The role of ECM components in the maintenance of epithelial cells and in the transition of mesenchymal cells to epithelial cells (MET) in nephrogenesis

Bachelor Scriptie voor “Ontwikkelingsbiologie en regenerative medicine”

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Abstract

The basal lamina (BL) which is located underneath epithelial cells does not only provide structural support, but has an important function in the development and maintenance of renal epithelial tubules. The basal lamina is composed of different molecules which include integrins; they serve not only as the connection between the ECM and the epithelial cells but also as initiator of signal transduction pathways. Through activation of kinases such as ILK and FAK these signal pathways are responsible for the survival, differentiation, proliferation and the migration of cells; the maintenance of (epithelial) cells.

The renal epithelial tubule cells originate from the mesoderm. Mesenchymal cells undergo significant expression changes to transform into epithelial cells, a process named MET (mesenchymal epithelial transition). During this process integrins and other BL components such as proteoglycans play a role. The expression of the integrin repertoire alters through out the entire MET; coinciding with the spatiotemporal expression of ECM ligand molecules. Despite the fact that integrin-ECM ligand communication is critical in the development of renal tubules there are two other vital communication types; GF and their receptors and proto-oncogenes and their ligands.

In order to create interventions to restore the function of damaged renal tubules understanding of the development of these cells is necessary otherwise one would be clueless about what to do to restore the cell's function.

Introduction

Multi-cellular organisms are formed from tissues; specialized cells gathered together. These cells interact not only with their environment (neighboring cells, and the extracellular matrix (ECM)) but also have signal transduction pathways which elapse inside of them (1). These interactions and signal transduction pathways are of utter most importance in the maintenance of healthy tissues, organs and thus a healthy multi-cellular organism. It is important to realize that not only the organization and processes of maintaining healthy tissues is of significance in regenerative medicine. The development of these specialized cells is another critical element that should be taken into account in regenerative medicine.

ARF (acute renal failure) is a cause of Renal I/R (renal ischemia-reperfusion) that causes high morbidity and mortality figures (11). The kidneys receive 25% of the cardiac output, signaling the importance of a proper blood flow to the kidneys (12). Due to circumstances (such as blockage of blood vessels, fluctuations in blood flow, transplantations) the kidneys can be restricted for a short period from blood; exposing them to a shortage of oxygen, glucose and other blood-borne molecules. When this occurs we speak of a renal ischemia-perfusion (11, 12). When there is a shortage of blood (flow) there is an unequal balance between the blood supply and the blood demanded by the kidney. The kidneys due to their vascular architecture have a high susceptibility of being exposed to ischemia. In particular the proximal tubes are highly susceptible to ischemia; the cortex receives most of the blood supply delivered to the kidneys whereas the medulla receives only a small portion, hence making them vulnerable to renal ischemia-reperfusion (12, 15). In short what happens after renal I/R is that the epithelial cells, that compose the tubules, loss their function, causing not only leakage of fluids but the activation of numerous inflammatory molecules. These in turn activate myofibroblasts which instead of replacing the epithelial cells secrete ECM products; leading to a further diminished function of the kidneys (11-15, 18). Interventions of replacing these damaged tubules don't exist yet but with the increase interested in regenerative medicine nothing is impossible.

In order to find interventions for cases as the one described above one has to have knowledge about organogenesis. Organogenesis is the organized development of many different cell types and their incorporation into complex three-dimensional assemblies (31). If it is known what signals, interactions, and or expression signals are needed to create specific specialized cells and it's know what interactions are damaged, one can combine the two concepts to try and create interventions which could possible 'regenerate' these interactions. In the case of restoring function of the epithelial tubules of the nephron we first have to understand how these specialized cells are formed before we can start thinking about possible interventions. This paper will focus on the role of ECM components in the transition of mesenchymal cells to epithelial cells (MET) in nephrogenesis.

First I'll briefly pass by and describe the foremost components of the basement membrane (MB) a specialized region of the ECM. Then I'll continue to portray the different adherens structures that keep the basement membrane and the above laying cells connected, as well as the adherens structures that hold neighboring epithelium cells together. A small section will also be dedicated to how these adherens structures are capable of initiating signal transductions in the above laying epithelial cell, and of what importance these signals are. Hoping that by then I have created an illustration of how the renal tubule appear in healthy state. Then I'll explain how these tubules form from the mesoderm with help of other cells and by means of reciprocal inductive signaling processes, specifying on the role of the ECM components.

Components of the basement membrane

The basement membrane, also named basal lamina (BL), is a part of the ECM which has specialized and underlies all epithelial tubes and cell sheets (41). The basal lamina is composed of several structural proteins. These proteins include collagen type IV, laminin, nidogen and proteoglycans and will be discussed below.

Collagen IV

Collagen type IV belongs to the family of fibrous proteins; the collagens are characterized by their triple-helical structure (1). All three chains are α chains which are wrapped around each other. Another important feature of the collagen family is that it contains vast amounts of GXY repeats, repeats that have glycine, proline and hydroxyproline amino acids respectively (44-45). Collagen IV is an exception on this for it lacks one third of the glycine which other collagens have. In other words the GXY repeats in collagen type IV are interrupted more often allowing it to form sheets and kinks in its helix. There are 6α chains known; the three α chains in collagen IV are in a ratio of 2:1 with α1 and α2 (30). The triple helical structure of collagen IV ends with a non collagous COOH domain, also called the C terminus (NC1). At the other terminus, the N terminus is a non collagous NH2 terminal domain, also known as the 7S domain. The C terminus allows the fibers to link head to head instead of aligning them parallel. This binding property also allows collagen IV to form sheets as they do in the basal lamina (44).

Laminin

Laminin is a non collagenous protein that is a major structural protein in the basement membrane (1). They are hetrotrimeric glycoproteins with an unique cruciform structure (1). Laminins are composed of three polypeptide chains, one α, one β, and one γ chain which are linked to each other through disulphide bonds. There are five α, three β and three γ chains known, which can form different combinations and thus different laminin types(1). The three short arms are formed from the N terminal segments of the α, β, and γ chain whereas the globule domain of the long arm is exclusively formed from the C terminal of the αchain

(1, 41). Because of its characteristic shape it has 4 binding sites, where it can bind to not only other laminin molecules but also to collagen IV, perlecan and integrin receptors (44).

Nidogen

Nidogen or also known as ectactin is family of the basement membrane glycoproteins. It consists of three globular domains, yet it contains no obvious α or β chains. It has been shown that it binds to laminin, collagen IV and perlecan. Data has indicated that it mediates the binding of laminin to collagen type IV, and this could be a major function of nidogen (1, 44).

Proteoglycans

Proteoglycans are glycoproteins (proteins that have oligosacharides attached to their polypeptides) that are attached to a core protein with one or more covalently attached glycoaminoglycan chains (GAGs) (41). They can be grouped into different groups depending upon their glycoaminoglycans. These GAGs include keratan sulfate and heparan sulfate. Hyaluronan is a special proteoglycan because it contains nonsulfated GAG chains. It has been shown, by electron microscopy that proteoglycans bind to the long arm of laminin, to regions in the collagen IV triple helix and to nidogen (1, 44). Additional functions include storage of growth factors, which bind to the GAGs. Perlecan is an example of a proteoglycan which is found predominantly in the basal lamina. It has a core protein with three GAGs (heperansulfate) attached to it. Perlecan binds to collagen IV, laminin and nidogen (44).

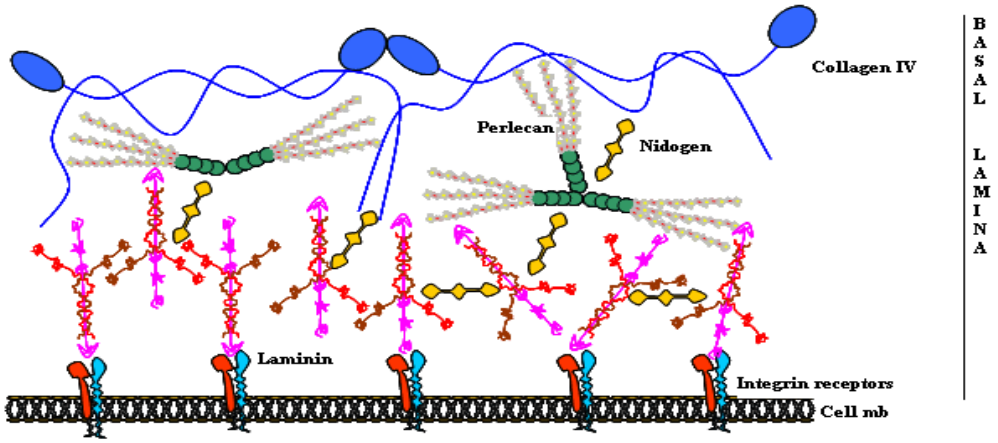


Figure 1 'Components of the basal lamina' (6)

Fibronectin

Fibronectin is another glycoprotein that plays an important role in binding other matrix molecules and cells (1). It consist of two identical polypeptide chains that are linked together by a pair of disulfide bonds at the C terminal; forming a V-shaped dimeric structure. Fibronectin contains four different binding sites, including one for fibronectin, collagen IV, perlecan and a binding site for (integrin) receptors on cell surfaces. They are the connecting

piece between collagen IV and integrin receptors and between the other molecules found in the basal lamina (44).

Figure 1 gives a schematic representation of the different molecules found in the basal lamina and how they bind to each other to form the basal lamina (NB fibronectin isn't shown in this representation (6).

Adhesion molecules

There are two main types of adhesion sites; namely those that adhere cells with the matrix molecules of the basal lamina and adhesion sites that adhere neighboring cells together (1, 3, 41).

Cell-cell adherens junctions

Neighboring cells are held together through a variety of different molecules. One complex of molecules that hold adjacent epithelial cells together are *adherens junctions;* they connect the actin filaments, located in the plasma membrane, of the two cells together (41). When several of these junctions are located close to each other, they form what is called an adhesion belt (3, 41). The actin filaments are connected through intracellular anchor proteins and through trans-membrane adhesion proteins. In cell to cell adherence the trans-membrane proteins belong to the cadherin family, whereas in the cell- matrix adherence the trans-membrane proteins belong to the integrin family (3, 41).

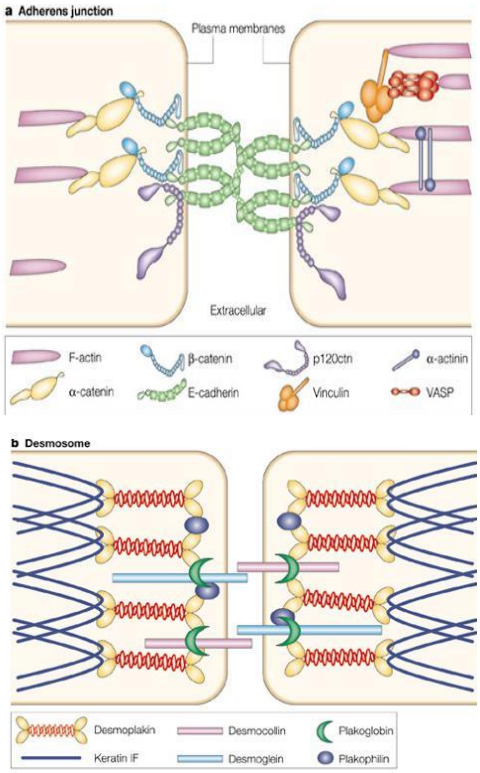


Figure 2'Schematic representation of cell-cell adherens structures' (36)

Another protein complex that hold adjacent cells to each other are *desmosomes*. These unlike the adherens junctions make use of the intermediate filaments, which like the actin filaments form the cytoskeleton of the cell (3, 41). In epithelial cells the intermediate filaments are of the keratin type. Desmosomes form two symmetric plaques across the plasma membrane of two adjacent cells. Similar to the adherens junction the trans-membrane proteins belong to the cadherin family. Examples of cadherins are desmoglein and desmocollin (3). Attached to these proteins are the intracellular anchoring proteins, which in desmosomes anchor to keratin filaments on one site and on the other side they are attached to a trans-membrane cadherin protein. Intracellular

anchoring proteins are called plakoglobin, desmoplakin and plakophilin (3, 41). Figure 2 gives a schematic representation of these two protein complexes that hold neighboring cells together (36).

Cell- matrix junctions

Adjacent epithelial cells aren't only connected to each other but are also attached to the basal lamina on which they rest. On these places they are connected by means of adherence molecules that also make use of the actin and intermediate filaments. Trans-membrane adherence proteins that use actin filaments are called *focal adhesions* and belong to the integrin family (3, 41). Examples of intracellular anchor proteins that connect integrin molecules with the actin filaments, are filamin, vinculin and α- actinin. The extracellular domains of the integrins connect the epithelial cells to the basal lamina by connectiing to laminins via fibronectin (3, 31).

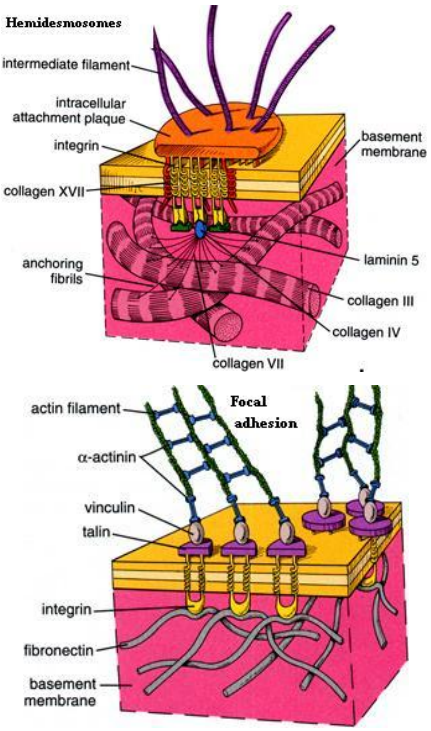


Figure 3 'Schematic representation of cell-matrix adherens structures' (50)

Hemidesmosomes are half desmosomes and use keratin intermediate filaments as anchor point to connect the cell to the basal lamina. Like the trans-membrane protein in focal adhesions, the trans-membrane proteins in hemidesmosomes belong to the intregrin family (3). Just as in all the other junctions the intracellular anchor proteins attach the trans-membrane protein to the keratine filaments. An example of an intracellular anchor protein is plectin. The extracellular domains of the integrins also bind to laminin via anchor fibrils which are composed of collagen VII. Figure 3 shows a schematic representation of a focal adhesion and a hemidsmosome (50).

Integrins

Integrins are trans-membrane proteins in focal adhesions and hemidesmosomes that also function as surface receptors (1, 3, 41). Integrins are thus not only involved in mediating adhesive interactions between the ECM and epithelial cells but also function as receptors that initiate important signal transduction pathways in the cell. The section below will describe the structure of integrin receptors and how they are capable of initiating signal transduction pathways (7-9).

Structure and adhesive functions

Integrins are composed of an α subunit and β subunit which are associated non-covalently forming a heteromer (1, 41). Over the years a number of isomers of the α subunits and β subunits have been discovered; at the moment there are eighteen α subunits and eight β

subunits known. These can form twenty-four different heteromers; allowing them to be subdivided into different subclasses according to the ligands to which they bind. There are four subclasses of integrins; (I) laminin binding integrins, (II) collagen binding intergrins, (III) RGD recognizing integrins and (IV) leukocyte binding integrins (8). The first three subclasses play an important role in adhesive interactions of the ECM and the above laying epithelium cells. Whereas leukocyte binding integrins plays an essential role in the immune system; allowing leukocytes to leave the bloodstream and enter the tissue (1, 7-10,48).

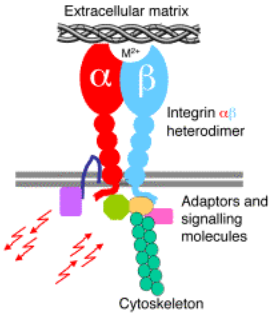


Figure 4 'General structure of an integrin receptor' (51)

Mentioned above was that the integrins where subdivided into different subclasses, the subclasses of integrins that bind to ECM molecules can be divided even further namely into the VLA family. The VLA family resembles all the integrin receptors that bind to ECM molecules and share the β1 subunit (4). Examples of these receptors are α1β1, α2β1, α3β1, α6β1, α7β1, α11β1 and α12β1. The integrin receptor α6β4 doesn't belong to the VLA family but still remains an important receptor for adhesive interactions between the epithelial cell and the ECM (8). The cytoplasmic tails of the intergrin are short and contain about 750 amino acids, the only exception on this is 4β which has 1,000 amino acids. This is explained by the fact that 4β contains four fibronectin III domains (48). Figure 4 gives a general sketch of what an integrin receptor looks like and how it can function to initiate signal transduction pathways and function as anchor structure between cells and the ECM (51).

Structure in initiating signal transduction pathways

Concentrated at the sites (focal adhesions) where ligand-integrin binding takes place are not only cytoskeleton and structural proteins but also signaling molecules (7, 8). The cytoskeleton and structural proteins include the actin filaments and the intracellular anchor proteins, whereas the signaling molecules can be a variety of different molecules. One of the molecules that is always present near integrin receptors is Focal Adhesion Kinase (FAK); all integerins can activate FAK (7). Besides FAK there are other signaling molecules present such as the Integrin Linking Kinase (ILK) (7-10, 22). These signal transduction pathways, going from the ECM to the cell mediate cell proliferation, migration, differentiation and survival of the cells. In the next section these signal transduction pathways will be elaborate on, this paragraphs' aim was to explain that molecules at the beginning of different signal cascades are co-localized at focal adhesion sites.

The signal pathways initiated by integrin receptors

Focal Adhesion Kinase (FAK)

Focal Adhesion Kinase is a cytoplasmic tyrosine kinase, that is recruited to focal adhesions by paxillin or talin; proteins that intracellular anchor the α integrin connect with either subunit respectively subunit or β integrin intracellular anchor (7, 20, 41). The the FAK and can also proteins are part of filamin, vinculin and include actin, recruited FAK tensin (7). The phosphorylate each molecules tyrosine (Tyr397); other on a specific generating a phosphotyrosine docking site for molecules of the Src family. C-src, like FAK, is a cytoplasmic tyrosine kinase, which recognizes the SH2 domains of the phosphorylated FAK molecules (7, 8, 41). These SH2 domains are recognition points for phosphotyrosine. C-src phosphorylates the FAK molecules on further tyrosines creating new docking points for other signal molecules such as the adaptor protein GRB2-SOS complex (growth-factor receptor bound protein). This adaptor protein is involved in activating Ras, which in its turn activates Rac which is the regulator of the signaling pathway of MAPK (mitogen activating protein kinase) or also known as ERK. MAPK regulate transcription factors such as cMyc and SRF which are involved in the growth and differentiation of cells (46). Figure 5 gives an overview of how this signal transduction pathway is connected (51)**.**

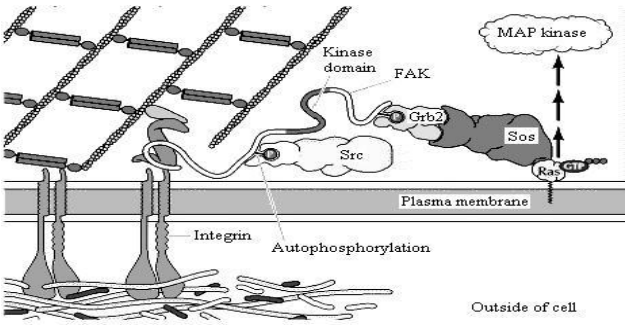


Figure 5 'FAK signal transduction pathway'(51)

Important tasks of FAK is the spreading and migration of cells early in the development, cell differentiation, cell death and accelerating the process from G1 to S phase in the cell cycle. FAK can be seen as a crucial component in the organization of focal adhesion structures that induces many signaling pathways; by recruiting associated signal transduction complexes(20, 41, 48).

Intergrin Linked Kinase (ILK)

ILK (intergrin linked kinase) is serine/threosine kinase which is also up regulated at focal adhesion sites. It regulates cell-matrix adhesions, fibronectin assembly and has been suggested to be involved in the Wnt and growth factor signaling pathways (22). In short it mediates the transduction of signals between the ECM and the intracellular compartment (7, 9). ILK contains three domains; (I)The C terminal is a site for protein catalytic kinase as well as the (II) binding site for the β1 cytoplasmic domain of the integrin receptor and (III) the N terminal has a PH domain. PH domains can bind PIP3 and up regulates kinase activity. ILK directly phosphorylates AKT which is also known as protein kinase B (7-10).

AKT enzymes are coded by three genes (Akt1, Akt2 and Akt3) and belong to the serine/threonine-specific protein kinase family. AKT is involved in several signal pathways that lead to cell survival, proliferation and metabolism (41). They have PH domains which bind to phosphoinositides such as PIP3 and P2 (phosphatidylinositol triphosphate/biphosphate), this interaction has as result that the AKT are correctly positioned in the membrane. AKT then phosphorylates by its own activating kinases, PDK1 and 2, (phosphatidylinositol dependent kinases). The PDKs phosphorylate specific serine and threonine sites of the AKT(21-22, 41). Other molecules such as ILK and MAPKAPK2 (mitogen activating protein kinase activating protein kinase 2) can play the role of PDK2 hence also continue AKT pathways. AKT is not the only signaling protein ILK can directly phosphorylate, GSK-3 can also be directly phosphorylated by ILK. Suggestions have been made that GSK-3 is involved in the cell survival and proliferation and can thus be involved in oncogenic transformations (9, 46, 48).

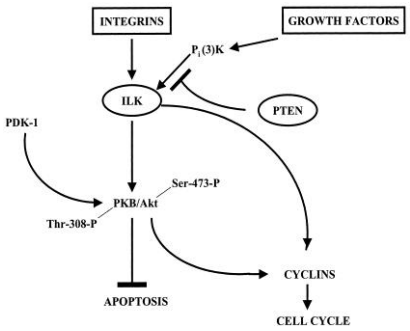


Figure 6 'Signal transduction pathway through ILK' (52)

Studies where they used ILK deficient mice show that ILK can also bind with other molecules than AKT. It can also interact with PINCH1 and 2 through the PH domain on the N terminus. PINCH 1/2 in turn creates a binding place for the SH2 domain containing adaptor molecule Nck2. Nck2 molecules are involved with vital cytoskeleton related molecules. PINCH 1 and 2 can be found near focal adhesion sites. Figure 6 shows the signal transduction pathway through ILK (52).

Nephrogenesis

The development of a multi-cellular organism originates from the three germ layers which are formed after gastrulation. These layers are the endoderm, ectoderm and the mesoderm and are responsible for the development of the entire organism (26). Epithelium tissue covers the entire surface of the body and is composed of closely packed cells that are one or more layers thick (41). Most of the epithelium in the adult organism is derived from the ectoderm, yet the epithelial cells in the renal tubules are an exception on this and are derived from the mesoderm (19, 26,28).

Nephrogenesis explains the process of kidney development. During the process of development three different kidneys form temporarily and in a specific order. The first ‘kidney’ to be developed is the pronephros at about day twenty three (26, 31, 39). It is seen as a segmenting of mesoderm to form nephrotomes; rounded mesoderm portions (24). These nephrotomes form pronephic tubules which empty in the pronephic duct which has formed at both sides. In humans the pronephic has no functional meaning (24,49).

The pronephros degenerate as the second kidney, mesonephros, starts to develop at about three and a half weeks (24). The pronephros tubules start to degenerate as the mesonephros develop at the causal ending of the pronephros (24). The mesonepheric duct expands so that it can fuse with the cloaca (26, 31).

The mesonepheric duct that fuses with the cloaca is also called the Wolffian duct (24). A diverticulum out of the mesonepheric duct is the first indication of the development of the third kidney, the metanephros (24). This projection is called the uteric bud (UB) and elongates into the metanephrogenic mesenchyme (MM), or undifferentiated mesoderm. The MM is induced upon interaction with the UB causing mesenchymal cells to undergo morphological changes and transfer to epithelial cells (19,24, 26, 31, 39).

The UB continues to split into symmetrical branches and will determine the number of nephrons that will develop and develops into the collecting ducts (24). The MM starts to condense and group around each subdivision of the UB. During this grouping the mesenchyme cells proliferate, differentiate en migrate to form the MM cap. Inside the MM cap lumen is produced in order to create a functional tubule. Slowly this cap elongates and forms comma-shaped and S-shaped bodies which represent the tubule of the nephron (24, 37-40, 42, 49). Figure 7 shows the reciprocal induction between the UB and the MM (44).

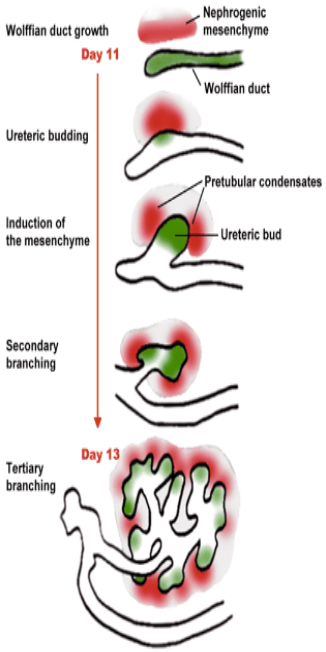


Figure 7 'Schematic representation of kidney development' (44)

Signals in the development of the kidney

Organogenesis, the development of organs, demands a coordinated development of many different cell types. As told above the reciprocal signaling between the UB and MM guides the development of the kidneys. Various experiments have shown that the UB and MM require the presence of each other for the development of the nephrons. One can only develop in the presence of the other (37-40).

Mesenchymal and epithelial cells

Present in the mesoderm are mesenchyme cells. These can be seen as mesenchyme stem cells for they are multipotent and can differentiate into a variety of cells, such as osteoblasts, adipocytes, myocytes and epithelial cells(23-26). Mesenchyme cells are extended and elongated in comparison to epithelial cells. Mesenchymal cells also form structures that are irregular and not uniform in shape, composition or in compactness; the opposite of what epithelial cells do (26). Mesenchymal cells also easily migrate individually due to the weak adhesions between neighboring cells, whereas epithelial cells move as entire sheet (28). Epithelial cells form sheets that are made of continuous cells that are not only attached to

each other, through adherens junctions, but are also tightly attached to the underlying basement membrane (24, 28). Another important characteristic is that the epithelial sheet is polarized; allowing that the apical and basal surfaces can adhere to different structures(24-26, 29).

Seeing that these two cell types differ drastically from each other one can understand that a complex process and drastic changes in gene expression are required to differentiate one into the other. These changes require a collection of macromolecules that organize the spatiotemporal and stage specific changes which these mesenchymal cells must undergo in order to form the tubules of the nephron. Important to comprehend is that besides differentiation, other vital processes such as migration and proliferation need to occur for the proper development of the kidneys.

Mesenchymal Epithelial Transition (MET)

The transfer from mesenchyme cells to epithelial cells is called MET, mesenchyme epithelial transfer, but is also referred to as branching epithelial morphogenesis (25). MET forces mesenchymal cells to undergo positional and morphological changes and increases the interactions with neighboring cells (28). This transition has several steps, the first crucial action is the condensation of the mesenchymal cells (26). These cells condense after being induced by the UB. The UB which grows out of the Wolffian duct is an epithelial lined tubular structure (24). The condensing mesenchymal cells reciprocate the UB; signaling the UB to branch. Through this reciprocal signaling, an exquisite example of paracrine-juxtacrine signaling, further modifications occur in gene expression and composition of the mesenchymal cells (24). These modifications occur through mechanisms such as (I) inductive signaling, (II)morphogenesis, (III)differentiation, (IV) proliferation and (V) migration of the cells. These mechanisms are regulated by a range of different molecules including adhesion molecules, soluble mediators and components of the ECM. The paracrine-juxtacrine signaling between the UB and the MM can be divided into three groups (I) GF and their receptors, (II) proto-oncogenes and their ligands, and (III) integrins and ECM molecules (29).

We'll be focusing out attention to the third type of communication; how the uteric bud epithelia interacts with the mesenchyme cells through ECM constituents such as integrin receptors and laminin.

Important to realize is that mesenchymal cells, as they are going through transition to epithelial cells, secrete ECM molecules, which in turn positively helps complete their transformation. This is facilitated by the fact that MET is controlled by profound changes in gene expression of various transcription factors (19).

ECM molecules and their contribution to MET

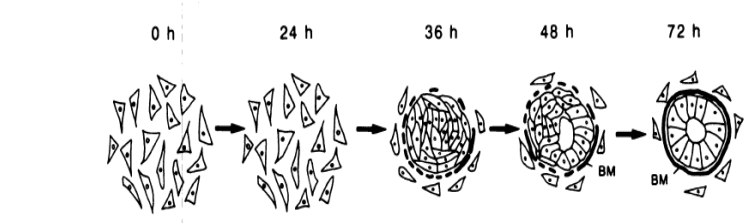
As mentioned above the mesenchymal cells start condensing after contact with the UB, invitro studies have shown that after day 1 (in mice embryos) significant changes in mRNA expressions can be detected (19).

These changes are seen in matrix proteins which are markers for mesenchymal and epithelial cells; hence are down and up regulated respectively (26). N CAM and collagen I and III, markers for mesenchymal cells are down-regulated where as epithelial markers such as the laminin chains β1 and γ1 are up-regulated. These laminin changes are detectable after one day since the UB and the MM made contact. At day three (in the invitro studies on mice embryo's) the transforming cells are starting to show signs of polarization, which coincides with the elevation in mRNA expression of laminin chainα 1. It appears that laminin 1(α 1, β1 and γ1 chains) modulates the polarization which characterizes epithelial cells.

In addition other adhesion molecules such as Ecad (an epithelial marker) and other ECM molecules for instance intergrin (α6) display an increase in mRNA expression (19). The ECM molecules have multiple binding domains, which works in their favor; allowing them to bind to surface receptors as well. This can be concluded from the fact that not only the expressions of ECM molecules differ through out the MET, but the integrin expression repertoire also changes through out the entire nephrogenesis (19, 24, 26, 28). Expression of the different molecules and receptors is asynchronous; reflecting the spatiotemporal expression importance which contributes to the MET.

Nidogen is produced by mesenchyme cells, and interacts with the γ1chain of laminin. It is been shown that nidogen is of crucial importance in the organization of the BM (19, 24). Next to secretion of nidogen, integrins (α6 β1) and dystroglycan are expressed by mesenchyme cells. Functioning as receptor for laminin 1, these two molecules share a similar α6 subunit. Experiments with integrin or dystroglycan deficient mice show inhibition in further kidney development.

Table 1 'Expression of molecules during MET' (26)



	0 h	24 h	36 h	48 h	72 h
Collagen I, III	+	-	-	-	-
Fibronectin	+	+ -	+ -	+	+
Tenascin	-	-	-	-	-
Laminin B	+	+	+	+	+
Laminin A	-	-	+	+	+
Uvomorulin	-	-	+	+	+
N-CAM	+	+	+	-	-

Proteoglycans (PGs) are cell surfaced protein that are expressed in the developing kidney. Not only do they bind to constituents of the ECM but they can act as low affinity receptors for growth factors, and other signal molecules such as the Wnts and other

transforming growth factors. These GF are found on their GAG chains and suggests that proteoglycans not only contribute to regulating adhesive interactions but modulate activity of signal molecules through their GAGs. During early development of the kidney these PGs are localized in the uteric bud tip which interacts with the MM. Suggesting that it ensures that there are enough growth factors and other molecules which can help induce the MM to transform. Crucial to note here is that numerous GFs in their turn can regulate the expression of PG's; creating a vital link between PGs and GFs; where they modulate each others activity. An example of an important PG is syndecan which disappears as the nephron matures signifying that it contributes to the early epithelial transformation stage (24, 26).

Table 2 'ECM proteins and their binding proteins/integrin' (24)

ECM Protein	Integrin Receptor(s)/Binding Protein(s)
Collagen IV (basal lamina)	$\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_v\beta_3$
Laminins (basal lamina)	$\alpha_1\beta_1$–$\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, $\alpha_7\beta_1$, $\alpha_v\beta_3$, dystroglycan
Perlecan (basal lamina)	Dystroglycan
Nidogen (basal lamina)	$\alpha_3\beta_1$
TIN-Ag (basal lamina)	$\alpha_3\beta_1$, $\alpha_v\beta_3$
Collagen I/III	$\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_v\beta_3$
Fibronectin	$\alpha_1\beta_1$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_v\beta_3$
Tenascin-C	$\alpha_8\beta_1$, $\alpha_v\beta_3$
Fibrillin-1	$\alpha_v\beta_3$
Nephronectin	$\alpha_8\beta_1$
Osteopontin	$\alpha_8\beta_1$, $\alpha_v\beta_3$

Integrins form an other key molecule in MET. They interact through their extracellular domain with ECM ligands and regulate transduction pathways with their cytoplasmic tail. Throughout the entire nephrogenesis the integrin expression repertoire changes, as does the expression of ECM molecules. The changes in expression of ECM molecules can be seen in table 1 (26). Whereas table 2 gives an overview of which ECM ligand binds with which integrin (24).

So far we have only discussed one main form of communication between the UB and MM, that between ECM molecules and the integrins, and left the other two forms for what they are. It is important to understand that these other two forms are of equal importance in the transition from mesenchymal cell to a mature nephron, and are often interrelated and linked to the communication pathway of the other forms. Meaning in other words that molecules contribute to their own communication pathway but also positively influence the other two communication pathways.

Discussion

As mentioned in the beginning of the paper multi-cellular organism are composed of different organs and tissues formed from specialized cells. The space between these cells is filled with locally secreted macromolecules that form an organized complex called the extracellular matrix. Regions of the ECM can specialize and form the basement membrane (or basal lamina) on which epithelial cells are attached. In an adult organism the basement membrane is not only of structural and adhesive importance but also a modulator of various cellular functions. These functions include differentiation, proliferation, migration and survival, which are modulated by integrins.

Integrins, are trans-membrane proteins which are attached to constituents of the ECM (laminin) and to the cytoskeleton of the epithelial cell. They function as a receptor for intracellular transduction signal pathways with the help of FAK and ILK. The functions initiated by these pathways are required for the regular functioning of the cell.

The ECM not only participates in the preservation of the above laying epithelial cells, it is also an important figure in the epithelial morphogenesis. The mesoderm and its mesenchymal cells are the origin of renal tubular epithelial cells. The mesenchymal cells undergo complex and spatiotemporal changes to eventually alter into epithelial cells. This transfer requires three main forms of communication between the epithelial cells of the UB and the mesenchymal cells of the MM. One of these communication forms is contributed by ECM molecules which are secreted by mesenchyme cells themselves. After induction of the MM by the UB, the mesenchymal cells start condensing and changing their expression. They show decreased mRNA levels in mesenchymal cell markers and increased levels in epithelial cell markers. It can be said that MET requires profound changes in gene expression (19, 24).

Proteoglycans which are also secreted by mesenchymal cells provide not only adhesive forces but can act as a reservoir for GF. This is seen at an early stage of transition; where the UB induces the MM. The UB tip is highly concentrated in proteoglycans and expression of proteoglycans diminishes as the nephrons mature.

Nidogen, a mesenchymal factor, is secreted at early stages of the MET. Nidogen is an extremely essential molecule in the assembly of the BM for it is connects the other components of the BM (collagen IV, perlecan, and integrin) with each other (1, 43).

Integrins as mentioned change their expression repertoire during the entire process of nephrogenesis, this coincides with the changes in expression of ECM molecules. Integrins are the connection between the ECM and the epithelial cell; hence it makes sense that this repertoire changes as the expression of different ECM molecules alters.

Though the ECM is not on its own capable of accomplishing the transition of mesenchymal to epithelial cells it does play a notable role. Despite the fact that further experiments are

needed to acquire more understanding of the ECM and its interactions with mesenchymal cells; many steps are already taken into a positive direction.

Suggestions for possible interventions could be to (I) stimulate damaged cells to start expressing adhesion molecules, (II) increase the levels of integrin receptor. This way you are trying to restore the adhesion interactions that keep the epithelial cells together and attached to the BL. A second suggestion could be to grow tubules invitro and transplant these; yet problems would be where to get MM and UB from to develop into tubules. A third proposal would be to study the process EMT closer (Epithelial mesenchymal transition) so that you can 'culture' mesenchymal cells and place these in the damaged tubules. Once these 'cultured' mesenchymal cells are located in the damaged kidneys you apply the appropriate signals (that are seen in nephrogenesis) and see if they turn into epithelial cells again. It is not that easy to think of interventions to restore the function of damaged organs because not only are there multiple factors involved but also because some interventions are not applicable due to ethical or practical reasons.

Of one thing we can be certain though; with the present knowledge and new steps taken every day, we will find an interventions in the near future that will enable us to restore epithelial cells in renal kidney failure.

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