Biochemical Journal (2021) **478** 1809–1825
https://doi.org/10.1042/BCJ20210083

Review Article

What we can learn from embryos to understand the mesenchymal-to-epithelial transition in tumor progression

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Epithelial plasticity involved the terminal and transitional stages that occur during epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET), both are essential at different stages of early embryonic development that have been co-opted by cancer cells to undergo tumor metastasis. These processes are regulated at multiple instances, whereas the post-transcriptional regulation of key genes mediated by microRNAs is gaining major attention as a common and conserved pathway. In this review, we focus on discussing the latest findings of the cellular and molecular basis of the less characterized process of MET during embryonic development, with special attention to the role of microRNAs. Although we take in consideration the necessity of being cautious when extrapolating the obtained evidence, we propose some commonalities between early embryonic development and cancer progression that can shed light into our current understanding of this complex event and might aid in the design of specific therapeutic approaches.

Introduction

Epithelial plasticity is defined as the ability of cells to change their epithelial phenotype to mesenchymal and vice versa. This characteristic is essential during embryonic development because the morphogenesis and organogenesis that generate the body plan depends on cells changing rapidly and reversibly among those phenotypes. Epithelial cells go through a process called epithelial-to-mesenchymal transition (EMT), which involves losing of apico-basal polarity, negative regulation of adhesion protein expression, and cytoskeleton reorganization to accomplish the extensive cellular movements that will give rise to the embryonic germ layers and later to tissues [1]. Mesenchymal migratory cells may occasionally initiate a reversible process called mesenchymal-to-epithelial transition (MET) which includes not only cellular repolarization but also increasing their tight and adherens junctions. During morphogenesis and organogenesis, progenitor cells would require multiple events EMT and MET allowing the precise creation of microenvironments required during cell–cell communication. Both mechanisms are conserved evolutionarily and it has been described that they do not conform to a binary system, but instead embryonic cells sometimes display attributes of both epithelial and mesenchymal phenotypes [2,3]. These transitions and intermediate stages do not only participate in normal development from gastrulation to complex organ formation, they are also involved in tissue regeneration during adulthood and co-opted by cancer cells to facilitate tumor metastasis [1,4,5].

Epithelial plasticity is tightly regulated at different levels, including regulation of gene expression by key transcription factors (known as EMT-TF or MET-TFs), changes on cell and tissue architecture and modulation in cellular levels of microRNAs (miRNAs). This well-studied class of small regulatory RNAs plays a critical role in the checkpoint through degradation of transcripts or inhibition of EMT/MET-TFs [6,7], providing a quick solution to the need for a specific temporal and spatial control of

Received: 8 February 2021
Revised: 6 April 2021
Accepted: 23 April 2021

Version of Record published: 14 May 2021

Biochemical Journal (2021) **478** 1809–1825
https://doi.org/10.1042/BCJ20210083

epithelial plasticity during development. Although EMT regulation has been highly characterized and extensively revised during embryonic development and under pathological conditions [8], very little is known related with the mechanistic processes driving MET.

In this review, we will focus on the understanding of the cellular and molecular basis of MET process during early development, with special attention to the role of microRNA as a possible platform to understand their contribution during cancer progression.

Epithelial plasticity: not only a binary state

During embryonic development, epithelial cells have a characteristic apico-basal polarity, low degree of individual motility due to strong intercellular adherence and tight junctions, desmosome complexes, and basal interaction with the extracellular matrix [9]. However, during the EMT process these structures are dismantled, epithelial cells change their phenotype and acquired mesenchymal characteristics in concomitance with a high degree motility. Specifically, expression levels of adhesion proteins decrease, epithelial genes begin to be repressed, and mesenchymal gene expression programs are activated. Finally, the actin cytoskeleton is reorganized, apical-basal polarity and interaction with the extracellular matrix are lost, but cells acquired a front-back polarity [1,10]. In some cases, mesenchymal cells initiate a re-epithelialization process through MET, which allows them to reestablish cell polarity, re-express adhesion proteins, such as certain type of cadherins and integrins, to finally recover their ability to form an epithelial tissue [2]. This process is essential to maintain the morphology of tissues and the establishment of the body plan in organisms, but if it is activated inappropriately, it will end in deleterious development pathologies and in cancer metastasis [9].

It is important to mention that in the last years the conceptual framework where EMT promotes metastasis and invasion was challenged and extensively discussed [11]. Several lines of evidence support this notion since the knockout of EMT-TFs (Twist or Snail) in mouse models of pancreatic and breast cancer does not significantly affect the incidence of metastasis [12,13]. These data may suggest that an overt or a complete EMT may reduce the metastatic capacity when some molecular and/or morphological epithelial traits are required to be maintained [14–16]. Tanking this in consideration, in the recent years epithelial plasticity was not only considered as a binary process where cells pass completely through EMT or MET, acquiring a mesenchymal phenotype or recovering epithelial characteristics, respectively [10,17,18]. However, it has been reported that in some cases there are cells which present epithelial and mesenchymal characteristics simultaneously [19,20], indicating that some characteristics of both phenotypes were lost or acquired. Cells with an intermediate phenotype generally have low levels of expression of adhesion proteins, but they do not completely lose adhesion capacity to neighboring cells. Likewise, they can retain some apico-basal polarity but cells still have the ability to migrate [21,22]. Interestingly, these spectrum of phenotypes or partial transition have been described not only during embryo development but also recently in wound healing, organ fibrosis and circulating tumor cells where these intermediate phenotypes present the greatest metastatic potential [5,23–25]. Nowadays, we know that partial transitional stages may represent the final outcome but not only an intermediate metastable stage [1]. Regarding this, the degree of 'epitheliality' or 'mesenchymality' is very variable and partial transition would depend of the initial epithelial state and the type of migration [19,20]. For example, migratory cells that maintain epithelial features but exhibit intermediate expression of mesenchymal markers, this would favor reversion to the epithelial state via MET compared with cells that have undergone full EMT [27]. On one hand, this is still a debate in cancer cells, because some authors proposed that partial or complete EMT contributes to their invasiveness [28,29]. On the other side, there are also a variable degree of mesenchymal states, very appreciable during collective or individual migration. As a general rule, the more cell–cell adhesion and junctions are presented between cells, the more 'collective' the migratory process will be [15,30]. However, this is a very simplistic assumption, many cell types that were thought to move individually because they have a complete EMT, also shown to make cell–cell contacts that influence each other's movements [31,32]. Moreover, collective migration of mesenchymal cells is also observed in absence of cell–cell contact where chemotaxis between neighboring cells plays a major role [32,33].

In sum, the spectrum of phenotypes, including intermediate metastable and stable stages, suggests that epithelial plasticity depends on cell context, extracellular signaling, epithelial/mesenchymal maturity and molecular changes that ultimate will affect the cellular machinery driving migration and invasion. Based on this, it is very difficult to define the 'epithelialization' or 'mesenchymalization' state in a group of cells by only analyzing a few markers since many initial, intermediate and final states are possible.

Biochemical Journal (2021) **478** 1809–1825
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Cellular and molecular basis of MET

Vertebrate epithelial cells typically contact each other through tight junctions near the apical surface along with adherent junctions, desmosomes and gap junctions at the lateral surface [34]. The cellular changes occuring during the 'mesenchymalization' included several stereotypical events: (1) The tight junction weakening is accompanied by a decrease in the expression of their components (Claudin and Occluding), and the relocation of the Zona Occludens 1 protein (ZO1) upon initiation of an EMT process. (2) E-cadherin (E-cad or CDH1) is transcriptionally repress by SNAIL, cleavage and degraded from the cell membrane [35]. Thus, β-catenin may no longer interacts with E-cad favoring either their degradation or protecting them in order to activate the downstream transcription [36]. (3) Desmosomes and gap junctions are disrupted. (4) Loss of epithelial cell polarity is altered by abolishing Par and Crumbs complexes localization at the cell junctions [37,38]. Typically, these complexes are associated with Lin-7 to define the apical compartment; whereas, Scribble complexes define the basolateral compartment [39].

On the other hand, the mechanisms driving MET or 're-epithelialization' are far less well understood than those underlying EMT, and it remains unclear how the mechanisms driving these processes relate one to another. How re-epithelialization is executed has been partly revealed by studying apico-basal polarity. However, it is important to mention that the order of events is likely to be highly dependent of the cell and tissue context. Stereotypical MET events involve the polarization that generates the apical membrane facing the lumen, and the basolateral membrane which ensures intercellular adhesion and interaction with the extracellular matrix [40,41] (Figure 1). Intercellular adhesion is established through the transmembrane protein Nectin, which builds scaffolds that recruits the actin-binding protein Afadin and E-cad [42,43]. E-cad forms calcium-dependent homo-trans-dimer with adjacent cells and interacts with β-catenin, p120 catenin, and α-catenin, which then links the protein to the actin cytoskeleton. Ultimately, this triggers downstream signaling by indirectly recruiting Rac and Rho [44–46]. Then, their effectors will promote cortical actin network formation and stabilization which, in turn, strengthens adherens junctions [42]. Cell–cell and cell–extra cellular matrix

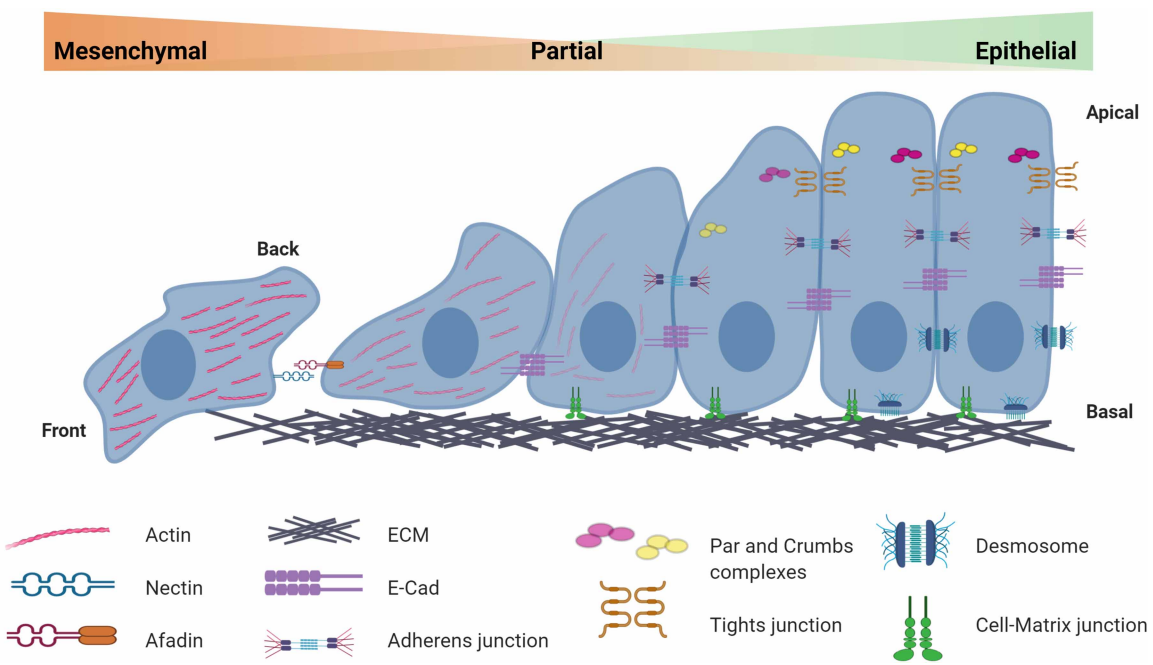

Figure 1. Major cellular changes occurring during mesenchymal-to-epithelial transition (MET).

Mesenchymal cells express Nectin and recruit the protein Afadin. The interaction between both proteins leads to the expression of E-cad and the interaction between adjacent cells. Adherent junctions and cell–matrix junctions are formed. Polarization gradually recovers with the remodeling of the actin cytoskeleton and the expression of Par and Crubs complexes in the future apical region. Finally, desmosomes and tight junction consolidate the apical-basal polarity. The figure was created with BioRender.com.

Biochemical Journal (2021) 478 1809–1825
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crosstalk are required during the establishment of adherens junctions through the action of Nectin family members [47–49] which recruits partitioning defective 3 and 6 (Par3 and Par6) to form the Par polarity in the apical domain. Par proteins recruit the apical determinant Crumbs complex and displace the basolateral determinant Scribble complex. Finally, desmosomes and tight junction, composed by occluding, ZO proteins and Claudins, consolidate the apical-basal polarity [2,9,50].

Some authors postulate that MET is trigger just by down-regulating the EMT-inducing signals, including the transforming growth factor β (TGF-β) [51,52] or master EMT-associated transcription factors (EMT-TF: SNAIL1/2, TWIST1/2 and ZEB1/2) [53,54]. In line with this, EMT-TFs are rarely detectable in adult epithelial tissues and several factors were recently shown to protect this epithelial state [55]. However, EMT-TFs down-regulation does not appear to be sufficient for MET induction during embryo development, where the interaction with neighboring tissues and the activation of specific sets of transcription factors or signaling pathways would be required [56–59]. Together these evidences put in consideration that the mechanisms driving MET may be quite distinct from those that were disassembled during EMT. Thus, emerging concepts are considering that the re-epithelialization of cells, during development or under pathological condition, represent an alternative pathway with new players where cell cross-talk may play a pivotal role. A recent revision proposed Grainy-head like family (GRHL1–3), Ovo-like family (OVOL1/2), Epithelium-Specific Ets-Domain containing factors (ELF3/5), Singleminded 2 (Sim2), and SRY-box 3 (Sox3) as a main MET-TF regulated in a tissue-specific manner to enable precise spatiotemporal control of MET event during development and in adult epithelial tissue maintenance [50]. After all, the identification of MET-TF is yet to be formally investigated in different developmental system and cellular context.

In the pathological context, it has been considered that both EMT/MET transitions are responsible for the progression of metastasis, but the most unfavorable stage in the development of the disease is the MET due to its fatal potential [54,60]. However, the study of tumor spread *in vivo* presents many difficulties due to its unpredictability in terms of where and when the secondary tumor is going to be established and also the low number of cells that colonize the new tissue. It is important to mention that during metastasis, cancer cells co-opted programs which the organism uses during early embryonic development [1]. For these reasons, insights from early development are very helpful in elucidating the mechanisms underlying MET during cancer progression.

Insights from early development the MET

Morphogenesis in animals require cell plasticity and in some cases several rounds of EMT–MET cycles, with MET being a critical step during embryonic development and stabilization of epithelial tissues. This ability of mesenchymal cells to revert to an epithelial phenotype during developmental condition would serve as an excellent platform to study the inter-conversion between mesenchymal and epithelial phenotypes with far reaching implications in pathological conditions. Principally due to the predictability of where and when these processes will occur during embryo development, compared with the unpredictability observed in cancer cells. In this section, we will comment on some aspects related to MET processes that occur in a few examples during embryogenesis, tissue and organ development (Figure 2A–G).

Epiblast formation

The first MET in embryonic development happens before gastrulation during the formation of the epiblast. This tissue contains a pluripotent population that will give rise to all cell lineages in an adult body. Loss of pluripotency coincides with the onset of gastrulation, when the epiblast initiates lineage differentiation by generating the three definitive germ layers (ectoderm, mesoderm and endoderm). In avian and humans, this occurs in a very similar series of molecular and morphogenetic events which includes: (1) molecular specification of epiblast precursors; (2) morphological aggregation of epiblast-fated cells; (3) full epithelialization of the epiblast; (4) morphogenesis of epithelialized epiblast; and (5) dissolution of epithelial structure during gastrulation through EMT [61]. A very recent article provided evidences that during this initial epithelialization to form the epiblast, cells initiated a pluripotency exit and correlated with a partial MET [62]. Specifically, the authors proposed that transitions from naïve-to-primed and the later pluripotency exit have a correspondence with two consecutive partial MET going from non-polarized epiblast to polarized epiblast and the latter fully epithelial epiblast (Figure 2A). These sequential steps of polarization are induced by integrin-mediated cell–extra cellular matrix (ECM) signaling. Specifically, β1-integrin and Dystroglycan are two of the mayor

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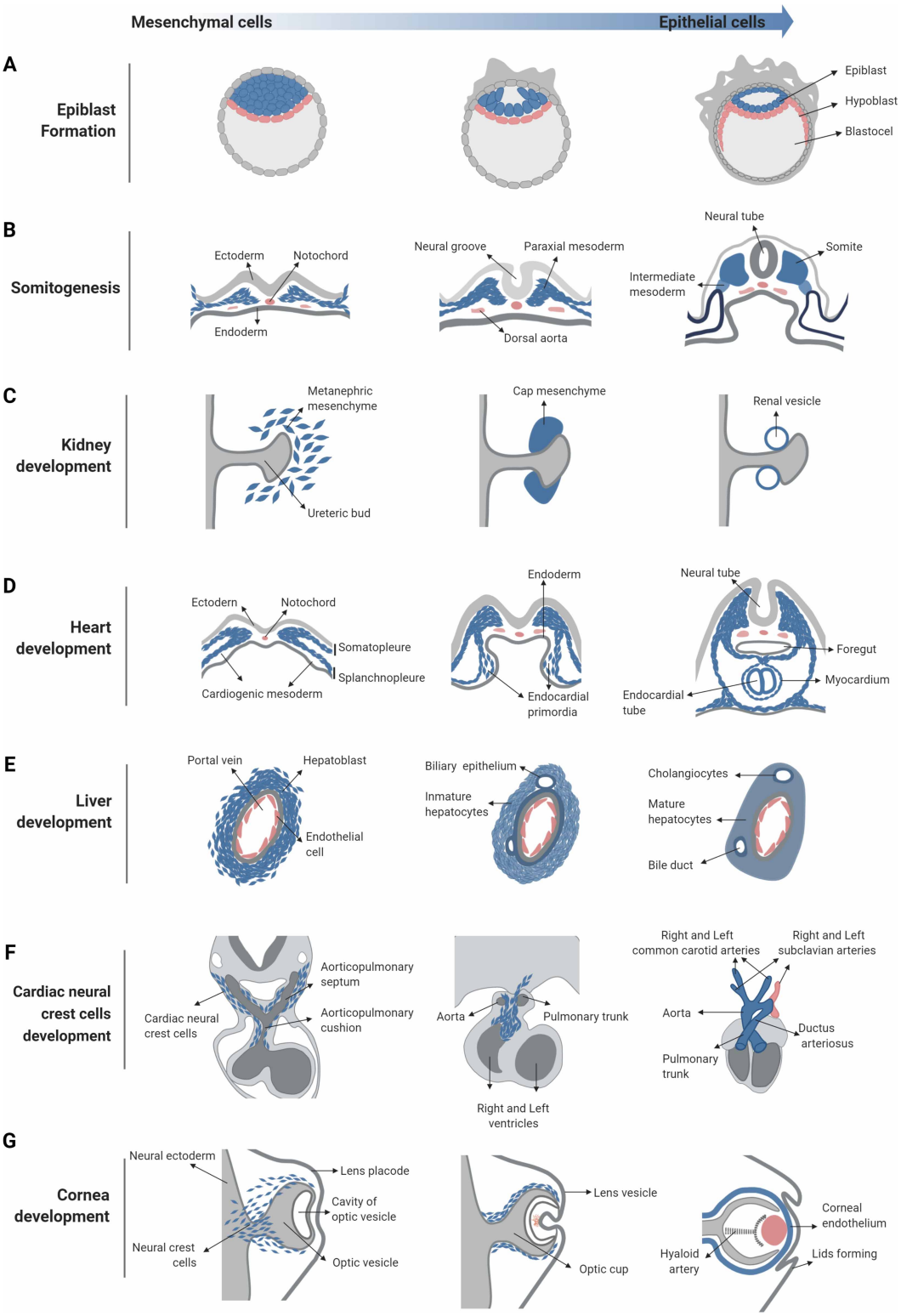

Figure 2. Examples of MET processes that occur during embryogenesis, tissue and organ development. Part 1 of 2

Epiblast formation. Epiblast cells traverse the MET after gastrulation. Somitogenesis. MET from the paraxial mesoderm gives rise to somites. Kidney development. METs of the adjacent metanephric mesenchyme giving rise first to the renal vesicles and then to the epithelial nephritic tubes. Heart development. The inner layer of the splanchnopleure will form the endocardial and myocardial progenitors through MET. Liver development. During bile duct formation, hepatoblasts undergo MET to differentiate

Biochemical Journal (2021) **478** 1809–1825
https://doi.org/10.1042/BCJ20210083

Figure 2. Examples of MET processes that occur during embryogenesis, tissue and organ development. Part 2 of 2
into cholangiocytes and hepatocytes. Cardiac neural crest development. The cardiac outflow tract septation is formed by cardiac neural crest that condense along the endocardial walls via MET. Cornea development. The cells of the neural crest are condensed to give rise to the cornea also through MET. Mesenchymal cells are represented as individual scattered cells, whereas epitheliated cells passing through MET are represented in blue. The figure was created with BioRender.com.

transmembrane proteins participating in the epiblast-ECM interaction during their polarization [63,64]. Adherens junction proteins, such as E-cad, also have an important role on epiblast cell–cell adhesion and cell–ECM interactions [65]. Moreover, the destabilization of integrin epiblast–ECM interaction prevents the efficient establishment of E-cad adhesion, probably through a regulation of their adhesive activity, among epiblast cells [66]. Taking together, a clear crosstalk among those type of interaction is crucial during epiblast MET [67]. These lines of evidence suggest that polarization of the epiblast, including the establishment of apico-basal polarity, epiblast-ECM interaction and the modulation of epiblast adherens junction, is involved in epiblast pluripotency regulation.

Epiblast cell polarity is maintained until primitive streak formation, where the cells initiated the EMT during gastrulation [68]. Newly formed mesenchymal cells will form the mesoderm which is subdivided into paraxial cells, made up of mesenchymal cells that are close to the neural tube, while those that are located laterally are organized into intermediate and lateral mesoderm. These populations will form transitory epithelium through MET (somites, notochord, primordia of the urogenital system, splanchopleura and somatopleura) that later will form specialized and differentiated tissues and organs. In the majority of these transient structures, cells will undergo a second round of EMT and in some cases several rounds of EMT/MET are needed to form the final tissue. Thus, demonstrating the high epithelial plasticity is required during embryonic development [10].

Somitogenesis

A prominent feature of the paraxial mesoderm lineage (cells destined to form the dermis, muscles and axial bones) is to giving rise to a transitory epithelialized structure called somites through MET (Figure 2B). The presomitic mesoderm, a pair of strips composed of mesenchymal cells, is localized laterally on both sides of the embryo along the anterior-posterior axis. The mesenchymal state of these cells is maintained by the expression of SNAIL1 and SNAIL2, whose expression is repressed when they move through tissues with high expression of FGF [69]. At this moment, cells initiated the MET concomitantly with a dynamic and periodic formation of tissue blocks that pinches off from the anterior end of the presomitic mesoderm [69,70]. Cells at the front of the prospective boundary for the next segment begin to reorganize their actin cytoskeleton, polarize, re-construct epithelial cell–cell adhesion and synthesize base-membrane proteins, ultimately resulting in the re-epithelialization of these mesodermal cells. Cdc42 and Rac1 play important and different roles during MET of vertebrate somitogenesis. High levels of Cdc42 maintain cells in a mesenchymal state and, opposite to that, when Cdc42 signaling was blocked hype-repithelialization was promoted [71,72]. Ephrin signaling have been also implicated in cell epithelialization during somite segmentation by regulating Cdc42 activity [73]. Proper levels of Rac1 are also important for somitegenesis, since its activation or inhibition evidenced defects during epithelialization [72]. Rac1 contributes to the induction of somitogenesis by acting post-transcriptionally on the basic-loop-helix transcription factor Paraxis [72], a well-known transcriptional factor essential for somite epithelialization [74]. In agreement with this, the overexpression of SNAIL2 in chick presomitic mesoderm blocked MET and somitogenesis by preventing the expression of Paraxis [69]. It is important to mention that there are clear specie-specific differences in the degree of somite epithelialization. Avian exhibit the most polarized example with clear apical-basal assembly [72,75]. In contrast, fish and amphibians, which undergo a faster larval development, presented a less epithelialized somites lacking apical junctions [76,77]. Somitogenesis in these species appear to be more sensitive to same factors that destabilize the somite epitheliality [78], suggesting that mesenchyme cells may undergo early phases of MET but do not complete the process, thus representing a partial MET.

Kidney development

From the caudal region of the intermediate mesoderm, the kidney is formed by the MET of the adjacent metanephric mesenchyme that gives rise to the epithelial nephritic tubes [79]. MET in kidney development is one

of the best characterized processes during organogenesis and the cellular and molecular basis of this transition have been already well reviewed [79]. Initially, the intermediate mesoderm becomes segregated into the epithelial nephric duct and the metanephric mesenchyme containing the cap cells undergo MET and give rise to part of the glomerulus (Figure 2C). MET in the cap cells is induced by Wnt4 and Wnt9b secreted from the lateral bud of the nephric duct [80]. These signals stabilize β-catenin and repress the Six2 transcription factor and Cited1 (Cbp/p300-interacting transactivator 1) to engage cap cells into renal epithelial differentiation. Polarization of renal tubule epithelia appears crucial for kidney development as perturbations give rise to defects such as polycystic kidney disease [81].

Heart development

Heart development involved several rounds of EMT and MET [82,83]. As the definitive germ layers emerge in the developing embryo, cardiac progenitors are amongst the first epiblast cells to undergo EMT and migrate out from the primitive streak. Then, at the trunk level of the embryo, the lateral mesenchyme is subjected to MET inducing the formation of a transient epithelia (splanchnopleura) that will contributes to the formation of the heart [84] (Figure 2D). Later, when the heart tube starts shaping through folding of the two lateral domains of the heart field, the inner layer of the splanchnopleure will then undergo EMT to generate endothelial progenitors and will subsequently form the endocardial and myocardial progenitors through MET. The outer layer of the splanchnopleura will give rise to pericardial progenitors, now forming a concentric epithelial-like structures (endo-, myo- and peri-cardium) that constitute the heart tube (part of the ventricle and most of the atria) [85,86]. A second wave of progenitors remains in contact with the endoderm and are maintained as a pool of undifferentiated cells. These cells will progressively infiltrate new cardiac precursors contributing to ventricle and outflow tract at the arterial pole, and part of the atria and inflow tract at the venous pole. Although the EMT aspects of heart development have been extensively studied [87], very little mechanistic insight regarding MET process is known in these tissue. What is known is that β-catenin, apical aPKC [84,88] and a belt of N-cad [89] are expressed during endocardial and myocardial progenitor epithelialization. Fibronectin is also deposited on the basal surface and plays a crucial role in establishing polarity and adherens junctions [88]. The epicardium also derives from a MET at the external surface of the myocardium. The zinc-finger Wilms tumor transcription factor WT1 participates in both EMT and MET regulation [82,83]. Interestingly, perturbation of actomyosin based cell contractility prior to heart tube epithelialization causes cardiac defects [92,93], while post-MET does not [89], implicating a crucial role of MET step for proper heart function.

Liver development

Some organs develop from the definitive endoderm, such as the lung, pancreas and liver. In the case of the latter, although it is known that its development requires EMT–MET cycles, the precise molecular basis are not well defined [94]. Hepatic buds are made up of endodermal cells that migrate into the mesenchyme via EMT through the induction of BMP4, controlled by GATA4, which represses E-cad [95]. Then, hepatoblasts go through MET to initiate liver morphogenesis and cell differentiation (cholangiocytes and hepatocytes) (Figure 2E). However, some differences on the final outcomes exist where perivenous hepatocytes forming the bile canaliculus express N-cad, while cholangiocytes and periportal hepatocytes are organized in a tubular structure expressing E-cad [94–96]. This stage is fundamental to orchestrate cell specialization, differentiation and assembly, ensuring correct liver morphogenesis.

Neural crest condensation

Neural crest cells are a multipotent group of embryonic cells that originate from the ectodermal epithelium and initiate their delamination via a well described EMT process [97]. At their destinations, some cells stop their pathways and undergo a condensation process to begin forming different tissues such as sensory, autonomic and enteric ganglia, cornea, outflow tract septation, adrenal glands and peripheral nerves sheaths [98]. This condensation step has been likened to MET, but we have to consider that during ganglia formation cells do not become completely epithelial, and so this transition has been named as mesenchymal-to-ganglionic transition. In this context, a very recent work revealed that many genes are specifically enhanced in neural crest-condensing cells, including macrophage migration inhibitory factor (MIF) functioning as a potent chemoattractant during trunk ganglia formation [99].

Cardiac outflow tract septation depends on the neural crest cells colonization, whose invasion and condensation along the endocardial walls forces its scission into two tubes, the aorta and pulmonary trunk [100]

Biochemical Journal (2021) **478** 1809–1825
https://doi.org/10.1042/BCJ20210083

(Figure 2F). This is a very important developmental process since account for 10% of all congenital anomalies [101]. A very recent work has shown that neural crest cells aggregation along the outflow tract closure depends on the BMP signaling gradient [102]. The authors found that CTDNEP1 (CTD Nuclear Envelope Phosphatase 1, also known as Dullard) tunes the BMP gradient amplitude and prevents the premature condensation of neural crest cells. The maintenance of its mesenchymal trait is caused by the repression of the aggregation factor Sema3c and activating the EMT-TF Twist1. Conversely, down-regulation of CTDNEP1 increases Smad1/5/8 activity concurrently with the down-regulation of mesenchymal markers (Snai2, Twist1, Rac1, Mmp14 and Cdh2) and up-regulation of Sema3c. This lead to a premature neural crest cell condensation and the consequent asymmetric septation of the outflow tract closure. In agreement with this notion, Sema3c promotes the aggregation of neural crest cells in primary cultures as well as cancer cells *in vivo* [103–106].

The cornea is transparent epithelium located at surface of the eye which consists of three main tissue layers, the outer stratified squamous epithelium, the intermediate stroma, and the inner endothelium. The stroma and endothelial layers are derived from the periocular mesenchyme, which consists of neural crest cells that goes through MET by bi-directional mesenchymal-epithelial signaling pathways coming from surrounding tissues [107] (Figure 2G). These signals included including the TGFβ, retinoic acid and the canonical Wnt/β-catenin pathway [108]. Pitx2 and Dkk2 factors have been identified to play an essential role in regulating Wnt/β-catenin signaling, thus determining the epithelial fate of mesenchyme cells [109–111].

Finally, we would like to stress that the complications related to MET investigations led to common errors in the characterization and description of the actors and the pathways implicated. During several years many authors speculated that similar and reversible mechanisms may be occurring during EMT and MET [60]. However, new evidence supports the idea that distinct factors function during EMT and MET. This is clearly evident in zebrafish embryos, where MET is dependent on the activation of α5-integrin and the extracellular matrix protein fibronectin during somite repolarization. However, none of these proteins appear to play an important role in the maintenance of epithelial status during the early embryogenesis [112]. Furthermore, while E-cad repression is critical for EMT during the movement of cells through the primitive streak in vertebrates; it is not required for MET during kidney morphogenesis [112]. Rather, new actors such as Afadin (a Nectin adapter protein) are required during repolarization of renal vesicles formation, acting upstream of the recruitment and/or stabilization of the predominant cadherin called R-cadherin [49]. Thus, in the few developmental systems that have been examined in detail, condensation and/or re-epithelialization appear to be driven by mechanisms other than those underlying the original transition to a mesenchymal state. Although we are still in our infancies to understand the events mediating the end of mesenchymal cells migration, the beginning of condensation and onset of epithelialization, studies on different developmental systems and the recent advances in high-throughput technologies will provided new markers and potential regulatory candidates that will shed some light during this event in normal and pathological conditions.

microRNAs control of MET in cancer and development

MicroRNAs (miRNAs) appear as excellent regulators of components that participate during MET, particularly by inhibiting EMT-TFs or mesenchymal related genes [6,109]. They bind to target genes by base complementarity at the 3′UTR end causing their down-modulation through multiple mechanisms, including translational repression and/or mRNA destabilization [113–115]. In general, several microRNAs can act on the same target gene, or few core microRNAs may regulate several genes on the same metabolic cascade [116], thus allowing a very precise transcriptional control during transitional stages [117]. In agreement with this notion, early studies demonstrated that miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) is a stereotypical epithelial marker and regulator of EMT by directly inhibit ZEB1/2 to maintain E-cad expression [112,118,119]. Contrarily, ectopic expression of miR-200a and miR-200c induce MET in mesenchymal cancer cells [120]. In turn, ZEB1/2 represses miR-200 transcription by binding its promoter in a negative feedback loop, ensuring a tight temporal control during EMT and MET [121,122]. Similarly, two other studies identified another feedback loop between miR-203 and SNAIL that regulates tumoral cell transitions [123,124]. Interestingly, miR-200 and miR-203 are both EMT suppressors and significantly down-regulated in mesenchymal cancer cells [125,126]. Moreover, there is a reciprocal regulation where SNAIL also represses miR-200 and ZEB also regulate miR-203 [123,127], positioning this module of microRNAs and EMT/MET-TF in a high hierarchical position that defined cellular epitheliality. In addition, miR-200 and miR-203 are both epigenetically regulated by DNA methylation [123–125]. There is an inverse correlation between miR-200 and miR-203 expression and

their promoter methylation status [128,129]. Notably, a recent study showed that DNA methylation of the miR-200 and miR-203 is dynamic and reversible and may play an important role in regulating plasticity between epithelial and mesenchymal states [130].

In addition to these pioneer miRNAs, other have been characterized as suppressors of EMT, thus possibly activating MET. Few of them have been studied during embryo development and presenting similar targets and functionalities (Figure 3). In this regards, a recent work from our group demonstrated that the epigenetic repression of miR-203 by DNA methylation is required to induce the expression of its direct targets Snail2 and Phf12 [131], both required to trigger the EMT in neural crest cells [97]. Moreover, we evidenced a similar feedback-loop where SNAIL2 itself is involved in the recruitment of the DNA methyltransferase 3B (DNMT3B) to methylate the miR-203 promoter and repress its expression prior to neural crest delamination [131]. On the other side, miR-200 family have been extensively studied regulating cell proliferation, migration and neuronal differentiation during vertebrate development [132]. Particularly, miR-200 has shown to control ZEB expression during brain development and a similar negative feedback loop between ZEB and miR-200 have been also described [133]. Interestingly, Gregory et al. [130] demonstrated using canine kidney cell line, that by manipulating the ZEB/miR-200 balance were able to repeatedly switch cells between epithelial and mesenchymal states. Furthermore, prolonged autocrine TGF-β signaling induced reversible DNA methylation of the miR-200 loci with corresponding changes in miR-200 levels.

Interestingly, an integrated analysis has identified a master miRNA regulatory network for the MET in ovarian cancer cells [134]. Eight node miRNAs, including miR-506, miR-200a, miR-141, miR-101, miR-29c, miR-128, miR-182 and miR-25 were predicted to regulate 89% of the genes implicated in MET. Particularly, miR-506 augmented E-cad expression, inhibited cell migration and invasion, and prevented TGFβ-induced EMT by targeting SNAI2.

Mir-34 and miR-30a were also describe as EMT suppressors by inhibiting SNAIL1 thus leading to E-cad repression and the initiation of MET in cancerous cells [135–137]. Similarly, miR-30 has been also implicated

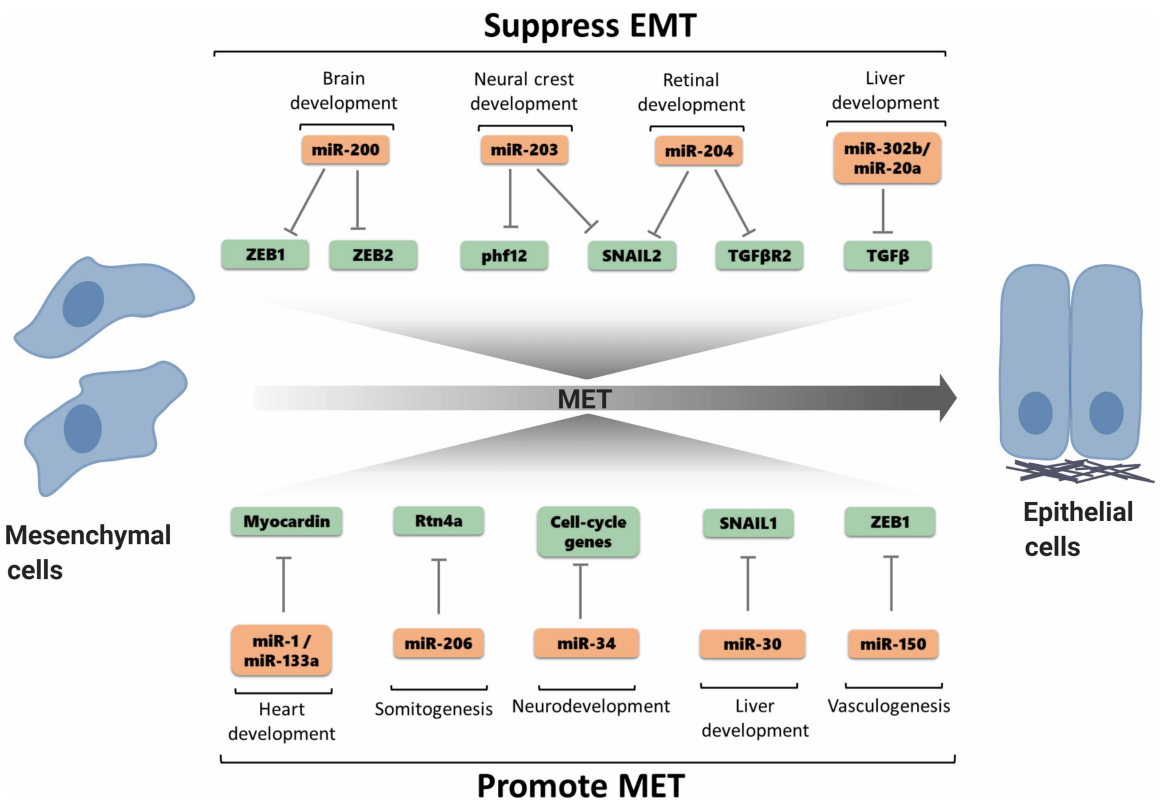

Figure 3. Summary of miRNAs playing an important role by promoting MET or inhibiting EMT during early development.
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Biochemical Journal (2021) **478** 1809–1825
https://doi.org/10.1042/BCJ20210083

in early development during hepatoblast migration and maturation by targeting SNAIL1, which result in the regulation of E-cad [138]. On the other side, p53 induces the expression of miR-34, as well as miR-200, thus determining the cell fate between epithelial, mesenchymal, or transitional stages [139]. Moreover, studies have shown that members of the miR-34 family enforced cells towards cell-cycle exit, which results in the generation of mature and differentiated cells [140]. This is in agreement with the observation that this family of microRNAs target multiple genes of the cell-cycle machinery such as cyclin D1, Cyclin E2, CDK4, CDK6, CDC25 and E2F3. On the other side, miR-34a have been described to restrict the cell fate potential of Embryonic Stem Cells (ESCs) and induced Pluripotent Stem Cells (iPSCs), possibly by affecting pruripotency genes (Nanog, Sox2 and N-Myc), which can convert into both embryonic and extraembryonic lineage [141]. The role of miR-34 family has also been studied extensively in neurodevelopment and neuronal apoptosis using different models [142–144], but not much attention was paid to their role on different embryonic cells passing through MET.

The expression of miR-302b and miR-20a have been implicated in hepatoblasts re-epitelialization to form the liver primordium [145]. Particularly, both miRNAs suppressed TGFβ signaling, thus repressing EMT and leading to liver morphogenesis and cell differentiation. It is interesting to note that the miR-302/367 family, comprising miR-367, miR-302a, miR-302b, miR-302c, miR-302d, and miR-371/373, is the most abundant miRNA family in human ESCs, and can promote somatic cell reprogramming [146]. It is important to mention that during somatic cells reprogramming, removal of repressive chromatin modifications and MET are required. In agreement with this notion, miR-302 members repressed chromatin modifying enzymes, such as lysine-specific histone demethylases and methyl-CpG binding proteins, leading to a genome-wide demethylation [146]. Lastly, miR-302 members also promoted BMP signaling and inhibited by TGFβ signaling thus lowering the MET barrier during reprogramming [147,148].

miR-204 has been also described as an EMT suppressor by regulating Snail2 and TGFβR2 [149]. In a similar manner, miR-204 maintains the epithelial integrity of the retinal pigment epithelium by regulating the same targets [149]. It is important to mention that TGFβ plays a key role, during pathological condition such as subretinal fibrosis (macular degeneration that lead to profound and permanent vision loss) by triggering the retinal EMT, leading to miR-204 repression [150]. Further investigations are needed to understand how miR-204 is activated during normal retinal development, a tissue formed by MET, which may open new opportunities in reversing subretinal fibrosis through MET reactivation to restore vision in adults.

miR-1 and miR-124 both target Snail2 and also act as EMT suppressors in tumoral cells [151,152]. Interestingly, deletion of miR-1/miR-133a gene cluster in mice results in embryonic lethality due to severe heart malformations. Particularly, the KO embryos exhibit a thinner ventricular wall due to prominent reduction in the number of cells at the compact layer of the heart and trabecular growth arrest [153]. Moreover, the authors identified Myocardin as one of the mayor target of miR-1/miR-133, but also evidenced a consistent down-regulation of Msx1 and Msx2 which are involved in cardiac EMT during atrioventricular myocardium formation [154]. Further studies are needed to evidence a possible role of miR-1 on the successive round of EMT/MET steps that are critical during heart development as we described before.

miR-150 expression was significantly lower in cancer tissues compared with adjacent non-cancerous tissues and correlated with tumor size, invasion capacity and poor prognosis [155]. *In vitro* assays showed ZEB1 as a new direct target of miR-150, thus acting as an EMT suppressor and leading to MET [155]. During development, miR150, together with miR-200c, play an important role in human endothelial lineage specification and chick embryonic vasculogenesis by targeting ZEB1 [156]. It is interesting to mention that endothelial tissue requires to go through a successive round of EMT/MET cycles during cardiovascular development and further studies are need to elucidate the role of particular miRNAs during those transitions.

Presomitic mesoderm, consisting primarily of mesenchymal cells, goes through MET to form somites in vertebrates. A recent study performed in zebrafish embryos demonstrated the role of miR-206 regulating MET during somite boundary formation [157]. Specifically, miR-206 mediates down-regulation of Reticulon4a (Rtn4a) during newly forming somites, leading to an increase in Cxcr4a/Thbs3a axis which is required during extracellular matrix formation and the epithelialization of somite boundary cells. A similar finding has been observed in *Xenopus laevis* embryos, where both knockdown and over-expression of miR-206 result in abnormal somite formation affecting cell adhesion [158]. Interestingly, miR-206 arrest cell cycle and suppress EMT acting as a suppressor in many cancers, including lung, colorectal, renal, and gastric cancers [159–162]. Although the role of miR-206 on those different type of cancer during MET has been demonstrated, its targets and the underlying mechanism are still obscure.

As we mentioned before, the existence of hybrid epithelial/mesenchymal phenotype has been increasingly recognized not only as a metastable or transient stage. Computational and experimental assays have positioned the miR-135 as one of the 'phenotypic stability factors' that can expand the existence of the hybrid epithelial/mesenchymal phenotype to a more stable stage [163]. This microRNA, together with other 'phenotypic stability factors', is involved in the stabilization of regulatory network governing epithelial/mesenchymal plasticity such as the miR-34/SNAIL and the miR-200/ZEB mutually inhibiting loops [139]. The author speculated that these 'phenotypic stability factors' may have a dual role during cancer metastasis as they can both enable collective migration of tumor cell clusters and confer these clusters with highly invasive properties. Both properties have been observed in many aggressive tumors since collective migration allows them to enter and exit the bloodstream more efficiently [164], are resistant to anoikis programmed cell death and form metastasis 50-times more frequently [30]. Thus, the hybrid epithelial/mesenchymal phenotype can pose a higher metastatic risk in patients as compared with the pure mesenchymal cells that complete EMT phenotype [165]. Therapeutically targeting the microRNAs that works on the stabilization of the hybrid phenotype can help to ameliorate the metastatic dissemination of tumoral cells.

Conclusion and perspectives

It is clear that without dissemination, metastases would not develop. However even when mesenchymal cells present high migratory capacities, they require to recover some epithelial characteristics to settle and resume proliferation at metastatic sites. The understanding of metastasis colonization *in vivo* presents many limitations due to its temporal and spatial unpredictability and the few cells that reach the metastatic sites. On the other side, MET is essential for normal embryogenesis. Taking this in consideration, the fields of cancer research and development appear to be converging. In this regards, some authors affirm that during metastasis, cancer cells co-opted the same programs as the ones that uses during early embryonic development [166]. Thus, developmental models enable the cellular and molecular manipulations with advantages of the *in vivo* approach and the predictability of the cellular behaviors.

In this review, we highlighted the role of miRNAs as central regulators of several aspects of MET during both tumor metastasis and development. The finding of new miRNAs and their targets during MET in embryonic development raised the possibility that cancer progression may mirror those mechanisms used by that tissue early in development. MET not only implies a reversion to the epithelial phenotype and many other non-MET-associated miRNAs may play an important role during cell proliferation, differentiation and communication, which are also important aspect during epithelialization that need to be taken in consideration for further studies.

An emerging challenge will be to decipher the heterogeneity of cells during transitional stages and how miRNA dynamics is represented on those cells. Excitingly, the increasing improvement of sequencing technology may help to further analyze miRNA expression at a single-cell resolution that will significantly contribute to the understanding of MET-associated core miRNA signature in different developmental systems. These studies may act as models and will help to comprehend the role of particular miRNAs in specific cancer types, thus serving later for prognosis and therapeutic approaches.

During decades researches and pharmaceutical companies has been placing all their effort to impede primary tumor to initiate EMT. However, based on the recent data on epithelial plasticity during metastasis, rather than preventing metastasis, inhibiting EMT may be counterproductive and favor the formation of secondary tumors from already disseminated cells. We are optimistic that this review will encourage further studies into the less characterized role of miRNAs during MET in early development and we propose that targeting tissue-specific MET-related miRNAs will help to define better therapeutic strategies to combat cancer metastasis.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

DNMT3B, DNA methyltransferase 3B; ECM, cell–extra cellular matrix; EMT, epithelial-to-mesenchymal transition; ESCs, embryonic stem cells; MET, mesenchymal-to-epithelial transition.

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Biochemical Journal (2021) **478** 1809–1825
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Biochemical Journal (2021) **478** 1809–1825
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