Dissecting Stages of Mesenchymal-to-Epithelial Conversion during Kidney Development

Kai M. Schmidt-Ott Debin Lan Benjamin J. Hirsh Jonathan Barasch

Department of Medicine, Columbia University College of Physicians and Surgeons, New York, N.Y., USA

Key Words

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Abstract

During embryonic development, the structures of the nephron from the glomerulus to distal tubule derive from the metanephric mesenchyme. The mesenchymal cells change their cell type and produce highly organized epithelia under the influence of signals from the ureteric bud. The morphological sequence of this conversion includes the formation of a corona of mesenchymal cells surrounding the tips of the ureteric bud, followed by the development of a pre-tubular aggregate, which evolves into preliminary forms of the segmented nephron. Currently, these stages are largely based on histomorphologic criteria and expression of marker molecules. However, to dissect the effects of inductive signals from the ureteric bud in more detail, a sophisticated readout of stages in the conversion process is required, based on the onset of epithelial polarity and the occurrence of vectorial transport. In this review, we discuss some of the new approaches in establishing the staging of the conversion process.

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Mesenchymal-to-Epithelial Conversion Is the Basis for Development of the Nephron

Development of the mammalian kidney is characterized by reciprocally inductive events between the ureteric bud – an outgrowth of the nephric duct – and the metanephric mesenchyme. While the mesenchyme induces the ureteric bud to extend and branch, the ureteric bud induces the formation of nephron epithelia from metanephric mesenchymal cells [1]. During this conversion, epithelial structures appear de novo. This differs from most epithelia in the embryo (airway, most of the urogenital system, exocrine glands, pancreas), which arise by the branching or the folding of pre-existing epithelial sheets or tubules, rather than from non-epithelial cells [2]. Renal morphogenesis consequently presents an opportunity to identify the sequence of polarization in mammalian epithelia.

Many aspects of renal development are well known, but there is surprising little information on the cellular level. Mesenchymal cells adjacent to the ureteric bud organize so that their long axis is perpendicular to the surface of the tubule, unlike more distant layers of mesenchyme and stroma, which are horizontal (fig. 1B). The perpendicular cells form a corona adjacent to the tip of the ureteric bud and they express Pax-2, cadherin-11 and $\alpha 8$ integrin (fig. 1A). These cells comprise a subpopulation of what is traditionally called ‘condensing mesenchyme’. Later, groups of cells organize to form a ‘pre-tu-

Fig. 1. Stages of epithelialization during kidney development. **A** The conversion of the metanephric mesenchyme is characterized by distinct morphological stages and expression of markers. **B** Early polarity can already be demonstrated at the coronal cell stage. Mesenchymal cells at the tip of the ureteric bud reveal a columnar appearance (arrows). **C** Uptake of rhodamine-transferin (red) in these cells suggests the presence of a basolateral trafficking compartment (arrows) in mesenchymal cells adjacent to the ureteric bud (marked by FITC cytokeratin staining). UB = Ureteric bud; M = metanephric mesenchyme.

bular aggregate' just beneath the tips of the ureteric bud at the juncture of the ampulla and stalk and they express Wnt-4 [3]. The pre-tubular aggregate then forms a 'renal vesicle' (epithelial-like cells), which is the immediate precursor of the nephron. Some of these stages can be identified by genetic markers (fig. 1A). However, when and where different components of epithelial polarity appear and how these cells are related is not clear at this point.

Identification of Triggers for Mesenchymal-to-Epithelial Conversion

It has been known for more than half a century that the ureteric bud delivers critical signals to the metanephric mesenchyme to induce the conversion process [4]. In the past decade, several ureteric bud-derived factors have been demonstrated to induce epithelial conversion in the metanephric mesenchyme.

Our group has taken two distinct approaches to identify ureteric bud-derived molecules that regulate conversion. In our original approach, we developed a ureteric bud-derived cell line [5] and assayed fractions for their potential to induce epithelialization in metanephric mesenchyme. This led to the identification of the interleukin-6 cytokine, leukemia inhibitory factor (LIF), as the first ureteric bud-derived inductive molecule [6]. LIF and other interleukin-6 cytokines signal through a composite receptor consisting of gp130, the LIF receptor as well as other components. This leads to STAT-3 phosphorylation in the mesenchyme and subsequent epithelial conversion over a time course of 7 days in vitro. A second inductive molecule we identified is neutrophil gelatinase-associated lipocalin (NGAL; lipocalin-2), which induces a distinct response in the mesenchyme and biases the induced epithelia toward a proximal nephron phenotype [7]. Additional inductive activities can be identified from ureteric bud cells and this approach holds promise for

future discoveries, yet the *in vitro* phenotype of these cells might differ from the ureteric bud *in vivo*.

We recently developed a new approach for an unbiased screening for ureteric bud derived factors *in vivo*. By microdissection, we isolated the tips of the ureteric bud, which contain the presumptive inductive proteins, and performed a genome-wide expression study using Affymetrix microarrays [8]. This led to the discovery of the interleukin-6 cytokine chaperone cytokine-like factor-1 (CLF-1) as a ureteric bud tip molecule. CLF-1 has been demonstrated to bind to the interleukin-6 cytokine cardiotrophin-like cytokine (CLC) and to serve as a shuttling molecule to permit secretion of CLC to the extracellular space [9]. Indeed, when CLF-1/CLC complex was applied to isolated rat metanephric mesenchymes, we observed epithelial induction [8]. However, CLC is expressed at low levels in the embryonic kidney pointing to the possibility of additional ligands of CLF-1. Also, CLF-1 has been shown to bind to a consensus motif that is also present in LIF, pointing to the possibility that CLF-1 may modulate the activity of the known inducer LIF [8].

Other investigators have identified additional inducers of mesenchymal to epithelial conversion. Wnt-6 is secreted from the ureteric bud and causes conversion of isolated metanephric mesenchymes when expressed in co-cultured cell lines [10]. In addition, genetic deletion of Wnt-9b leads to an arrest of epithelialization in the metanephric mesenchyme before occurrence of Wnt-4-positive pretubular aggregates and epithelialization can be rescued in Wnt-9b^{-/-} animals by overexpressing Wnt-1 from the ureteric bud, indicating that a Wnt signal is essential and permissive for epithelialization [11]. These findings indicate that inductive signaling from the ureteric bud probably constitutes a complex set of secreted factors and several ureteric bud-derived signals may be required in a temporal and spatial sequence for the proper execution of the induction program.

Identification of Stages of Mesenchymal-to-Epithelial Conversion

The current assay for inductive activity of a given factor in metanephric mesenchyme is crude: it relies on the ability of this factor to induce established renal epithelial marker proteins (such as E-cadherin) and tubules in isolated metanephric mesenchyme. Yet, this 'terminal assay' does not detect factors that may have stage-specific effects by inducing the transition between intermediates rather than the complete program of epithelialization. Fa-

cilitation of markers (fig. 1A) is one opportunity to improve our capability to detect stage-related changes. However, the known markers are rarely specific to only one stage. In addition, our description of stages is based on histomorphological criteria, and not functional aspects of epithelialization.

Our current knowledge about establishment of epithelial polarity in a developing vertebrate organism is derived from studies of blastocyst formation during early mouse embryogenesis and cultured epithelia undergoing phenotypic rearrangements [12, 13]. In the initial stage of epithelialization (e.g. the 8-cell stage mouse embryo) intercellular adhesion mediated by the cadherin/catenin system is activated, causing the cells to undergo compaction. The cadherin-based adherens junction has a central role in this system as it initiates a coordinated change in cell organization that converts cells from a non-polar to a polarized, proto-epithelial phenotype. Cell polarization at this stage includes the formation of apical and basolateral membrane domains with associated changes in the structure of the underlying actin cytoskeleton, polarization of cytoplasmic organelles, and asymmetrical patterning of the actin and microtubule cytoskeletons. Only then begins the step-wise assembly of the tight junctions, which are permeable to ions and water initially (e.g. 16-cell morula during blastocyst formation), but become impermeable later (32-cell early blastocyst) indicating epithelial maturation.

Morphological studies on epithelia formation in the developing kidney suggest a similar sequence of events leading to the formation of epithelia of the nephron (fig. 1A). However, the timing of cellular compaction in the kidney, which initiates epithelialization, is currently enigmatic. The pre-tubular aggregate may represent the first occurrence of compaction in mesenchymal-to-epithelial conversion. However, another possibility is that the columnar coronal cells surrounding the ureteric bud already represent compacted pre-epithelia (fig. 1B). In fact, our recent studies on the uptake of fluorescent transferrin into these coronal cells revealed an apicobasal asymmetry in the distribution of rhodamine-transferrin in relation to the adjacent basement membrane of the ureteric bud (fig. 1C), suggesting the possibility that cells at an early stage of development (before the expression of Wnt-4) are already polarized.

These observations stress the importance of developing criteria and markers for functional stages during mesenchymal-to-epithelial conversion. We are currently conducting studies to achieve these goals. Epithelia create polarity by retaining subsets of proteins on specific cell

surfaces. The mechanisms include cell–cell and cell–matrix interactions, and these interactions have long distance effects in the cell. Based on this principle, we are developing a functional readout of apicobasal polarity *in vivo* using genetically modified animals that target fluorescent proteins to the basolateral or apical domain, respectively. This tool will allow us to determine the first onset of epithelial polarity in the derivatives of the metanephric mesenchyme. Also, these animals will aid us in developing a more sophisticated readout of inductive signals from the ureteric bud, since they detect the onset of the mechanisms of vectorial transport, rather than expression of mature marker proteins.

A second approach for improving our understanding of the stages in the conversion process is to follow patterns of gene expression during conversion *in vitro* using microarray analysis. We have identified the global profile of gene expression at different stages of epithelial conversion, beginning with freshly isolated mesenchyme already containing the coronal cells, up to the appearance of mature renal epithelia after 7 days of culture with inductive growth factors (our unpublished data). Because we can apply purified inducers to mesenchyme, we can follow synchronous waves of gene expression in the coronal cells, and because we can apply more than one single inducer, we can deduce a pattern of common genes induced by these different factors. Therefore, the analysis highlights a group of genes that are typical of the conversion process, rather than typical of a given inductive signal.

This whole-genome screening approach holds several promises: first, it identifies regulators of epithelial polarity prior to the onset of morphological changes. It may allow us to differentiate between proteins involved in *de novo* epithelialization and those that contribute to the maintenance of the epithelial phenotype, which is not possible in established epithelial cell culture models (e.g. MDCK cells). In one example, we found that MAL2, an essential component of vectorial transport, is upregulated only after the appearance of polarized cells in the metanephric mesenchyme suggesting that this transcytotic pathway plays a role in maintenance rather than the establishment of polarization (unpublished data). Secondly, different tissues utilize distinct members of a given class of molecules (e.g. adherens junction components, cytoskeletal proteins etc.), which ultimately determines the tissue-specific phenotype. The gene expression approach allows us to determine the class members specific to forming nephron epithelia, establishing a functional catalog of molecular events. For example, in the cadherin family of cell adhesion molecules we find a temporal sequence: Dur-

ing the early stages of conversion, we find a transient up-regulation of cadherin-13, followed by protocadherin-7, while E-cadherin and cadherin-16 are only activated with the appearance of mature epithelia (unpublished data). Third, individual molecules can be assigned to classes or pathways based on their time course of regulation. It is likely that molecules that are closely co-regulated in the course of epithelialization are functionally related. Several methods for clustering genes based on their expression pattern have been developed in recent years. The use of divisive neural network-based algorithms (e.g. SOTA) has greatly improved the possibilities of delineating classes of genes [14]. In addition, the statistical tools to identify genes that are significantly regulated in a time course experiment have been improved so that the number of false positives and false negatives can be minimized, whereas common statistical tests (e.g. ANOVA) would neglect the inherent temporal relationships [15]. These are not trivial issues taking into account the enormous amount of data generated by microarrays that target thousands of genes compared over several time points and conditions. Finally, the tools for functional annotation of genes and their assignment to classes and pathways are much more powerful than only a few years back. Extensive catalogs of gene function (e.g. Gene Ontology Consortium, Kyoto Encyclopedia of Genes and Genomes) are growing rapidly and are updated on a regular – often daily – basis. Consequently, new published insights into gene function can be immediately translated into hypotheses concerning the role of a specific gene or group of genes in the process of epithelialization.

In summary, our current understanding of stages in the conversion process from the metanephric mesenchyme to a mature nephron is largely based on morphological criteria and marker molecules. In the future, a more advanced staging will rely on functional aspects that characterize the maturation process of the epithelial cell. In addition, a more comprehensive identification of critical molecules using whole-genome approaches on converting mesenchyme will deepen our understanding of this fundamental process in kidney development.

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