Intermediate Filament Proteins as Distinguishing Markers of Cell Type and Differentiated State in Cultured Human Urinary Tract Epithelia

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The epithelia are remarkably diverse with respect to cell morphology, tissue structure, and modes of growth regulation, befitting the wide range of functions they perform. Identification of the growth factors, hormones, and nutritional components that a variety of epithelial cell types require for serial cultivation is permitting investigation of the mechanisms regulating their growth and synthesis of differentiation-related proteins. It is now known that the keratins (subunit proteins of the epithelial cell-specific type of intermediate filament) are a large family of about 20 different proteins. A subset of from two to ten of these keratins is expressed by each epithelial cell type in vivo and in culture\(^1\)\(^-\)\(^8\) as a stable feature of its differentiated state, although often subject to extrinsic modulation.\(^9\)\(^-\)\(^12\)

Recently this laboratory has been studying the mesothelial cell—a simple squamous epithelial cell type, derived from embryonic mesoderm—that lines the pleural, pericardial, and peritoneal cavities and covers the organs contained within these cavities. Mesothelial cells contain four keratins, different from those present in epidermal cells (Figure 1).\(^9\) In vivo, normal mesothelium has a high keratin and a low, but detectable, vimentin content.\(^11\)\(^,\)\(^13\)\(^,\)\(^14\) During rapid growth in culture, mesothelial cells shut off their keratin synthesis and become virtually devoid of keratin filaments, while synthesizing and accumulating high levels of vimentin.\(^11\) When they become quiescent at confluence, especially when their required mitogen epidermal growth factor (EGF) is absent from the medium, mesothelial cells again synthesize and accumulate keratin and reduce their synthesis of vimentin.\(^11\) The mechanism of this switching involves a change in levels of cytoplasmic mRNA for these proteins.\(^14\)

This reversible de-differentiation by mesothelial cells, so different from the continual keratin synthesis by keratinocytes during serial culture, suggests that mesothelial cells are much more dependent upon extrinsic signals for expression of even their most basic epithelial characteristics than are other epithelial cell types studied to date. Curious about the behavior of other mesoderm-derived epithelial cell types, we have begun to examine the urinary tract epithelia. We have cultured different regions of
FIGURE 1. Human epithelial cell types in culture distinguished by colony morphology and patterns of keratin and vimentin synthesis. (a and b) epidermal keratinocytes; (e and d) mesothelial cells; (e and f) urothelial cells. (a, c, and e) Phase contrast microscopy, 300× magnification. Mitomycin-treated 3T3 feeder cells, needed for optimal growth of keratinocytes and urothelial cells, are indicated by X in (a) and (e). Keratinocytes form stratifying colonies; the outline of a terminally differentiating cell flattened over the top of the colony is indicated by an arrow in (a). Urothelial cells form adherent, epithelioid colonies but do not stratify under these conditions of culture. Mesothelial cells adopt a stubby, fibroblastoid morphology and form dispersed colonies during rapid growth in culture. (b, d, and f) The Triton/high salt-insoluble fraction of [35S]methionine-labeled cell cultures separated in two dimensions by non-equilibrium pH gradient electrophoresis (NEPHGE) in the horizontal dimension and SDS-polyacrylamide gel electrophoresis in the vertical dimension. Numbers indicate keratin proteins according to the nomenclature of Moll et al. and correspond to the keratins we previously named on the basis of size (e.g., "40K" (40 kilodaltons)) and whether they were synthesized by epidermal or mesothelial cells (subscript E or M) as follows: #5/58Ke, #6/56Ke, #7/55Km, #8/52Km, #13/52Km, #14/50Km, #17/46Km, #18/44Km, and #19/40Km. Vimentin is indicated by V and the small amount of total cellular actin that is Triton/high salt-insoluble is indicated by an arrowhead. Proteolytic breakdown products of vimentin and keratin #8 are indicated by asterisks in (d). Spots directly beneath the arrow in (b) and (f) are keratins that were dissociated incompletely during NEPHGE and consequently migrated at intermediate charge as complexes of basic and acidic keratins.
the urinary tract from 20-week-old human fetuses, a stage of development at which fully mature structures have formed. A full report on kidney cortex and urine-derived epithelial cells will be published elsewhere (O'Connell and Rheinwald, in preparation). This article briefly summarizes this work and updates the preliminary observations we had reported as part of an earlier monograph.14

Culture of explants and single cell suspensions of urinary bladder, ureter, and renal pelvis—all regions lined with the stratified, transitional epithelium known as “urothelium”—yielded epithelial cells having the colony morphology and pattern of keratin synthesis shown in Figure 1(e and f). The cells from all three regions synthesized the four simple epithelial keratins (#7,8,18,19) and five of the stratified squamous epithelial keratins (#6,13,17 and small amounts of #5 and 14), which we had reported earlier as the unique “fingerprint” of bladder urothelial cells.6 Urothelial cells from all three regions displayed stringent in vitro growth requirements for a 3T3 feeder layer and a complex set of growth factors, hormones, and other supplements, as we had reported for bladder urothelial cells.6

In contrast, cells that grew from single cell suspensions of renal cortex were primarily of two morphological types (Figure 2, a), both distinguishable from urothelial cells. Adherent, swirling, epithelioid Type I colonies predominated in most primary cultures of cortex. Dispersed colonies of stubby, fibroblastoid Type II cells became predominant with serial passage because these cells subcultured with much higher colony forming efficiencies and usually had longer lifespans than did Type I cells. Type I and II kidney cells formed colonies and grew progressively in a simple medium without 3T3 feeder cells or added growth factors (although they grew more rapidly in the presence of EGF). Thus their growth requirements are different from those of either urothelial or mesothelial cells.6,11,14

Clones of Type I kidney cells examined in primary or later passage culture were somewhat heterogeneous with respect to keratin and vimentin synthesis (Figure 2, b and d). All cells forming Type I colonies synthesized the four simple epithelial keratins, but some synthesized no vimentin at all and some appeared to synthesize small amounts of keratin #17. (We did not detect any synthesis of keratins #5, 6, or 13 in primary cultured populations of renal cortex cells or in Type I clones from our two most recent preparations. Possibly the earlier Type I culture in which we detected keratins #5, 6, and 13 and on which we based a preliminary report of the keratins in Type I cells,14 contained some contaminating renal pelvis urothelial cells.) Indirect immunofluorescence microscopy of kidney cortex epithelial cell colonies in either primary or later passage culture also disclosed heterogeneity in keratin and vimentin content among Type I colonies (Figure 3, a-d).

Type II kidney cells always synthesized very high levels of vimentin and usually very low, but sometimes moderate, levels of the four simple epithelial keratins (Figure 2, e). Accordingly, they had low to moderate keratin, but high vimentin, contents (Figure 3, e and f).

Normal urine contains cultivable epithelial cells.15-19 Typical colonies arising in primary cultures of urine-derived cells are shown in Figure 4 (a and c). We have analyzed these cells for their growth requirements and intermediate filament proteins. The cells grow equally well in the presence or absence of 3T3 feeder support and exhibit no stringent growth factor or medium requirements. They synthesize only simple epithelial keratins (Figure 4, b and d). We have found no colonies having either morphology or keratin pattern characteristic of urothelial cells.

Two colony morphologies predominate in primary cultures of either male or female urine-derived cells. One type resembles Type I renal cortex epithelial cells (Figure 4, a), and also has a high keratin and very low vimentin content (Figure 5, a
FIGURE 2. Colony morphology and intermediate filament protein synthesis of two epithelial cell types cultured from human renal cortex. (a) Phase contrast microscopy, 300× magnification, of Type I (upper left) and Type II (lower right) colonies growing in the primary culture of a 20-week fetal kidney cortex (HKi-12). Note the adherent, swirling, unstratified epithelioid morphology of the Type I cells and the stubby, fibroblastoid morphology and dispersed growth of the Type II cells. Autoradiograms of two-dimensional NEPHGE gels of the Triton/high salt-insoluble fractions of [35S]methionine-labeled Type I and II kidney cells in secondary and later passage culture. Keratins #7,8,18, and 19 are indicated by arrows, vimentin by V, and actin by arrowheads. (b) Type I cells, secondary culture of HKi-10. Note lack of vimentin and small amount of keratin #17. (c) Type II cells, third passage of HKI-10. Note high level vimentin and low level keratin synthesis. (d) Secondary culture of a Type I clone picked from the primary culture of HKi-12. Note the synthesis of vimentin and the relatively higher keratin synthesis, which was maintained during serial culture of this clone.
FIGURE 3. Keratin and vimentin immunofluorescence of Type I and Type II kidney cortex epithelial cells. Colonies arising from single cells in the third passage of renal cortex population HK-10. (a, c, e, and g) Rhodamine-coupled anti-keratin staining, using a rabbit antiserum raised against human epidermal stratum corneum keratins. (b, d, f, and h) Fluorescein-coupled anti-vimentin staining, using a guinea pig antiserum against vimentin (kindly provided by Dr. W. Franke, Heidelberg). (a and b) Type I colony possessing a high keratin content and no detectable vimentin. (c and d) Type I colony in which the cells possess a moderate keratin content and variable vimentin content from low to moderate. (e and f) Type II colony, exhibiting very low keratin and high vimentin content. Note the star-shaped pattern of vimentin and the rare cells with moderate keratin content. (g and h) A small Type II colony to the right of a Type I colony.
FIGURE 4. Intermediate filament proteins synthesized by epithelial cell types cultured from normal human urine. Cells were collected by centrifugation from the urine of a normal adult male and plated in DME/F12 (3:1 vol/vol) medium + 15% fetal calf serum + 0.4 μg/ml hydrocortisone (HC) + 10 ng/ml epidermal growth factor (EGF). Colonies or cell populations were photographed, then labeled with [35S]methionine and the Triton/high salt-insoluble proteins extracted, separated by NEPHGE, and autoradiographed. Magnification of photographs is 300×. Arrows indicate keratins #7, 8, 18, and 19; V indicates vimentin; and arrowheads indicate actin. (a and b) An adherent, swirling epithelioid colony (reminiscent of Type I kidney cortex epithelial cells) arising in primary culture. Note the synthesis of keratins #7, 8, 18 and 19 and the absence of vimentin synthesis. (c and d) An adherent triangular epithelioid colony arising in primary culture. Note that vimentin synthesis is greater than keratin synthesis. We interpret this cell type as a pre-Type II, because upon subculture the cells grow with either a dispersed, fibroblastic morphology, as in (e), or a slightly flattened, football-shaped morphology (not shown) and reduce their synthesis of keratins while synthesizing high levels of vimentin (f). Cells cultured from the urine of a normal adult female had the same characteristics as those described in this figure.
FIGURE 5. Keratin and vimentin immunofluorescence of urine-derived epithelial cells. Colonies grown from cells obtained from the urine of a normal adult male. (a, b, and c) staining for keratin as in Figure 3. (d, e, and f) staining for vimentin as in Figure 3. (a and d) Type I-like colony growing in the presence of 3T3 feeder cells. Note the high keratin content and absence of vimentin. (b and e) A presumptive pre-II colony growing in the presence of 3T3 feeder cells. Note the low-to-moderate levels of both keratin and vimentin and the rather high level of vimentin in a star-shaped distribution in some of the cells. Vimentin-positive 3T3 cells are indicated by a 3 in (d) and (e). (c and f) Cells of a pre-Type II colony replated in secondary culture and grown to saturation density. Note the very low level or absence of keratin and the high level of vimentin in most cells.
and d). The other displays an adherent, epithelioid morphology in primary culture (Figure 4, c), but the cells are more triangular and less swirling, and have a somewhat lower keratin and higher vimentin content than Type I cells (Figure 5, b and e). When subcultured, this second urine-derived cell type grows with either a dispersed, fibroblastoid morphology (Figure 4, e) or with an ovoid or football shape (not shown), both of which we have also observed among clones of Type II renal cortex epithelial cells. In second and later passages such Type II urine-derived cells synthesize very high levels of vimentin and usually very little keratin (Figure 4, f). Many of the cells in Type II clones contain little or no keratin by the secondary culture (Figure 5, c and f), although the clones of football-like cellular morphology often retain a moderate keratin synthesis rate and content (not shown).

We conclude that the colony-forming cells present in urine are kidney tubule epithelial cells that somehow become shed from the nephrons. The cell type shown in Figure 4c is apparently a pre-Type II cell, which de-differentiates more completely after reinitiating growth in secondary culture. During primary culture of fetal kidney cortex, cells of Type II morphology usually become evident later than those of Type I morphology, and we are currently seeking a pre-II epithelioid cell type in kidney cultures. Serial cultivation of several clonally derived Type I kidney cell populations disclosed no conversion of Type I cells to Type II cells.

We do not know the regions of the nephron from which Type I and II kidney cells originate, although it has been reported recently that cultured human kidney cells of Type I morphology are proximal tubule cells. The entire nephron, from glomerulus to distal tubule, is formed during embryogenesis from a single vimentin-positive, keratin-negative precursor cell type in the mesoderm, the nephrogenic mesenchyme, as a consequence of induction by contact with the ureteric bud. Type I cells may be a specialized cell of the nephron that is less dependent upon extrinsic signals for expression of its differentiation program than is the nephron cell type from which Type II cells originate. Alternatively, Type II cells may be an incompletely induced nephrogenic mesenchyme cell. Further investigation is necessary to distinguish between these hypotheses.

REFERENCES