156 (1996) 202–216.
- [49] J.D. Aplin, C.J. Jones, L.K. Harris, Adhesion molecules in human trophoblast – a review. I. Villous trophoblast, *Placenta* 30 (2009) 293–298.
- [50] L.K. Harris, C.J. Jones, J.D. Aplin, Adhesion molecules in human trophoblast – a review. II. extravillous trophoblast, *Placenta* 30 (2009) 299–304.
- [51] N. Gjorevski, C.M. Nelson, Integrated morphodynamic signalling of the mammary gland, *Nat. Rev. Mol. Cell Biol.* 12 (2011) 581–593.
- [52] C.J. Watson, W.T. Khaled, Mammary development in the embryo and adult: a journey of morphogenesis and commitment, *Development* 135 (2008) 995–1003.
- [53] P. Cowin, J. Wysolmerski, Molecular mechanisms guiding embryonic mammary gland development, *Cold Spring Harb. Perspect. Biol.* 2 (2010) a003251.
- [54] E. Tomaskovic-Crook, E.W. Thompson, J.P. Thiery, Epithelial to mesenchymal transition and breast cancer, *Breast Cancer Res.* 11 (2009) 213.
- [55] B.A. Howard, In the beginning: the establishment of the mammary lineage during embryogenesis, *Semin. Cell Dev. Biol.* 23 (2012) 574–582.
- [56] D. Nanba, Y. Nakanishi, Y. Hieda, Changes in adhesive properties of epithelial cells during early morphogenesis of the mammary gland, *Dev. Growth Differ.* 43 (2001) 535–544.
- [57] E.J. Ormerod, P.S. Rudland, Cellular composition and organization of ductal buds in developing rat mammary glands: evidence for morphological intermediates between epithelial and myoepithelial cells, *Am. J. Anat.* 170 (1984) 631–652.
- [58] J. Muschler, C.H. Streuli, Cell-matrix interactions in mammary gland development and breast cancer, *Cold Spring Harb. Perspect. Biol.* 2 (2010) a003202.
- [59] A.J. Ewald, R.J. Huebner, H. Palsdottir, J.K. Lee, M.J. Perez, D.M. Jorgens, A.N. Tauscher, K.J. Cheung, Z. Werb, M. Auer, Mammary collective cell migration involves transient loss of epithelial features and individual cell migration within the epithelium, *J. Cell Sci.* 125 (2012) 2638–2654.
- [60] A.J. Ewald, A. Brenot, M. Duong, B.S. Chan, Z. Werb, Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis, *Dev. Cell* 14 (2008) 570–581.
- [61] S.E. Mutsaers, Mesothelial cells: their structure, function and role in serosal repair, *Respirology* 7 (2002) 171–191.
- [62] B. Zhou, Q. Ma, S. Rajagopal, S.M. Wu, I. Domian, J. Rivera-Feliciano, D. Jiang, A. von Gise, S. Ikeda, K.R. Chien, W.T. Pu, Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart, *Nature* 454 (2008) 109–113.
- [63] S.T. Baek, M.D. Tallquist, Nf1 limits epicardial derivative expansion by regulating epithelial to mesenchymal transition and proliferation, *Development* 139 (2012) 2040–2049.
- [64] A. von Gise, B. Zhou, L.B. Honor, Q. Ma, A. Petryk, W.T. Pu, WT1 regulates epicardial epithelial to mesenchymal transition through beta-catenin and retinoic acid signaling pathways, *Dev. Biol.* 356 (2011) 421–431.
- [65] J. Que, B. Wilm, H. Hasegawa, F. Wang, D. Bader, B.L. Hogan, Mesothelium contributes to vascular smooth muscle and mesenchyme during lung development, *Proc. Natl. Acad. Sci. USA* 105 (2008) 16626–16630.
- [66] B. Wilm, A. Ipenberg, N.D. Hastie, J.B. Burch, D.M. Bader, The serosal mesothelium is a major source of smooth muscle cells of the gut vasculature, *Development* 132 (2005) 5317–5328.
- [67] J. Karl, B. Capel, Sertoli cells of the mouse testis originate from the coelomic epithelium, *Dev. Biol.* 203 (1998) 323–333.
- [68] N. Auersperg, A.S. Wong, K.C. Choi, S.K. Kang, P.C. Leung, Ovarian surface epithelium: biology, endocrinology, and pathology, *Endocr. Rev.* 22 (2001) 255–288.
- [69] C.F. Liu, C. Liu, H.H. Yao, Building pathways for ovary organogenesis in the mouse embryo, *Curr. Top. Dev. Biol.* 90 (2010) 263–290.
- [70] J.M. Mora, M.A. Fenwick, L. Castle, M. Baithun, T.A. Ryder, M. Mobberley, R. Carzaniga, S. Franks, K. Hardy, Characterization and significance of adhesion and junction-related proteins in mouse ovarian follicles, *Biol. Reprod.* 86 (2012) 153.
- [71] G.R. Dressler, The cellular basis of kidney development, *Annu. Rev. Cell Dev. Biol.* 22 (2006) 509–529.
- [72] G.R. Dressler, Advances in early kidney specification, development and patterning, *Development* 136 (2009) 3863–3874.
- [73] M.H. Little, A.P. McMahon, Mammalian kidney development: principles, progress, and projections, *Cold Spring Harb. Perspect. Biol.* 4 (2012) a008300.
- [74] J. Pieczynski, B. Margolis, Protein complexes that control renal epithelial polarity, *Am. J. Physiol. Renal Physiol.* 300 (2011) 589–601.
- [75] M.A. Schluter, B. Margolis, Apical lumen formation in renal epithelia, *J. Am. Soc. Nephrol.* 20 (2009) 1444–1452.
- [76] K.M. Schmidt-Ott, D. Lan, B.J. Hirsh, J. Barasch, Dissecting stages of mesenchymal-to-epithelial conversion during kidney development, *Nephron Physiol.* 104 (2006) 56–60.
- [77] P. Wang, F.A. Pereira, D. Beasley, H. Zheng, Presenilins are required for the formation of comma- and S-shaped bodies during nephrogenesis, *Development* 130 (2003) 5019–5029.
- [78] D.K. Marciano, P.R. Brakeman, C.Z. Lee, N. Spivak, D.J. Eastburn, D.M. Bryant, G.M. Beaudoin 3rd, I. Hofmann, K.E. Mostov, L.F. Reichardt, P120 catenin is required for normal renal tubulogenesis and glomerulogenesis, *Development* 138 (2011) 2099–2109.
- [79] K. Si-Tayeb, F.P. Lemaigre, S.A. Duncan, Organogenesis and development of the liver, *Dev. Cell* 18 (2010) 175–189.
- [80] R. Bort, M. Signore, K. Tremblay, J.P. Martinez Barbera, K.S. Zaret, Hex homeobox gene controls the transition of the endoderm to a pseudostratified, cell emergent epithelium for liver bud development, *Dev. Biol.* 290 (2006) 44–56.
- [81] L. Lokmane, C. Haumaitre, P. Garcia-Villalba, I. Anselme, S. Schneider-Maunoury, S. Cereghini, Crucial role of vHNF1 in vertebrate hepatic specification, *Development* 135 (2008) 2777–2786.
- [82] P.S. Vestentoft, P. Jenes, B.M. Hopkinson, B. Vainer, K. Mollgard, B. Quistorff, H.C. Bisgaard, Three-dimensional reconstructions of intrahepatic bile duct tubulogenesis in human liver, *BMC Dev. Biol.* 11 (2011) 56.
- [83] F.C. Pan, C. Wright, Pancreas organogenesis: from bud to plexus to gland, *Dev. Dyn.* 240 (2011) 530–565.
- [84] G.K. Gittes, Developmental biology of the pancreas: a comprehensive review, *Dev. Biol.* 326 (2009) 4–35.
- [85] G. Kesavan, F.W. Sand, T.U. Greiner, J.K. Johansson, S. Kobberup, X. Wu, C. Brakebusch, H. Semb, Cdc42-mediated tubulogenesis controls cell specification, *Cell* 139 (2009) 791–801.
- [86] A. Villaseñor, D.C. Chong, M. Henkemeyer, O. Cleaver, Epithelial dynamics of pancreatic branching morphogenesis, *Development* 137 (2010) 4295–4305.
- [87] J.M. Rukstalis, J.F. Habener, Snail2, a mediator of epithelial–mesenchymal transitions, expressed in progenitor cells of the developing endocrine pancreas, *Gene Exp. Patterns* 7 (2007) 471–479.
- [88] M. Gouzi, Y.H. Kim, K. Katsumoto, K. Johansson, A. Grapin-Botton, Neurogenin3 initiates stepwise delamination of differentiating endocrine cells during pancreas development, *Dev. Dyn.* 240 (2011) 589–604.
- [89] L. Cole, M. Anderson, P.B. Antin, S.W. Limesand, One process for pancreatic beta-cell coalescence into islets involves an epithelial–mesenchymal transition, *J. Endocrinol.* 203 (2009) 19–31.
- [90] T. Otonkoski, M. Banerjee, O. Korsgren, L.E. Thornell, I. Virtanen, Unique basement membrane structure of human pancreatic islets: implications for beta-cell growth and differentiation, *Diabetes Obes. Metab.* 10 (Suppl 4) (2008) 119–127.
- [91] Z. Granot, A. Swisa, J. Magenheimer, M. Stolovich-Rain, W. Fujimoto, E. Manduchi, T. Miki, J.K. Lennerz, C.J. Stoeckert Jr., O. Meyuhas, S. Seino, M.A. Permutt, H. Piwnicka-Worms, N. Bardeesy, Y. Dor, LKB1 regulates pancreatic beta cell size, polarity, and function, *Cell Metab.* 10 (2009) 296–308.
- [92] L.R. Nyman, K.S. Wells, W.S. Head, M. McCaughey, E. Ford, M. Brissova, D.W. Piston, A.C. Powers, Real-time, multidimensional in vivo imaging used to investigate blood flow in mouse pancreatic islets, *J. Clin. Invest.* 118 (2008) 3790–3797.
- [93] G.C. Weir, S. Bonner-Weir, Islets of Langerhans: the puzzle of intraslet interactions and their relevance to diabetes, *J. Clin. Invest.* 85 (1990) 983–987.
- [94] G. Nikolova, N. Jabs, I. Konstantinova, A. Domogatskaya, K. Tryggvason, L. Sorokin, R. Fassler, G. Gu, H.P. Gerber, N. Ferrara, D.A. Melton, E. Lammert, The vascular basement membrane: a niche for insulin gene expression and beta cell proliferation, *Dev. Cell* 10 (2006) 397–405.
- [95] L. Orci, B. Thorens, M. Ravazzola, H.F. Lodish, Localization of the pancreatic beta cell glucose transporter to specific plasma membrane domains, *Science* 245 (1989) 295–297.
- [96] N. Takahashi, T. Kishimoto, T. Nemoto, T. Kadowaki, H. Kasai, Fusion pore dynamics and insulin granule exocytosis in the pancreatic islet, *Science* 297 (2002) 1349–1352.
- [97] B.G. Timms, Prostate development: a historical perspective, *Differentiation* 76 (2008) 565–577.

- [98] M.M. Shen, C. Abate-Shen, Molecular genetics of prostate cancer: new prospects for old challenges, *Genes Dev.* 24 (2010) 1967–2000.
- [99] G.R. Cunha, The role of androgens in the epithelio-mesenchymal interactions involved in prostatic morphogenesis in embryonic mice, *Anat. Rec.* 175 (1973) 87–96.
- [100] P.C. Marker, Does prostate cancer co-opt the developmental program?, *Differentiation* 76 (2008) 736–744.
- [101] K. Aigner, B. Dampier, L. Descovich, M. Mikula, A. Sultan, M. Schreiber, W. Mikulits, T. Brabletz, D. Strand, P. Obrist, W. Sommergruber, N. Schweifer, A. Wernitznig, H. Beug, R. Foisner, A. Eger, The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity, *Oncogene* 26 (2007) 6979–6988.
- [102] S. Spaderna, O. Schmalhofer, M. Wahlbuhl, A. Dimmler, K. Bauer, A. Sultan, F. Hlubek, A. Jung, D. Strand, A. Eger, T. Kirchner, J. Behrens, T. Brabletz, The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer, *Cancer Res.* 68 (2008) 537–544.
- [103] E.L. Whiteman, C.J. Liu, E.R. Fearon, B. Margolis, The transcription factor snail represses *Crumbs3* expression and disrupts apico-basal polarity complexes, *Oncogene* 27 (2008) 3875–3879.
- [104] M.I. Koster, D.R. Roop, Mechanisms regulating epithelial stratification, *Annu. Rev. Cell Dev. Biol.* 23 (2007) 93–113.
- [105] X. Wang, H. Ouyang, Y. Yamamoto, P.A. Kumar, T.S. Wei, R. Dagher, M. Vincent, X. Lu, A.M. Bellizzi, K.Y. Ho, C.P. Crum, W. Xian, F. McKeon, Residual embryonic cells as precursors of a Barrett's-like metaplasia, *Cell* 145 (2011) 1023–1035.
- [106] J.P. Thiery, Epithelial–mesenchymal transitions in tumour progression, *Nat. Rev. Cancer* 2 (2002) 442–454.
- [107] L. Huang, S.K. Muthuswamy, Polarity protein alterations in carcinoma: a focus on emerging roles for polarity regulators, *Curr. Opin. Genet. Dev.* 20 (2010) 41–50.
- [108] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, *Cell* 144 (2011) 646–674.