BioMol Concepts, Vol. 3 (2012), pp. 333–344 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/bmc-2011-0065

Review

Role of extracellular matrix in regulating embryonic epithelial-mesenchymal transition

Francesca Zito

Istituto di Biomedicina e Immunologia Molecolare 'Alberto Monroy', Consiglio Nazionale delle Ricerche, Via Ugo La Malfa 153, I-90146 Palermo, Italy

e-mail: zito@ibim.cnr.it

Abstract

Embryogenesis and morphogenesis are characterized by complex cell rearrangements and movements which require appropriate interactions of cells with the surrounding extracellular matrix (ECM) by means of specific membrane receptors. Interest in the identification and purification of ECM components, as well as in conducting functional studies of them, including their ligands and other molecules involved in cell-matrix adhesion, has intensified in recent years, increasing our knowledge of developmental machinery. Cellular movements play an important role during the epithelial-mesenchymal transition (EMT) events, which are key processes in normal embryogenesis as well as in pathological conditions, such as fibrotic diseases and cancer. Thus, to more fully understand mechanisms underlying the EMT process, and for better knowledge of the embryonic defects related to this process, it would be useful to study the substrates on which EMT cells move during embryo development. This review focuses on a few different embryonic systems, taking into account the cell migration that occurs during EMT and highlighting, in particular, studies describing the direct involvement of ECM molecules.

Keywords: basement membrane; cell migration; heart development; neural crest; sea urchin embryo.

Introduction

The developing embryo undergoes a highly complex sequence of events devoted to generating the adult body. Among such events, epithelial-mesenchymal transition (EMT) is a key embryological process driving the remodeling of epithelial layers by converting steady-state cells into individual and motile mesenchymal cells. From a single-layered epithelial embryo, this process produces a three-germ-layered organism with an external ectoderm and internal mesoderm and endoderm which will give rise to tissues and organs. EMT also plays a crucial role in pathogenesis, occurring in fibrotic diseases as well as in cancer progression. Thus, for obvious reasons, numerous studies are currently focused on EMT and are providing a considerable amount of data about its basic mechanisms. From a clinical viewpoint, the results obtained so far offer a great promise for the development of new therapies. At the same time, they advance our knowledge about essential developmental processes.

Although EMT is a widely studied topic, it is likely that not all aspects of this process have been elucidated so far. Considerable knowledge has been accumulated regarding cell morphological changes, and the regulation of several genes and various signaling pathways have been partially understood. Still, little is known about the role of the extracellular matrix (ECM) as a cell substrate during EMT, and cell motility is probably the least understood aspect of the whole process.

Many events in developing embryo are associated with cell migration, which consists of the coordinated movements of several different cells over short or long distances throughout the embryo. The molecular mechanisms underlying cell migration seem to be similar among different cell types, and the major actors appear to be functionally conserved in evolution. Significant progresses in understanding the basic principles of cell migration have been made, primarily through studies of *in vitro* cell cultures in which cells usually move on two-dimensional surfaces. During embryogenesis, however, cells migrate through a three-dimensional (3D) network of the ECM which possesses distinctive physical and chemical properties, thus introducing additional levels of complexity to the system. Cell-ECM interactions, as well as ECM deposition and assembly, are essential to shaping the developing embryo and to supporting cell polarization, short- and long-range cell migrations, and modifications in cell behavior.

A link among embryonic EMT, cell movements, and ECM molecules underlying these movements has rarely been emphasized in the recent literature. Indeed, many issues remain unresolved, such as: (i) what signals initiate cell movements; (ii) how cells undergoing EMT 'move across' the basement membrane; (iii) which ECM molecules are involved; and (iv) what role they have, and so on. An understanding of basic mechanisms underlying embryonic EMT, the identification and characterization of embryonic cell-ECM adhesion molecules, and the growing understanding of their role during EMT in many different systems will certainly provide answers to many of these questions.

Details of EMT processes will not be extensively addressed in this review; rather, the purpose is to focus on the cell migration during EMT in a few different embryonic systems, with special emphasis on those studies which describe the direct involvement of ECM molecules in EMT. Due to space restrictions, citations primarily refer to key original

articles or to review articles with more extensive and relevant bibliographies.

ECM and embryonic development: a few general concepts

Research on ECM molecules and their interactions with living cells is fundamental for many different disciplines, such as developmental biology, cancer research, and tissue engineering. The wide range of syndromes resulting from genetic abnormalities of ECM proteins demonstrates the great importance of the ECM for normal living cells (1).

The ECM is a multifunctional 'meshwork of fibers embedded in a gel-like ground substance' (2), whose components are synthesized by the resident cells of each tissue and secreted via exocytosis.

It seems difficult in this context to delineate a single model for the structure of the ECM. In fact, the composition of the ECM is highly variable among tissues and organs due to a number of factors, including the presence of multiple forms of individual molecules and the expression of several molecules in different tissues (2). Moreover, the physical status of the ECM varies at different stages of development; its composition is modified, either enzymatically or non-enzymatically, and remodeled in its 3D organization (both in terms of architecture and density), thus forming many different natural 'scaffolds' upon which embryonic cells can organize themselves in order to build tissues and organs. However, in a very general way, we can state that the ECM primarily exists in two forms: as an interstitial matrix filling the intercellular space, and as the more specialized basement membrane, a thin layer of ECM underlying an epithelium.

The major structural and functional components of the ECM are fibrillar and non-fibrillar collagens, glycoproteins, proteoglycans, and glycosaminoglycans. Each class of these molecules has characteristic structures and functions (2, 3). Collagens are a heterogeneous class of proteins which are the most abundant in the vertebrate body. In addition to being the main structural scaffold of most types of ECM, forming long cable-like structures (fibrillar collagens) or mesh-like structures (non-fibrillar collagens), collagens play important roles in cell attachment and spreading, thus influencing cell movements. Glycoproteins are characterized by multiple binding domains which enable them to attach to cells as well as to a variety of other ECM proteins. These molecules provide a substrate for cell adhesion and play an essential role during cell movement and differentiation. Many proteoglycans and glycosaminoglycans help in regulating matrix density by controlling hydration and spatial organization of ECM and the function of several growth factors. The availability of complete genome sequences for many organisms, coupled with the accumulated data about ECM proteins, allows the compilation of a reasonably complete list of all ECM proteins in any given matrix, namely, the 'matrisome' (3).

Implicit in this description of the ECM components is the idea that the ECM does not function solely as a passive scaffold of fibers and networks for cells, providing only structural and positional information. In fact, the fundamental knowledge that has emerged in recent years is the active role of ECM molecules in influencing (sometimes simultaneously) many aspects of cellular behavior, including cell shape and polarization, cell motility, cytoskeleton organization, cell proliferation and differentiation (4–6). Rozario and DeSimone propose a conception of the ECM as a 'morphogenetic language or code' that is interpreted by cells in contact with it (6). These ECM functions are carried out by at least three different mechanisms, involving: (i) the composition of the ECM; (ii) cell surface receptors (briefly described in a following section); and (iii) interactions with soluble growth factors to regulate their distribution, activation, and presentation to cells (7).

One of the current goals of developmental biologists is to understand, through various approaches, how ECM molecules accomplish all these functions. As underlined by Dorothy Hodgkin (Nobel Prize in Chemistry, 1964), 'knowledge of structure is critical to an understanding of function.' Thus, the identification of ECM molecules and their characterization, both at the biochemical and molecular levels, is the first step to be taken. Then, it is important to localize them in whole-mount embryos and to study their role *in vivo*, through functional assays, such as perturbation with specific antibodies or molecular probes, or the more sophisticated approach of generating mutant embryonic strains. Although the *in vivo* functional assays have significantly advanced our understanding of the role of the ECM in developmental processes, care should be taken in interpreting ECM loss-of-function phenotypes. Multiple processes may be compromised due to the multifunctional role of single ECM components, and the perturbation of one element can sometimes induce a cascade of changes involving more than one molecule. Thus, especially in mutant embryos with complex phenotypes, more in-depth analyses are required to obtain extensive descriptions of the function of a disrupted gene (8).

EMT during embryonic development: a general overview

EMT is a physiological process occurring during embryonic development which drives a remodeling of epithelial layers by converting steady-state cells into individual and motile mesenchymal cells (9). Intriguingly, EMT appears to be a conserved process throughout the animal kingdom, as many common features at both cellular and molecular levels are shared by many different species (10).

A number of complex molecular and cellular mechanisms underlie EMT, which usually converge in the activation of master genes, which in turn control the appropriate expression of genes inducing and supporting the EMT program. Several steps have been identified and some of which occur concurrently (11–13). From a morphological viewpoint, the first steps are the disruption of the intercellular adhesion complexes and the loss of the characteristic apico-basal polarity of the epithelial cells, with a decreased expression of epithelial markers. Radical cytoskeleton remodeling, together with

the breakdown of the basement membrane by ECM protease activity, allows delamination and movements of the cells undergoing EMT. These cells show a front-back end polarity, with an elongated morphology and some filopodia. A concomitant increase in the expression of mesenchymal markers occurs in these cells which acquire invasive properties and start to migrate through the ECM toward their final destination. It is worthwhile to emphasize that the term EMT refers to the shape and adhesive properties of the cells, including cellular and molecular aspects, but it does not indicate cell specification or final cell fate acquisition (11, 12).

Emerging evidence suggests that EMT also has a crucial role in adult organisms during wound healing and tissue repair as well as in pathogenesis, such as the fibrotic processes occurring during organ degeneration, carcinogenesis, and metastatic progression. However, its implication during carcinoma progression is a matter of debate (14, 15). For further discussion of EMT in pathology, see reviews by Kalluri and Neilson (16), Thiery (17), and Yang and Weinberg (18).

Classically, EMT is associated with modifications in the cell-cell and cell-ECM interactions, thus reflecting both changes in the expression levels of cell-surface markers and alterations in ECM composition; this results in a repositioning of EMT cells from a basement membrane into a fibrillar ECM. Basement membranes are highly specialized layers of ECM underlying different epithelia which separate cells from the interstitial matrix, enabling them to orientate their apical/basal polarity and guide and support migrating cells (19). During development, basement membranes change their composition, which mainly includes members of the collagen and laminin families together with entactin/nidogen, glycosaminoglycans, and proteoglycans. Fibronectin and tenascin are also sometimes associated with basement membranes. In general, proteoglycans may have a structural role, regulating the permeability of the basement membrane, the adhesive properties of which are usually attributed to the glycoproteins. Current knowledge ascribes an important role to cell-basement membrane interactions in the EMT, as has been recently shown with targeted disruptions of basement membrane components. For example, using gene targeting methods, numerous mutant mouse strains have been generated, which have significantly contributed to the understanding of how the ECM components function *in vivo* (20). In general, the idea is that the basement membrane prevents the EMT and the early differentiation of ectoderm toward mesoderm.

A critical step in many EMT processes appears to be the regulated degradation of the basement membrane underlying the epithelial cells. The degradation of ECM substrates by proteases, in fact, has different roles in the regulation of cell migration: (i) a structural role, removing matrix 'barriers' during migration and remodeling intercellular junctions; and (ii) a functional role, producing biologically active fragments and releasing important signaling molecules, such as growth factors. Extensive evidence proves that several families of proteases are actively secreted by migrating cells, including matrix metalloproteases, serine, and cysteine proteases (21). In spite of this evidence, there is a current dispute in the field regarding whether cell migration completely depends on proteolytic events. Indeed, clinical trials using matrix protease inhibitors as anticancer drugs produced disappointing results, leading to a re-evaluation of the role of proteases during cell migration and invasion (22). Subsequently, a new concept of a plasticity mechanism used by tumor cells to migrate in a proteolysis-independent manner emerged, which accounts for a putative escape strategy in tumor cell dissemination after the abrogation of pericellular proteolysis (23). Nevertheless, it remains difficult to determine whether these conflicting results stem from technical or conceptual factors and whether they should also be applied to embryonic cell migration. In general, for the successful completion of embryonic EMT, coordination among the regulated disruption of cell surface-basement membrane interactions, EMT-related morphological changes of the cell, and the ECM alterations is critical.

Cell migration – an important aspect of embryonic EMT

Migration is one of the important characteristics attributed to cells undergoing EMT, and it is definitely the most common function attributed to ECM during embryonic development. A fundamental requirement of cell migration is the competence of a cell to recognize and interact with specific ECM molecules. Dynamic interactions between ECM and cells, via specific cell surface receptors, support cell adhesion and de-adhesion to the substrate, thus facilitating the movement of isolated or grouped cells, such as those cells undergoing EMT. From the 1970s to the mid-1980s, a number of studies focused on the migratory behavior of fibroblasts, as one of the problems was to understand how cells move on the substrate and how they behave during such movements. These early observations were performed with the 'state of the art' tools available at the time, which included light and electron microscopy; thus, they were mostly morphologically based. In the early 1970s, a cyclical three-stage model of cell migration was proposed, consisting of the protrusion of the leading edge, the formation of anterior matrix adhesions and retraction of the cell, and the consequent detachment of the trailing edge (24).

In the same period, Huxley proposed an interesting theory, suggesting an explanation of how the fibroblast might use its cell matrix contacts to migrate on the substrate (25). According to this author, the cell surface ('plasmalemma and actin cortex') does not move during fibroblast locomotion. Indeed, the 'fixed cortex' theory postulates that 'the myosin-rich endoplasm of the fibroblast slides forward along an actin cortex that is stably attached to matrix via actin-binding proteins and ECM receptors.' What is intriguing is that this theory has been re-evaluated and applied to the mesenchymal cell during EMT. Thus, from studies of the emigration of neural crest cells from isolated neural tube under *in vitro* conditions, it seems that the presumptive mesenchymal cell constantly makes a new front end on its base, capped with filopodia, into which its myosin-rich endoplasm slides. This part of the cell also contains all the cell organelles, actin, and mRNA. To get away from the epithelium, the cell withdraws

its rear end, leaving blobs of cytoplasm behind, as is evidenced by the numerous cytoplasmic pieces observed at the sites where cells have detached from the epithelium (26). A later study elucidated the cellular migratory behavior in great detail, following the migration of individual avian neural crest cells labelled with DiI by means of time-lapse video microscopy (27). The authors developed a novel explant culture system which permits the tracking of migrating cells in an environment closely mimicking the intact embryo. Thus, the trajectories of individual cells and their migration rates were calculated in normal conditions or after manipulation of the molecular environment.

A few model systems to study ECM during EMT

In general, the involvement of ECM molecules in the EMT process has been easily studied using *in vitro* cell cultures, under conditions in which epithelial cells are plated on various substrates and stimulated to exhibit a mesenchymal phenotype a few days later. Through this approach, a large number of EMT participants have been listed; however, this seems rather superficial if the real aim is to study the process in detail. One of the numerous examples of these *in vitro* studies is the use of rat lens epithelial explants cultured on various types of substrata, including fibronectin, laminin, and vitronectin (28). In this study, the explants were monitored for cell migration on the different ECM molecules plated and for the concurrent expression of specific EMT markers. The authors demonstrated that vitronectin and fibronectin promote EMT in lens epithelial explants in a different way than the other ECM molecules taken into consideration, which represent good substrata for cell migration but do not promote EMT markers expression. However, these results still raise questions concerning the role of vitronectin and fibronectin in *in vivo* lens biology.

Clearly, it is more difficult to obtain data on the role of the ECM as a substrate during *in vivo* embryonic EMT. Studies on the spatiotemporal expression of ECM molecules in embryonic sites of EMT, where markers for EMT are contemporarily expressed, have provided evidence of this kind. In what follows, a few examples of model systems will be described in which the involvement of ECM molecules in EMT has been clearly shown (summarized in Table 1).

Gastrulation and neural crest formation: classic examples of embryonic EMT

The best-studied embryonic EMTs occur during gastrulation, the earliest EMT process in animal development, and during the neural crest formation (29).

Gastrulation is an ancient process, observed in all metazoans, which leads to the generation of three germ layers: the ectoderm, usually forming the skin and nervous system; the mesoderm, which forms the skeletal, cardiac muscle, and other derivatives; and the endoderm, forming mainly the gut of the adult organism. Therefore, gastrulation is an essential process devoted to place the mesoderm and endoderm inside the embryo (Figure 1A).

Table 1 ECM molecules involved in the embryonic EMT processes described in the text.

Process	EMT transition	ECM molecules		References
		Name	Function	
Gastrulation[a]	Epiblast→mesoderm/endoderm derivatives	Laminin	Directionality of migration	(32, 33)
Neural crest formation[a]	Neural tube→peripheral/enteric nervous system; facial bone/cartilage; many other derivatives	Fibronectin	Support adhesion/migration	(38)
		Laminin	Support adhesion/migration	(38)
		Tenascin-C	Support adhesion/migration	(39)
		Versican	Support adhesion/migration	(38)
		Collagen II	Support weak adhesion	(38)
		Collagen IX	Support weak adhesion	(38)
		Aggrecan	Prevent adhesion/migration	(38)
		Vitronectin	Not involved	(38)
PMCs ingression[b]	Ectoderm→skeleton	Fibronectin	Support adhesion	(47)
		Hyalin	Prevent adhesion	(48)
		Echinonectin	Prevent adhesion	(48)
		Pl-nectin	Prevent adhesion	(54)
		Laminin	Prevent adhesion	(47)
		Collagen	Prevent adhesion	(47)
Heart formation[a]	Cardiac endothelium→cardiac valves	Collagen VI	Support migration	(58)
		Fibulin	Support migration	(59)
		Laminin	Support migration	(57)
		Hyaluronan	Support migration	(56)
		Versican	Support migration	(63)
		Periostin	Support migration	(64)
		Fibronectin	Do not support migration	(57)

[a]Refers mainly to the chicken embryo.
[b]Refers to the sea urchin embryo.

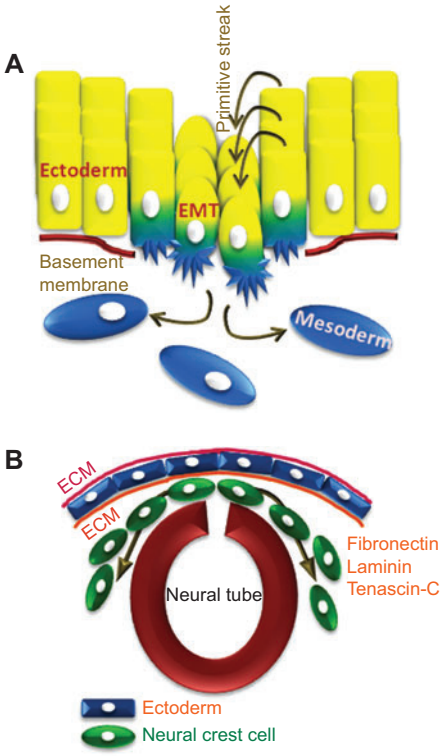

Figure 1 Embryonic EMT.
(A) EMT during vertebrate gastrulation. Ectodermal cells undergoing EMT ingress through the primitive streak and form mesenchymal cells, which migrate into the underlying tissue becoming the primary mesoderm. (B) Delamination of neural crest cells from the dorsal neural tube. The neural crest cells are formed from an EMT of ectodermal cells during the closure of the neural tube. After invading the underlying tissue, neural crest cells migrate throughout the developing embryo giving rise to diverse tissues.

Among amniotes, the chicken embryo is a relatively transparent embryo and can be easily cultivated *in vitro* – properties which make it well suited for the direct observation of morphogenetic processes. In particular, gastrulation occurring in the chicken embryo seems an appropriate model to analyze the molecular mechanisms controlling the interaction of the basement membrane with the basal surface of epithelial cells and its subsequent disruption during EMT. Therefore, the following discussion will refer primarily to studies using the chicken embryo, unless otherwise mentioned. The deposition of ECM molecules, including fibronectin and laminin, and their assembly as a fibrillar network in the blastocoel occurs very early during embryonic development, preceding the extensive cellular migrations of gastrulation (2, 30). Numerous studies over the years have established a strong correlation between certain ECM molecules and migrating cells through microscopic observations, including immunofluorescence and scanning and transmission microscopy (31). For example, by electron microscopy, a direct interaction has been described between migrating cells, through their filopodia, and the ECM, in particular with laminin-containing fibrils (2). Laminin, one of the major components of basement membrane, has a highly conserved basic structure among diverse species, and its early deposition in the embryo has been well documented (2, 32). A direct demonstration of laminin involvement in cell migration has been made by treating chick embryos with antibodies specific to this molecule, with the aim to inhibit its function. Based on the results, laminin does not seem to interfere with the triggering of cell movements, but rather it influences the directionality of cell migration (33). Although the current concept of the EMT postulates that basement membrane disruption is a process opening a path for epithelial cell migration, with little knowledge about its physiological significance, recent studies provide a new perspective on the subject. In fact, the knockout of *laminin γ1* in mouse embryonic stem cells inhibits the basement membrane deposition, with a consequent failure of ectoderm epithelialization and an acceleration of mesoderm differentiation (34). These results indicate the possibility of a regulative function of basement membrane on EMT progression. The loss of contact of the epithelial cells with the basement membrane could be one of the stimulators of the EMT.

The neural crest is an evolutionary upgrade with respect to gastrulation and is crucial for the development of a complex peripheral nervous system and the head in vertebrates (35). What is interesting in the context of this review is that neural crest cells are a typical example of cells which undergo EMT followed by extensive migrations from the neural tube, from which these cells arise, along ECM pathways to reach many targets throughout the embryo (Figure 1B). Signaling mechanisms for neural crest EMT have been fully described, and a substantial amount of literature has been produced regarding different organisms (36). More to the point, recent studies, combining a method for culturing explants from whole chicken embryo with high-resolution static and confocal time-lapse imaging, have provided interesting information about the migratory behavior of neural crest cells; they describe the movements of each single cell and the contacts with other cells or the ECM that neural crest cells maintain or create while migrating (37). Interestingly, a considerable amount of data indicates that changes in supramolecular organization of the basement membrane may govern the initial migration of neural crest cells from the neural tube during their EMT. Immunochemical and *in situ* hybridization studies have provided a rather extensive map of the ECM components expressed at various phases of neural crest development in different species. Many of these molecules have been tested *in vitro*, either alone or in various combinations, in the attempt to establish their potential role as migration-promoting components. Thus, ECM molecules have been arbitrarily divided in three different categories: (i) permissive, i.e., those that support extensive neural crest cell attachment and migration, such as fibronectin or laminin; (ii) non-permissive, i.e., those that promote a weak cell adhesion but do not sustain significant locomotion, such as collagens II and IX; and (iii) inhibitory, i.e., aggrecan, member of the family of large chondroitin sulfate proteoglycans which directly block neural crest cell adhesion and movements on a number of motility-promoting substrates (38). Moreover, indications about the *in vivo* role of some of these ECM molecules have been provided by gene deletion studies. Thus, for example, the *in vivo* knockdown of

tenascin-C expression by means of morpholino antisense oligonucleotides causes defects in the migration of neural crest cells in chicken embryos; on the one hand, most of these cells remained in clusters on or near the surface of the neural tube, pointing to a significant role for tenascin-C in the early phase of neural crest development (39). On the other hand, several other ECM molecules appear to be of lesser importance as their misfunction does not affect neural crest cell behavior: homozygous null mice completely deficient in vitronectin show normal development (40).

The role of ECM receptors is also of interest and a short paragraph will be devoted thereafter.

EMT and sea urchin embryo

The sea urchin embryo is one of the model systems in which a role of some ECM molecules as cell migration substrates during EMT has been clearly demonstrated.

The sea urchin embryo has been widely used as a valuable model, especially for developmental biology studies, taking advantage of a number of features particularly helpful for researchers: (i) its rather rapid embryogenesis, which takes a few days; (ii) its transparency, which simplifies observations and experimental manipulations; and (iii) its reasonable simplicity in shape and organization. The recent sequencing of the entire sea urchin genome has greatly advanced our knowledge on the molecular basis of its embryonic developmental processes (41). Moreover, the entire basic 'basement membrane ECM toolkit' (collagens IV, XV/XVIII, laminins, nidogen, and perlecan) is present, as it is in all metazoans whose genomes have been analyzed, although it is a little expanded (42). Taking into account all these features, the sea urchin embryo provides an excellent experimental 'laboratory' for performing detailed step-by-step analysis of embryonic EMT, at phenotypic and molecular levels (43).

The first event of EMT in the sea urchin embryo occurs before gastrulation and leads to the formation of primary mesenchyme cells (PMCs) (Figure 2). These are the first cell population to begin migration and are of great importance for the development of this embryo as they are the only cells committed early on to the synthesis of the larval skeleton. The gene regulatory network (GRN) that underlies the specification and differentiation of skeletogenic cells in the sea urchin embryo is currently understood in great detail. In addition, the GRN activating the EMT process of PMCs is relatively well-known, as most of the genes involved have been identified and characterized (44). From a molecular point of view, it has been shown that the main EMT genes, including snail, twist, cadherin, and others, which are expressed in the sea urchin embryo, are homologous with those found in other organisms. What is interesting for the scope of this review is the identification of the ECM molecules involved in the EMT of PMCs, localized both in the extraembryonic matrices and inner ECM as described in detail by Wessel and Katow (43). As early as Gustafson and Wolpert's pioneering time-lapse micro-cinematographic studies, it was hypothesized that many morphogenetic events occurring during sea urchin

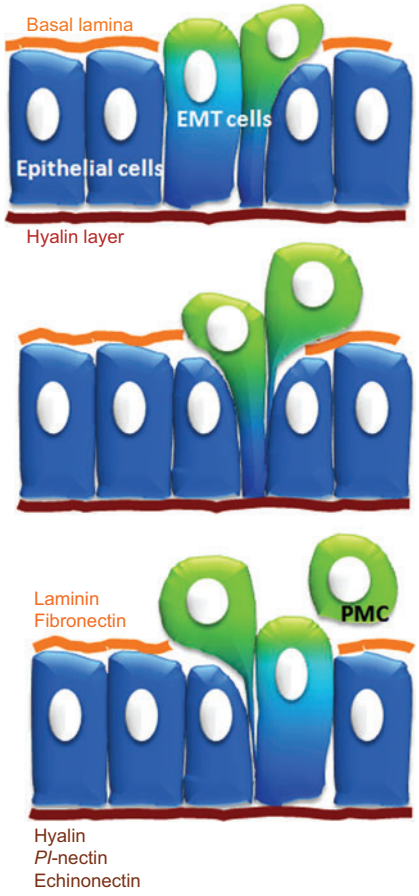

Figure 2 Ingression of PMCs.
Epithelial cells of the blastocoel wall undergo an EMT giving rise to the PMCs. These cells, during their ingression into the blastocoelic cavity, lose adhesion to the hyaline layer components and increase their adhesion to the basal lamina substrate.

development could be explained by changes in the cell-cell and the cell-substrate contacts: 'the mechanism of entry of the primary mesenchyme into the blastocoel…involves a pulsatory activity of the cell surfaces towards the blastocoel, and their loss of adhesion to each other and to the hyaline layer' (45). Moreover, descriptive studies by Katow and Solursh showed 'the fibrous nature of blastocoelic material that may serve as a substrate for cell movement' (46). Subsequently, thanks to advances in scientific technology, it has been possible to identify some of the ECM molecules to which PMCs lose or acquire adhesive affinity during EMT. These studies have been performed by means of *in vitro* adhesion assays, by which the affinity of isolated micromeres (the precursors of PMCs) or PMCs for ECM components have been measured (47). Thus, while micromeres adhere well to hyaline and echinonectin (48), which are two components of the extraembryonic ECM, PMCs lose adhesive affinity for both proteins during their ingression and gain a specific adhesive affinity for fibronectin, which is a known component of the basal lamina. Moreover, ingressed PMCs show reduced adhesion to laminin, which is also a component of the basal lamina, and no longer express the αSU2 integrin, which is involved

in binding the laminin of the basal lamina (49). At the same time, when ingression begins, the PMCs rapidly endocytose cadherins from their cell surface, thus destroying adherens junctions and losing their affinity for other cells of the blastula wall (50). As these numerous cell adhesion properties change, PMCs become motile – it takes about 15–30 min for an individual PMC to change its epithelial phenotype into a mesenchymal one.

In the sea urchin embryo, it has not yet been clearly shown whether matrix metalloproteases are involved in degrading basal lamina through which PMCs have to move for ingression. Electron microscopy studies have shown the disappearance of the basal lamina under ingressing cells, suggesting digestion or mechanical disruption, probably by the PMCs themselves (51).

Although several of the molecular changes leading to the EMT of PMCs have been documented, and because all of them occur at roughly the same time, it is not yet known how the cells coordinate these synchronous molecular changes to initiate ingression.

Additional information has been obtained indirectly by inhibitor assays with living embryos in which the inhibition or modification of some ECM molecules either caused or did not cause the inhibition of PMC ingression into the blastocoelic cavity. For example, continuous treatments of embryos with a specific inhibitor of lysyl oxidase, which is a collagen cross-linking enzyme, do not affect early development, allowing them to reach the mesenchyme blastula stage. Thus, considering that PMC ingression occurs regularly, collagen does not seem to be a substrate for PMC migration during EMT (52). This result is consistent with previous studies which demonstrated no significant affinity changes of PMCs for the collagen substratum (47, 53). Similarly, continuous treatment of blastula embryos with monoclonal antibodies to *Pl*-nectin, a glycoprotein localized in the extraembryonic ECM, does not affect the ingression of PMCs or their movements inside the blastocoel, although an inhibition of skeleton development is observed (54, 55).

EMT and embryonic heart development

Another instructive model is the embryonic heart, in which several EMT processes take place (56). A large number of studies over the years have focused on this system, especially on the EMT that gives rise to the mesenchymal cells from the endothelial cells lining the atrioventricular (AV) canal. Indeed, these cells invade the cardiac jelly and form the endocardial cushion, namely, the primordia of the valves and cardiac septa of the adult heart (Figure 3). As abnormal development of endocardial cushion tissues is linked to many congenital heart diseases, which sometimes exhibit disrupted ECM organization, the understanding of heart development will provide new insights into the pathogenesis of such diseases (56). This system is especially important for the aims of this review because of the variety of different approaches that, paying particular attention to the ECM and its role in embryonic heart EMT, have generated significant outcomes (57).

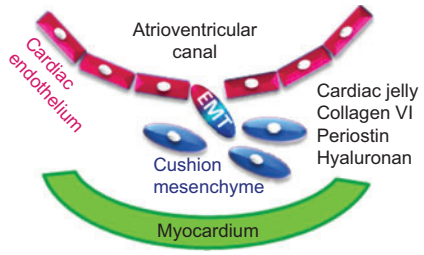

Figure 3 Formation of the cushion mesenchyme in the developing heart from the AV canal.
The cardiac endothelial cells that overlie the cardiac jelly undergo an EMT giving rise to the cushion mesenchymal cells. These cells disperse within the cushions and undergo terminal differentiation into valvular fibroblasts, which are the precursor of the cardiac valves.

The study of the spatiotemporal distribution and organization of the ECM components of the myocardial basement membrane has involved the use of microscopy, including light, confocal, scanning, and transmission electron microscopy, as well as a variety of histochemical procedures. Among the ECM proteins described in normal heart development, collagen VI (58) and fibulin (59) are particularly expressed where EMT occurs, i.e., within the endocardial cushions and the developing AV canal, in a pattern paralleling cell migration and septum-valve remodeling. In agreement with the important role hypothesized for collagen VI in cushion cell migration and differentiation, a molecular screening of Down syndrome infants has shown a correlation between aberrant expression of *collagen VI* and congenital heart defects, although a cause-effect link has been recently ruled out (60).

To study the functional role of the ECM during heart development, one of the initial approaches was the isolation of ECM from the heart and testing for its activity on the AV canal endothelium in culture. In particular, an ethylenediaminetetraacetic acid (EDTA)-soluble extract of the embryonic myocardial basement membrane was sufficient to induce EMT in a monolayer of competent endothelial cells cultured in a collagen gel system. Furthermore, an antiserum prepared against the 'inductive ECM fraction,' i.e., the EDTA-soluble extract, inhibited the formation of mesenchyme cells from the cardiac endothelium cells, indicating once again a relationship between ECM molecules and EMT. An analysis of EDTA extracts of embryonic hearts revealed the presence of fibronectin among about 40 unidentified proteins (61).

An approach used to explore cellular and molecular aspects of EMT in heart development more deeply is the use of a 3D collagen gel culture system (62). Using primary cardiac explants from chicken embryos, it has been possible to record mesenchymal outgrowth and show the presence of multiple adhesion mechanisms during cardiac mesenchymal cell migration. In fact, perturbation assays by particular inhibitors, including specific peptides mimicking either the binding domains of fibronectin or laminin and function-blocking antibodies specific for integrins, caused different effects: both laminin-related peptides and antibodies against integrin inhibited mesenchymal cell migration, while fibronectin-related peptides had no significant effect on migration (57).

The introduction of genetically engineered embryos has provided a significant advance in such studies. For example, an active role for hyaluronan in EMT has recently been shown in heart explant tissues from genetically engineered mouse models; embryos defective in the expression of *hyaluronan synthase-2* die of severe heart defects due to a failure of endocardial cushion cells to undergo EMT, with a consequent lack of heart valve formation. In general, hyaluronan is highly expressed during embryogenesis and plays two complementary roles: (i) expansion of the extracellular space, providing a hydrated matrix for cell migration; and (ii) stimulation of EMT in cardiac endothelial cells (56).

The expression and distribution of versican and aggrecan, already mentioned as two known antiadhesive proteoglycans in many cell types, has been studied in the aortic wall during chicken embryo development and during *in vitro* EMT (63). The two molecules have different spatial and temporal patterns of expression and have also shown differences in their content in all the examined stages, thus suggesting that they play different roles during the remodeling of the aortic wall. Interestingly, strong versican expression was detected in *in vitro* aortic explants in association with EMT, especially in detaching and migrating cells and in the newly synthesized and deposited ECM. Moreover, the presence of sulfated glycosaminoglycan chains is not required for versican to function in the EMT process in terms of cell detachment and migration, but they might contribute by providing a hydrated microenvironment together with other molecules (63).

Another interesting and noteworthy ECM component known to have a role in cell migration and EMT is periostin – a known valvulogenic matrix maturation mediator. It belongs to the fasciclin family and is able to interact with ECM scaffold proteins, such as fibronectin, collagen I, collagen V, tenascin-C and heparin, as well as with some integrins, thus modulating cell-matrix interactions, affecting the ability of cells to migrate and facilitating EMT. It shows dynamic expression in areas with ongoing cellular reorganization during both embryonic development and pathological conditions. Regarding embryonic development, periostin is expressed in the developing endocardial cushions of the heart and the mature valves, as well as in the periosteum and periodontal ligament – all are sites of EMT processes. By means of chick primary culture assays, periostin has been shown to promote AV cushion mesenchyme cell migration and regulate collagen remodeling during matrix maturation (64). Moreover, the spatiotemporal expression of periostin, both at the mRNA and protein level, has been recently analyzed during mouse palatal fusion which results in the confluence of palatal mesenchyme. The protein is strongly expressed in the basement membrane underlying epithelial cells during the onset of EMT, as shown by the concurrent expression of *twist1*, an EMT marker (65). In general, the majority of data available on periostin suggest that it has a common function in both embryonic development and disease, as a potent regulator of cellular reorganization and extracellular homeostasis (64).

ECM receptors as mediators of embryonic EMT

It is virtually mandatory to briefly refer to ECM receptors, with a particular attention to their role as mediators of EMT. These molecules are fundamental in mediating the extensive cell-ECM interactions during development, but, in this context, only three examples will be briefly described: integrins, discoidin domain receptor (DDR), and dystroglycan.

Many of the ECM receptors belong to the integrin family which is composed of at least 24 different α/β transmembrane heterodimers (66). Their role is to transduce the cell-ECM 'information flow' in a bidirectional manner. The integrin-ECM binding triggers the downstream activation of cytoplasmic signaling cascades, including variations in calcium concentrations, phosphorylation events, modifications of cytoskeleton organization, and assembly of focal contacts (66). The proteins connecting the cytoplasmic domains of integrins to the cytoskeleton are multiple and their interactions are complex. Multiple integrins are known to be co-expressed on the surface of neural crest cells and, for some of them, their role has been shown. Indeed, function blocking antibodies (67) or specific antisense oligonucleotides (68, 69) have been used to functionally knock out integrins, resulting in the inhibition of neural crest adhesion *in vitro* and migration *in vivo* and in the abnormal EMT in *in vivo* chicken embryos.

In addition to integrin, the non-integrin receptors form a varied group of molecules, some of which are probably involved in embryonic EMT.

DDRs are collagen-specific receptors, with an intrinsic tyrosine kinase function, which have been shown to regulate cell migration upon binding to collagen. In particular, DDR2 is found in the chicken AV canal during EMT – it is expressed in both 'activated' endothelial cells, i.e., those cells that are preparing to lose their cell-cell contacts, and migrating mesenchymal cells *in vivo* (70). DDR2 function has been shown by targeted deletion or knockdown with siRNA of the gene, in mouse embryo and in cell lines, respectively. Fibroblasts from *DDR2*-null mice are unable to migrate through an artificial basement membrane and show reduced MMP2 activity and collagen I expression (70). Moreover, knockdown of *DDR2* expression in cells cultured *in vitro* inhibits the EMT directly induced by collagen I, which established a critical role for collagen-dependent DDR2 signaling in the regulation of EMT (71).

Dystroglycan is a heterodimeric adhesion receptor widely distributed in a multitude of cell types and tissues and is involved in several diseases (72). In the chicken embryo, its expression is restricted to the baso-lateral membrane of epiblast cells and its function has been widely associated with the epithelial integrity. Dystroglycan expression is downregulated in cells undergoing EMT during gastrulation, thus it seems involved in the regulated disruption of the basement membrane occurring in many EMT processes, even though its exact function in EMT is not well understood (73).

Expert opinion

A large number of studies have clearly demonstrated that embryonic EMT is an evolutionary conserved mechanism,

although the molecular details often differ among different species. It is commonly held that the mechanisms driving embryonic EMT share some common features with those inducing EMT during tumor progression. However, implication of EMT during carcinoma progression has been a matter of debate until recently, as not all the properties described in embryonic EMT have been observed or are obvious in culture systems or in cancer cell lines (14–17). Nevertheless, for those who support a role for EMT in carcinogenesis, it seems very likely that the occurrence of EMT in adulthood depends on the reactivation of similar developmental programs, but with harmful consequences for the organism. For this reason, it is supposed that a deep knowledge of the molecular mechanisms of embryonic EMT may help us understand the mechanisms governing EMT in cancer, thus facilitating the design of anti-invasive or antifibrotic drugs (29, 74, 75).

The challenge in the coming years will be to link all the data obtained by different approaches to *in vitro* cell cultures and whole-mount embryos, combining life imaging techniques with biochemical, cellular, and molecular studies. A fundamental controversy remains regarding whether the adhesion mechanisms described in isolated cells cultured *in vitro* have any similarities to those found in the same cells in *in vivo* organisms. The observed discrepancies in cell behavior between the *in vitro* and *in vivo* systems have led, as a consequence, to an increasing tendency to switch to *in vitro* 3D models. These better represent the microenvironment of living tissues, while still maintaining the indispensable advantage of studying mechanisms by isolating and defining the specific contributions of individual factors to the overall process (76). In addition, advances in imaging techniques in recent years have vastly improved the study of cell adhesion in live embryos, thus contributing a great deal to the understanding of physiological and pathological motility and migratory processes in their correct context, including detailed analysis of the molecular composition and dynamics of the structures involved (77). Further improvements in life imaging techniques, together with the power of genetics to identify novel candidate molecules as EMT-ECM markers and the possibility to test for their function both in *in vitro* cell cultures and live embryos, will be instrumental to the further analysis of these processes and may lead to a blueprint of EMT movements.

Outlook

The complexity of biological regulatory mechanisms has led more and more to the use of computational modeling, thus increasing the interest in developing *in silico* models of *in vivo* and *in vitro* morphogenesis. In particular, a recent study was devoted to the construction of a simple computational model of EMT-driven rearrangements of cells during cardiac cushion tissue formation (78). The aim was to identify the key elements of these processes and study their relationship using *in silico* experiments. This was the first attempt to model cardiac cushion tissue formation by simultaneously taking into account different aspects, such as differential adhesion, EMT, cell proliferation, and matrix production by mesenchymal cells. Among the results obtained, which are in qualitative agreement with available experimental data, it seems that EMT is promoted more successfully by an increase in cell-substrate adhesion than by a decrease in cell-cell adhesion.

Interdisciplinary approaches, such as the 'omic' technologies, i.e., transcriptomics and proteomics, allow the study of the expression profiles of multiple targets simultaneously. Recently, transcriptomic and proteomic techniques have also been exploited in EMT research, revealing novel markers potentially involved in cancer progression, although several ECM players remain largely unknown (79). Thus, developments in proteomic technologies will permit the in-depth comprehensive characterization of the protein components within the ECM, including post-translational modifications, protein secretion, interactions, and cellular signaling. The challenge will be to relate this proteomic data to the results obtained by functional studies and to interpret them in the context of embryonic or pathophysiological EMT processes.

Another rapidly expanding field of research is focused on microRNA (miRNA). These are a class of very short, non-coding RNA molecules (about 22 nucleotides), which function as post-translational regulators of gene expression and are involved in a variety of physiological and pathological processes. Emerging evidence shows that miRNAs play important roles in many aspects of cell migration, being involved in the ECM remodeling, cell adhesion, signaling controlling cell movements (80). Moreover, several studies have identified miRNA families which are key regulators of embryonic and pathological EMTs (81). There does not seem to be any data describing miRNAs regulating cell migration of EMT cells.

In general, the successful integration of newly developed high-throughput approaches, from molecular biology, mathematics, physics, and engineering, to the study of embryonic EMT biology is promising for a deeper understanding of complex processes, such as cancer, and may help to develop effective strategies for therapies.

Highlights

In native tissues, cells are constantly and dynamically communicating with and influencing the surrounding ECM. Interactions between cells and ECM molecules play key roles in regulating cell migration both in physiological and pathological events. This review has sought to point out that there are several different kinds of molecules involved in EMT, and ECM components are among these. Changes in the ECM composition during EMT have important biological significance, as they support different migratory behaviors among EMT cells. The small number of concepts selected for discussion was proposed, keeping in mind the need to provide evidence of the important role of ECM on embryonic cells undergoing EMT.

Although many important factors involved in these processes have been identified and characterized, the GRNs and mechanisms employed to regulate the migration of cells during

EMT remain far from fully understood. One of the questions remaining is to determine how the changes in cellular adhesion and cell motility are coordinated. Undoubtedly, knowing how the timing of cell movements is controlled during embryonic EMT and which ECM components are chronologically involved would be a crucial outcome. Moreover, it remains to be clarified what happens at the molecular level when EMT cells bind to ECM. In general, cell migration on different ECM molecules is known to activate complex signaling pathways through surface receptors, whose major categories of signaling molecules include focal adhesion kinase, Src-family kinases, phosphoinositide 3-kinase, protein kinase C, and the Rho family of small guanosine triphosphatases. Until now, these pathways have not been described for EMT migrating cells.

However, as all the discussed mechanisms may well be conserved functions of cells undergoing EMT, either in normal or pathological conditions, they would be obvious and promising targets for pharmacological treatments of cancer cells and organ-degenerative diseases, namely, cell movements and ECM molecule inhibitors.

Acknowledgments

The author warmly thanks all the members of the lab for their continuous encouragement and support during the writing of this review. In addition, she wishes to apologize to everyone if some time has been taken off from the lab. The author also thanks the anonymous reviewers who made positive and valuable suggestions. She also deeply apologizes for the many important works whose references could not be included owing to space restrictions.

References

1. Järveläinen H, Sainio A, Koulu M, Wight TN, Penttinen R. Extracellular matrix molecules: potential targets in pharmacotherapy. Pharmacol Rev 2009; 61: 198–223.
2. Zagris N. Extracellular matrix in development of the early embryo. Micron 2001; 32: 427–38.
3. Hynes RO, Naba A. Overview of the matrisome – an inventory of extracellular matrix constituents and functions. Cold Spring Harbor Perspect Biol 2012; 4: a004903.
4. Adams JC, Watt FM. Regulation of development and differentiation by the extracellular matrix. Development 1993; 117: 1183–98.
5. Tsang KY, Cheung MCH, Chan D, Cheah KSE. The developmental roles of the extracellular matrix: beyond structure to regulation. Cell Tissue Res 2010; 339: 93–110.
6. Rozario T, DeSimone DW. The extracellular matrix in development and morphogenesis: a dynamic view. Dev Biol 2010; 341: 126–40.
7. Hynes RO. The extracellular matrix: not just pretty fibrils. Science 2009; 326: 1216–9.
8. Gustafsson E, Fässler R. Insights into extracellular matrix functions from mutant mouse models. Exp Cell Res 2000; 261: 52–68.
9. Hay ED. An overview of epithelio-mesenchymal transformation. Acta Anat 1995; 154: 8–20.
10. Shook D, Keller R. Mechanisms, mechanics and function of epithelial – mesenchymal transitions in early development. Mech Dev 2003; 120: 1351–83.
11. Morali OG, Savagner P, Larue L. Epithelium-mesenchyme transitions are crucial morphogenetic events occurring during early development. In: Savagner P, editor. Rise and fall of epithelial phenotype: concepts of epithelial-mesenchymal transition. Eurekah.com (Accessed June 2011). Kluwer Academic/Plenum Publishers, 2005: 12–28.
12. Hay ED. EMT concept and examples from the vertebrate embryo. In: Savagner P, editor. Rise and fall of epithelial phenotype: concepts of epithelial-mesenchymal transition. Eurekah.com (Accessed June 2011). Kluwer Academic/Plenum Publishers, 2005: 1–11.
13. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 2006; 7: 131–42.
14. Tarin D. The fallacy of epithelial-mesenchymal transition in neoplasia. Cancer Res 2005; 65: 5996–6001.
15. Thompson EW, Newgreen DF. Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? Cancer Res 2005; 65: 5991–5.
16. Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implication for fibrosis. J Clin Invest 2003; 112: 1776–84.
17. Thiery JP. Epithelial-mesenchymal transitions in tumor progression. Nat Rev Cancer 2002; 2: 442–54.
18. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 2008; 14: 818–29.
19. Timpl R. Structure and biological activity of basement membrane proteins. Eur J Biochem 1989; 180: 487–502.
20. Smyth N, Vatansever HS, Murray P, Meyer M, Frie C, Paulsson M, Edgar D. Absence of basement membranes after targeting the LAMC1 gene results in embryonic lethality due to failure of endoderm differentiation. J Cell Biol 1999; 144: 151–60.
21. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol 2007; 8: 221–33.
22. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002; 295: 2387–92.
23. Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, Deryugina EI, Strongin AY, Bröcker EB, Friedl P. Compensation mechanism in tumor cell migration: mesenchymal-amoeboid transition after blocking of pericellular proteolysis. J Cell Biol 2003; 160: 267–77.
24. Abercrombie M, Heaysman JE, Pegrum SM. The locomotion of fibroblasts in culture. I. Movements of the leading edge. Exp Cell Res 1970; 59: 393–8.
25. Huxley HE. Muscular contraction and cell motility. Nature 1973; 243: 445–9.
26. Hay ED. Theory for epithelial-mesenchymal transformation based on the 'fixed cortex' cell motility model. Cell Motil Cytoskeleton 1989; 14: 455–7.
27. Krull CE, Collazo A, Fraser SE, Bronner-Fraser M. Segmental migration of trunk neural crest: time-lapse analysis reveals a role for PNA-binding molecules. Development 1995; 121: 3733–43.
28. Taliana L, Evans MDM, Ang S, McAvoy JW. Vitronectin is present in epithelial cells of the intact lens and promotes epithelial-mesenchymal transition in lens epithelial explants. Mol Vision 2006; 12: 1233–42.
29. Acloque H, Adams MS, Fishwick K, Bronner-Fraser M, Nieto MA. Epithelial-mesenchymal transitions: the importance of changing cell state in development and disease. J Clin Invest 2009; 119: 1438–49.
30. Harrisson F. The extracellular matrix and cell surface, mediators of cell interactions in chicken gastrulation. Int J Dev Biol 1989; 33: 417–38.

31. Duband JL, Thiery JP. Appearance and distribution of fibronectin during chick embryo gastrulation and neurulation. Dev Biol 1982; 94: 337–50.
32. Zagris N, Stavridis V. The expression of the genes for laminin in the early embryo. In: Zagris N, Duprat AM, Durston A, editors. Organization of the early vertebrate embryo. New York: Plenum Press, 1995; 279: 169–82.
33. Zagris N, Chung AE. Distribution and functional role of laminin during induction of the embryonic axis in the chick embryo. Differentiation 1990; 43: 81–6.
34. Fujiwara H, Hayashi Y, Sanzen N, Kobayashi R, Weber CN, Emoto T, Futaki S, Niwa H, Murray P, Edgar D, Sekiguchi K. Regulation of mesodermal differentiation of mouse embryonic stem cells by basement membranes. J Biol Chem 2007; 282: 29701–11.
35. Le Douarin NM, Kalcheim C, editors. The Neural Crest, 2nd ed. Cambridge: Cambridge University Press, 1999: 445.
36. Newgreen DF, McKeown SJ. The neural crest: a model developmental EMT. In: Savagner P, editor. Rise and fall of epithelial phenotype: concepts of epithelial-mesenchymal transition. Eurekah.com (Accessed June 2011). Kluwer Academic/Plenum Publishers, 2005: 29–39.
37. Teddy JM, Kulesa PM. In vivo evidence for short- and long-range cell communication in cranial neural crest cells. Development 2004; 131: 6141–51.
38. Perris R, Perissinotto D. Role of the extracellular matrix during neural crest cell migration. Mech Dev 2000; 95: 3–21.
39. Tucker RP. Abnormal neural crest cell migration after the in vivo knockdown of tenascin-C expression with morpholino antisense oligonucleotides. Dev Dyn 2001; 222: 115–9.
40. Zheng X, Saunders TL, Camper SA, Samuelson LC, Ginsburg D. Vitronectin is not essential for normal mammalian development and fertility. Proc Natl Acad Sci USA 1995; 92: 12426–30.
41. Sea Urchin Genome Sequencing Consortium, Sodergren E, Weinstock GM, Davidson EH, Cameron RA, Gibbs RA, Angerer RC, Angerer LM, Arnone MI, Burgess DR, Burke RD, Coffman JA, Dean M, Elphick MR, Ettensohn CA, Foltz KR, Hamdoun A, Hynes RO, Klein WH, Marzluff W, McClay DR, Morris RL, Mushegian A, Rast JP, Smith LC, Thorndyke MC, Vacquier VD, Wessel GM, Wray G, Zhang L, Elsik CG, Ermolaeva O, Hlavina W, Hofmann G, Kitts P, Landrum MJ, Mackey AJ, Maglott D, Panopoulou G, Poustka AJ, Pruitt K, Sapojnikov V, Song X, Souvorov A, Solovyev V, Wei Z, Whittaker CA, Worley K, Durbin KJ, Shen Y, Fedrigo O, Garfield D, Haygood R, Primus A, Satija R, Severson T, Gonzalez-Garay ML, Jackson AR, Milosavljevic A, Tong M, Killian CE, Livingston BT, Wilt FH, Adams N, Bellé R, Carbonneau S, Cheung R, Cormier P, Cosson B, Croce J, Fernandez-Guerra A, Geneviève AM, Goel M, Kelkar H, Morales J, Mulner-Lorillon O, Robertson AJ, Goldstone JV, Cole B, Epel D, Gold B, Hahn ME, Howard-Ashby M, Scally M, Stegeman JJ, Allgood EL, Cool J, Judkins KM, McCafferty SS, Musante AM, Obar RA, Rawson AP, Rossetti BJ, Gibbons IR, Hoffman MP, Leone A, Istrail S, Materna SC,Samanta MP, Stolc V, Tongprasit W, Tu Q, Bergeron KF, Brandhorst BP, Whittle J, Berney K, Bottjer DJ, Calestani C, Peterson K, Chow E, Yuan QA, Elhaik E, Graur D, Reese JT, Bosdet I, Heesun S, Marra MA, Schein J, Anderson MK, Brockton V, Buckley KM, Cohen AH, Fugmann SD, Hibino T, Loza-Coll M, Majeske AJ, Messier C, Nair SV, Pancer Z, Terwilliger DP, Agca C, Arboleda E, Chen N, Churcher AM, Hallböök F, Humphrey GW, Idris MM, Kiyama T, Liang S, Mellott D, Mu X, Murray G, Olinski RP, Raible F, Rowe M, Taylor JS, Tessmar-Raible K, Wang D, Wilson KH, Yaguchi S, Gaasterland T, Galindo BE, Gunaratne HJ, Juliano C, Kinukawa M, Moy GW, Neill AT, Nomura M, Raisch M, Reade A, Roux MM, Song JL, Su YH, Townley IK, Voronina E, Wong JL, Amore G, Branno M, Brown ER, Cavalieri V, Duboc V, Duloquin L, Flytzanis C, Gache C, Lapraz F, Lepage T, Locascio A, Martinez P, Matassi G, Matranga V, Range R, Rizzo F, Röttinger E, Beane W, Bradham C, Byrum C, Glenn T, Hussain S, Manning G, Miranda E, Thomason R, Walton K, Wikramanayke A, Wu SY, Xu R, Brown CT, Chen L, Gray RF, Lee PY, Nam J, Oliveri P, Smith J, Muzny D, Bell S, Chacko J, Cree A, Curry S, Davis C, Dinh H, Dugan-Rocha S, Fowler J, Gill R, Hamilton C, Hernandez J, Hines S, Hume J, Jackson L, Jolivet A, Kovar C, Lee S, Lewis L, Miner G, Morgan M, Nazareth LV, Okwuonu G, Parker D, Pu LL, Thorn R, Wright R. The genome of the sea urchin Strongylocentrotus purpuratus. Science 2006; 314: 941–52.
42. Whittaker CA, Bergeron KF, Whittle J, Brandhorst BP, Burke RD, Hynes RO. The echinoderm adhesome. Dev Biol 2006; 300: 252–66.
43. Wessel GM, Katow H. Regulation of the epithelial-to-mesenchymal transition in sea urchin embryos. In: Savagner P, editor. Rise and fall of epithelial phenotype: concepts of epithelial-mesenchymal transition. Eurekah.com (Accessed June 2011). Kluwer Academic/Plenum Publishers, 2005: 77–100.
44. Wu SY, Ferkowicz M, McClay DR. Ingression of primary mesenchyme cells of the sea urchin embryo: a precisely timed epithelial-mesenchymal transition. Birth Defects Res, Part C 2007; 81: 241–52.
45. Gustafson T, Wolpert L. Cellular movement and contact in sea urchin morphogenesis. Biol Rev Cambridge Philos Soc 1967; 42: 442–98.
46. Katow H, Solursh M. Ultrastructure of blastocoel material in blastulae and gastrulae of the sea urchin Lytechinus pictus. J Exp Zool 1979; 210: 561–7.
47. Fink RD, McClay DR. Three cell recognition changes accompany the ingression of sea urchin primary mesenchyme cells. Dev Biol 1985; 107: 66–74.
48. Burdsal CA, Alliegro MC, McClay DR. Tissue-specific, temporal changes in cell adhesion to echinonectin in the sea urchin embryo. Dev Biol 1991; 144: 327–34.
49. Hertzler PL, McClay DR. αSU2, an epithelial integrin that binds laminin in the sea urchin embryo. Dev Biol 1999; 207: 1–13.
50. Miller JR, McClay DR. Changes in the pattern of adherens junction-associated β-catenin accompany morphogenesis in the sea urchin embryo. Dev Biol 1997; 192: 310–22.
51. Katow H, Solursh M. Ultrastructure of primary mesenchyme cell ingression in the sea urchin Lytechinus pictus. J Exp Biol 1980; 213: 231–46.
52. Wessel GM, McClay DR. Gastrulation in the sea urchin embryo requires the deposition of crosslinked collagen within the extracellular matrix. Dev Biol 1987; 121: 149–65.
53. Katow H. Behavior of sea urchin primary mesenchyme cells in artificial extracellular matrices. Exp Cell Res 1986; 162: 401–10.
54. Zito F, Tesoro V, McClay DR, Nakano E, Matranga V. Ectoderm cell – ECM interaction is essential for sea urchin embryo skeletogenesis. Dev Biol 1998; 196: 184–92.
55. Zito F, Costa C, Sciarrino S, Poma V, Russo R, Angerer LM, Matranga V. Expression of univin, a TGF-β growth factor, requires ectoderm-ECM interaction and promotes skeletal growth in the sea urchin embryo. Dev Biol 2003; 264: 217–27.
56. Hinton RB, Yutzey KE. Heart valve structure and function in development and disease. Annu Rev Physiol 2011; 73: 29–46.
57. Runyan RB, Heimark RL, Camenisch TD, Klewer SE. Epithelial-mesenchymal transformation in the embryonic heart. In: Savagner

P, editor. Rise and fall of epithelial phenotype: concepts of epithelial-mesenchymal transition. Eurekah.com (Accessed June 2011). Kluwer Academic/Plenum Publishers, 2005: 40–55.
58. Tan H, Junor L, Price RL, Norris RA, Potts JD, Goodwin RL. Expression and deposition of fibrous extracellular matrix proteins in cardiac valves during chick development. Microsc Microanal 2011; 17: 91–100.
59. Spence SG, Argraves WS, Walters L, Hungerford JE, Little CD. Fibulin is localized at sites of epithelial-mesenchymal transitions in the early avian embryo. Dev Biol 1992; 151: 473–84.
60. Davies GE, Howard CM, Farrer MJ, Coleman MM, Bennett LB, Cullen LM, Wyse RK, Burn J, Williamson R, Kessling AM. Genetic variation in the COL6A1 region is associated with congenital heart defects in trisomy 21 (Down's syndrome). Ann Hum Genet 1995; 59: 253–69.
61. Nakajima Y, Yamagishi T, Hokari S, Nakamura H. Mechanisms involved in valvuloseptal endocardial cushion formation in early cardiogenesis: roles of transforming growth factor (TGF)-β and bone morphogenetic protein (BMP). Anat Rec 2000; 258: 119–27.
62. Hoffman RM. Three-dimensional histoculture: origins and applications in cancer research. Cancer Cells 1991; 3: 86–92.
63. Arciniegas E, Neves CY, Candelle D, Parada D. Differential versican isoforms and aggrecan expression in the chicken embryo aorta. Anat Rec, Part A 2004; 279: 592–600.
64. Conway SJ, Molkentin JD. Periostin as a heterofunctional regulator of cardiac development and disease. Curr Genomics 2008; 9: 548–55.
65. Kitase Y, Yamashiro K, Fu K, Richman JM, Shuler CF. Spatiotemporal localization of periostin and its potential role in epithelial-mesenchymal transition during palatal fusion. Cells Tissues Organs 2011; 193: 53–63.
66. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002; 110: 673–87.
67. Bronner-Fraser M. Alterations in neural crest migration by a monoclonal antibody that affects cell adhesion. J Cell Biol 1985; 101: 610–7.
68. Kil SH, Lallier T, Bronner-Fraser M. Inhibition of cranial neural crest adhesion in vitro and migration in vivo using antisense oligonucleotides. Dev Biol 1996; 179: 91–101.
69. Tucker RP. Antisense knockdown of the β1 integrin subunit in the chicken embryo results in abnormal neural crest cell development. Int J Biochem Cell Biol 2004; 36: 1135–9.
70. Vogel WF, Abdulhussein R, Ford CE. Sensing extracellular matrix: an update on discoidin domain receptor function. Cell Signal 2006; 18: 1108–16.
71. Walsh LA, Nawshad A, Medici D. Discoidin domain receptor 2 is a critical regulator of epithelial-mesenchymal transition. Matrix Biol 2011; 30: 243–7.
72. Moore CJ, Winder SJ. Dystroglycan versatility in cell adhesion: a tale of multiple motifs. Cell Commun Signal 2010; 8: 3.
73. Nakaya Y, Sukowati EW, Alev C, Nakazawa F, Sheng G. Involvement of dystroglycan in epithelial-mesenchymal transition during chick gastrulation. Cells Tissues Organs 2011; 193: 64–73.
74. Thiery JP, Acloque H, Huang RYJ, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell 2009; 139: 871–90.
75. Nieto MA. Epithelial-mesenchymal transitions in development and disease: old views and new perspectives. Int J Dev Biol 2009; 53: 1541–7.
76. Even-Ram S, Yamada KM. Cell migration in 3D matrix. Curr Opin Cell Biol 2005; 17: 524–32.
77. Roy P, Rajfur Z, Pomorski P, Jacobson K. Microscope-based techniques to study cell adhesion and migration. Nat Cell Biol 2002; 4: E91–6.
78. Neagu A, Mironov V, Kosztin I, Barz B, Neagu M, Moreno-Rodriguez RA, Markwald RR, Forgacs G. Computational modeling of epithelial-mesenchymal transformations. Biosystems 2010; 100: 23–30.
79. Mathias RA, Simpson RJ. Towards understanding epithelial-mesenchymal transition: a proteomics perspective. Biochim Biophys Acta 2009; 1794: 1325–31.
80. Huang S, He X. microRNAs: tiny RNA molecules, huge driving forces to move the cell. Protein Cell 2010; 1: 916–26.
81. Gregory PA, Bracken CP, Bert AG, Goodall GJ. MicroRNAs as regulators of epithelial-mesenchymal transition. Cell Cycle 2008; 7: 3112–8.

Received December 7, 2011; accepted March 23, 2012