Keratin Profiling in the Developing Human Prostate. A Different Approach to Understanding Epithelial Lineage

MARLEEN TROMPETTER[1], FRANK SMEDTS[2], JAN VAN DER WIJK[1], COEN SCHOOTS[2], HANS-JURIEN DE JONG[2], ANTON HOPMAN[3] and JEAN DE LA ROSETTE[4]

[1]*Department of Urology, Refaja Hospital Stadskanaal;*
[2]*Department of Pathology, Foundation of Collaborating Hospitals of Eastern Groningen, Winschoten;*
[3]*Department of Molecular Cell Biology, Research Institute for Growth and Development, University of Maastricht;*
[4]*Department of Urology, Academic Medical Centre, University of Amsterdam, The Netherlands*

Abstract. *Background: Keratin profiling studies in the developing human prostate have characterized cells thought to be stem cells and so-called intermediate cells. In a series of human prostates of various gestational ages, we extended on these studies using a comprehensive panel of keratin antibodies. Materials and Methods: Autoptic tissue from 19 fetal prostates, gestational ages between 16 and 40 weeks, were immunostained with a panel of keratin antibodies: these recognize the luminal type keratins 7, 8, 18, 19, 20 and the basal/squamous type keratins 5, 6, 13, 14, 17. Results: Keratin8 and vimentin were important constituents of the cytoskeleton of budding tips in early gestational age fetuses. Very early in gestation, additional expression of keratins 5 and 13 was noted and, with time, increasing expression of keratins 7, 18 and 19, and also incidentally keratins 14 and 17. With differentiation into basal cell and luminal cell compartments and the formation of prostate acini, the keratin complement of basal and luminal cells became more pronounced, but only partial compartmentalization of keratin expression occurred. Conclusion: We suggest that prostate stem cells may contain only keratin8 and not 5 or 14. The acquisition of other keratins could be indicative of the function the cells will eventually acquire.*

Keratins are a family of intermediate filament proteins found in epithelial cells. They span the cell from the nucleus to the cell junctions and have a scaffold function important in the maintenance of cellular integrity and internal cellular transport (1). The keratin family consists of at least 20 members. Different types of epithelia contain up to 6 specific different keratin subtypes. The so-called simple keratins 7, 8, 18, 19 and 20 are found in columnar cells. The epidermis has a more complex keratin expression pattern: keratins 5 and 14, "basal cell" keratins, are present in the basal cell compartment, while keratins 1 and 10, associated with keratinisation, are found in the keratinising compartment. Simple keratins are not found in this type of epithelium. Non-keratinising epithelia do not contain keratins 1 or 10, but do contain 4 and 13. Vimentin, also an intermediate filament protein has the same function as the cytokeratins but is found mainly in mesenchymally derived tissues (2).

Studies reporting the distribution of keratins in the adult human prostate (3-9) and the developing human prostate (10-12) suggest the following model for epithelial cell lineage. A small subpopulation of stem cells present in the basal cell compartment of the prostate epithelium contain keratins 5, 14, 8, 18 and 19. If these cells give rise to basal cells they lose their expression of simple keratins 8, 18 and 19, resulting in the expression of keratins keratins 5 and 14 only. If luminal cells develop, they lose basal cell keratins 5 and 14. Initially keratin 19 is expressed along with keratins 8 and 18 in luminal cells, but eventually this keratin is lost.

The investigation of the keratin distribution in the developing human prostate epithelium, may be of value in ascertaining the relationships between the different types of epithelial cells in the prostate (10), particularly as it is thought that stem cells are more abundant during early prostate development. Using a large panel of monoclonal keratin antibodies we extend previous studies in a relatively large series of fetal prostates of various gestational ages.

Materials and Methods

Specimens. Formalin-fixed, paraffin-embedded blocks from autopsy prostates of 19 fetuses (16-40 weeks of gestation) were used. Prostate tissues from two adults aged 21 and 39 years were

Correspondence to: Prof. Dr. J. de la Rosette, Department of Urology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, P.O. Box 22660, 1100 DD Amsterdam, The Netherlands. Tel: +31 20 5666030, Fax: +31 20 5669585, e-mail: j.j.delarosette@amc.uva.nl

Key Words: Embryology, cell differentiation, intermediate filaments, keratin, prostate.

Table I. *Antibodies used in the study their source and the antigen retrieval method used to obtain optimal immunostaining.*

Intermediate filament detected	Clone		Antigen retrieval step pH	Source
k5, k6	D5/16 B4	citr.	7.3	Boehringer Mannheim, Mannheim, Germany
k1, 5, 10, 14	34βE12	citr.	6.0	Dako Cytomation, Denmark
k5+	RCK103	citr.	7.3	Mubio bv, Maastricht, The Netherlands
k5	XM-26	Tris/EDTA	9.0	Lab Vision, Fremont, CA, USA
k6	KA12	citr.	6.0	Mubio bv
k7	OV-TL 12/30	protease		BioGenex, San Ramon, CA, USA
k8 (k7)	CAM 5.2	protease		Becton Dickinson, Franklin Lakes, NJ, USA
k13	1C7	citr.	6.0	Lab Vision
k14	LL002	citr.	6.0	BioGenex
k17	E3	citr.	6.0	Lab Vision
k18	RCK106	citr.	6.0	Mubio bv
k19	RCK108	protease		Mubio bv
k20	IT.Ks 20.8	protease		Neomarkers, Fremont, CA, USA
Vimentin	V9	citr.	6.0	Dako Cytomation

K, keratin; citr., citrate buffer.

used as controls. Postmortem delay varied between 5-48 h. Gestational age was determined using a foot-length table combined with histologic parameters. In 5 fetuses, abortion was induced because of chromosomal abnormalities (trisomy 21, n=2; trisomy 18, n=3). In the other cases, autopsy revealed that intrauterine death was due to infection, placental pathology or unknown causes. Sixteen consecutive 4-μm-thick sections were cut from each paraffin block, mounted on pretreated slides (Superfrost*/Plus; Menzel-Glaser, Germany) and dried overnight. The first and last sections were stained for haematoxylin-eosin (H&E). The remaining slides were stained with the keratin antibodies as shown in Table I.

Scoring keratin immunoreactivity. The epithelial compartment of the prostate was divided into the following three developing compartments: *i) Budding tips:* the most primordial epithelial compartment in the prostate, defined as the peripheral part of the branching prostate duct, consisting of an entirely solid nodule composed of cells that are basaloid in the peripheral part and centrally spindle-shaped (*e.g.* Figure 1I). ii) *Lumininzing acini*: defined as more intermediately located structures lined peripherally by basal cells with a central lumen, lined by either columnar or cubiod cells (*e.g.* Figure 1J). iii) *Periurethral acini*: tubular structures directly adjacent to the urethra with a central lumen lined by columnar cells and a peripheral rim of basal type cells (*e.g.* Figure 1K).

In these structures the following epithelial compartments were evaluated: i) The luminal cell compartment, defined as epithelial cells lining the lumen. The lumen in the budding tip was absent and the most centrally located cells were scored. ii) Cells between the luminal cell layer and the basement membrane. In ducts, this layer was one or more layers thick, while in budding tips it was usually more than 4 layers thick (*e.g.* Figure 1L).

In each slide we evaluated staining in the compartments semiquantively. We estimated the number of cells staining from sporadic staining of isolated cells: ±, between 1-25% of cells; (+), 26-50% of cells; ++, 51-75% of cells; +++, 76-100% of cells; ++++, no stain was indicated as 0. Furthermore we determined the intensity of immunoreactivity: 0, no staining; (+), weak staining; ++, moderate staining; +++, intense staining. Slides were evaluated by MT and FS. TH reviewed the results.

Results

Fetuses. During autopsy no abnormalities of the urogenital tract were diagnosed. Fetuses with genetic abnormalities showed no differences in keratin expression compared to genetically normal fetuses.

Immunostaining. Immunostaining results are summarized in Table II and briefly highlighted below; they are also illustrated in Figure 1. At a gestational age of approximately 22 weeks, there seemed to be a significant change in keratin phenotype; this was noted for most of the antibodies in the majority of fetuses.

Keratin8 (cam 5.2) stained all cells in all compartments intensely, irrespective of gestational age (Figure 1A). Keratin20 (IT.keratins 20.8) was not detected (Figure 1B). Keratin6 (KA 12) was also not detected in any of the prostates (results not shown in the table).

Basal cell markers

Keratin5. Most of the sera used to detect keratin5, detect this keratin along with other keratins.

34βE12 (keratins 1, 5, 10 and 14) and RCK103 (keratin5 and other keratins). These stained all cells irrespective of cell compartment in fetuses with gestational age <22 weeks (Figure 1C). In the older fetuses, of gestational age >22 weeks, luminal cells stained less intensely and were sometimes unstained (Figure 1D, E and F).

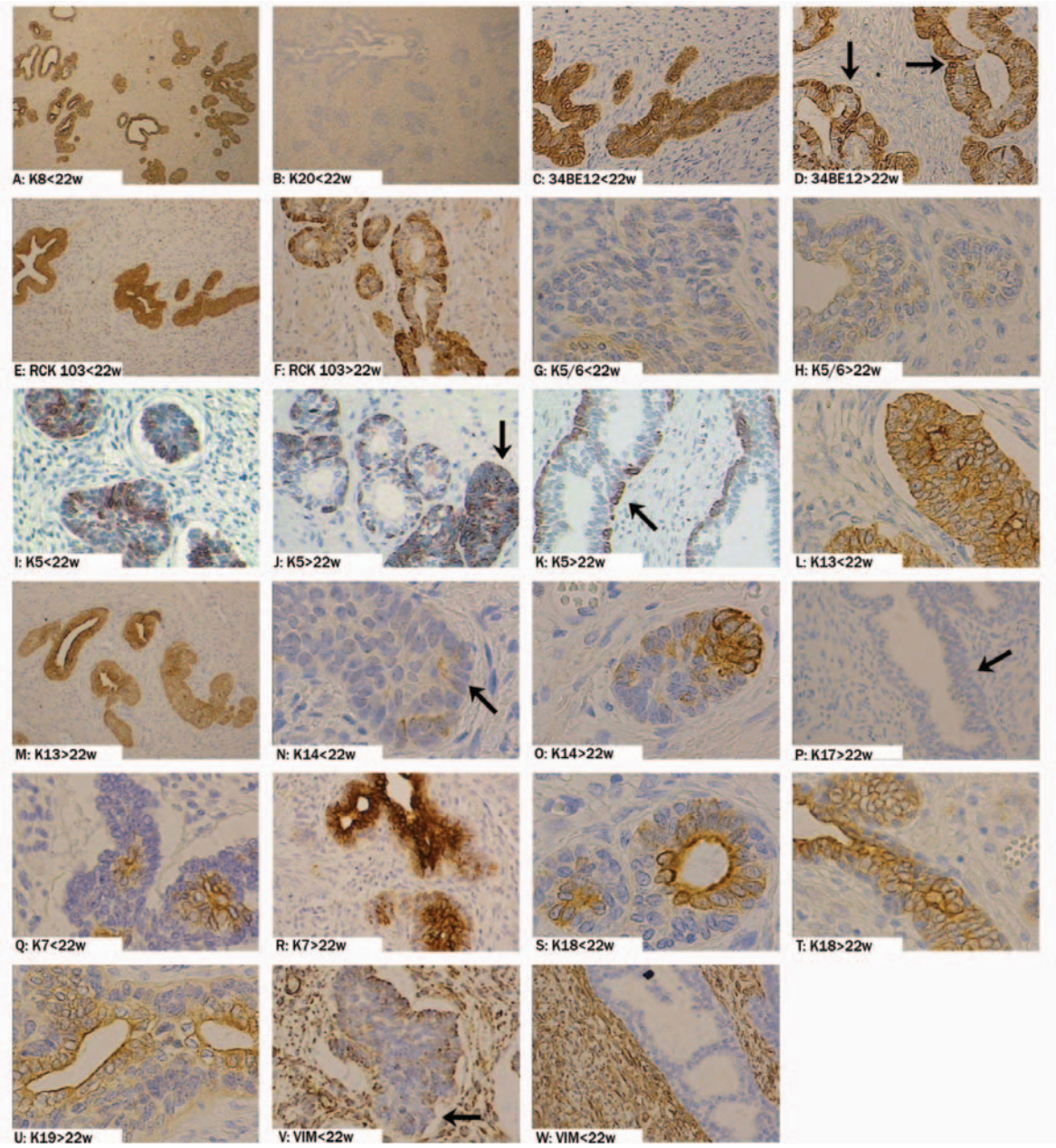


Figure 1. *Microphotographs of developing prostate epithelial compartment after staining with the various keratin (k) antibodies. A) K8 antibody staining the budding tips and the luminizing acini in a foetus of gestational age <22 weeks (w). B) K20 antibody non-reactive in all prostates. C) 34βE12 (k5+) immunostaining the entire epithelial compartment of a fetal prostate of gestational age <22 w. In fetuses >22 w compartmentalization of staining starts to occur, with more intense expression in the basal cell compartment (arrows, D). E) RCK103 (k5+) staining all epithelial cells in the prostate of a fetus of gestational age <22 w. In fetuses >22 w compartmentalization of staining occurred (F). G) K5 and 6 staining only weakly in a fetus of gestational age < 22 w. Staining increases slightly in the second half of gestation (H). I) K5 antibody showed some staining in budding tips of fetuses of gestational age <22 w. Older fetuses showed more intense staining in budding tips (arrow, J). Luminizing and periurethral glands expressed this k5 mainly in basal cells (arrow, K). L) K13 antibody is present in the whole epithelial compartment of the prostate during the entire gestational period (M). N) K14 weakly staining some cells in the budding tips of some fetuses of gestational age <22 w (arrow); more staining is noted in the older fetuses (O). P) K17 is focally weakly expressed in some basal cells in luminizing acini at late gestational age (arrow). K7 is present mainly in luminally located cells of early gestational age fetuses (Q). In the second half of gestation there is considerable immunoreactivity in all compartments (R). S) K18 in early gestational age fetuses there is considerable luminal staining and only some basal cell immunoreactivity. After 22 weeks' gestation, immunostaining increases dramatically and basal cells also stain (T). K19 is found in luminal and basal cells in periurethral acini in late gestational age fetuses (U). V) Vimentin staining in basal cells of budding tips (arrow). In perurethral acini no staining is noted (W). Original magnification: x40: A, B; x100: E, M; x200: C, D, F, J, P ,R, W; x400: G, H, I, L, N, O, Q, S, T, U, V.*

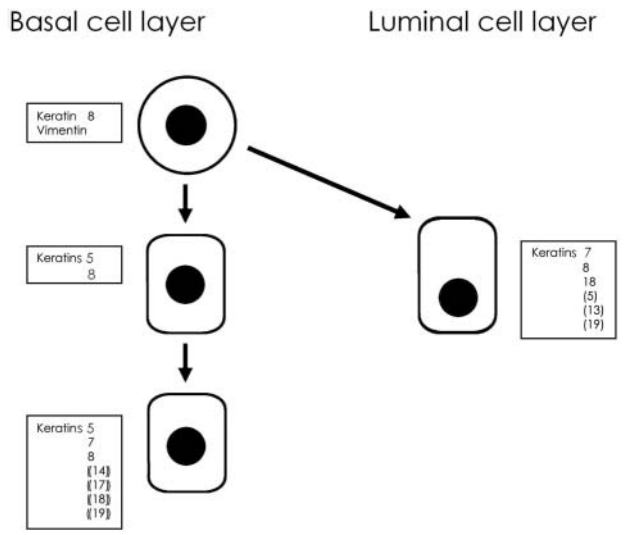


Figure 2. *A model for epithelial cell lineage in the prostate incorporating the observed keratin expression patterns. The round cell signifies a stem cell. This type of cell is discernable during early gestation. Initially it expresses keratin8 and vimentin. With increasing gestational age, cells with this phenotype, are rapidly replaced by cells containing other keratins, particularly keratin5, signifying the appearance of a basal cell compartment. The rectangular box with a central nucleus represents a basal cell. Keratin expression in basal cells is diverse; different keratin combinations are possible. We suggest that basal cells originate from the stem cells, thereby gaining a large number of keratins. Differentiation into luminal type cells may follow two pathways either via basal cells that partly lose their basal cell keratin component, or directly from stem cells during which process they acquire keratins 7, 18 and 19. The rectangular box with an eccentric nucleus is a luminal type cell. (): Indicates that the corresponding keratin is present in a substantial portion of cells. ((): Indicates that the corresponding keratin is present in a minor portion of cells.*

D5/16 B4 (keratins 5 and 6). This was weakly expressed in budding tips in approximately 50% of cells in half of the fetuses with a gestational age less than 22 weeks (Figure 1G). In the other cell compartments, only isolated cells were weakly positive. In the fetuses older than 22 weeks, more cells stained (Figure 1H). An increase in basal cell staining was also noted in luminizing acini and periurethral acini.

XM-26 (k5). Some groups of solid acini stained while in others staining was weak to absent (Figure 1I), suggesting a heterogeneous distribution of keratin5. Centrally located cells were frequently positive. Basal cells in periurethral glands were frequently positive and there was minor positivity in sporadic luminal cells. In the older gestational age fetuses, the budding tips showed more intense staining and all cases stained (Figure 1J). Luminizing acini and periurethral glands showed the same expression as the early gestational age fetuses (Figure 1K).

K13 (AE8). A majority of the early gestational age fetuses expressed this keratin in most cells, in both luminal and basal cell compartments (Figure 1L). In the fetuses with a gestational age above 22 weeks, all cells, irrespective of the epithelial compartment, stained intensly (Figure 1M).

Keratins 14 and 17 (LL002 and E3). In fetuses less than 22 weeks of age, keratin14 was found in sporadic basal cells in the budding tips in fewer than half of cases (Figure 1N). In the fetuses with a gestational age older than 22 weeks, levels of immunoreactivity were higher (Figure 1O). Keratin17 was not found in fetuses with a gestational age <22 weeks. Basal cells in luminizing acini of fetuses >22 weeks gestation were sporadically weakly positive in half of the cases (Figure 1P).

Simple keratins.
K7 (OVTL12/30). Keratin7 was weakly expressed in some of the basal cells and luminally located cells in fewer than half of the budding tips of fetuses with a gestational age less than 22 weeks (Figure 1Q). In luminizing acini, an identical staining pattern was observed, while in the periurethral acini with distinct lumina staining of luminal cells was intense and most of the underlying basal cells also stained. After 22 weeks of gestation, there was distinct strong positivity of centrally located cells in budding tips, lumenally located cells in luminizing and periurethral acini. Staining intensity increased with gestational age (Figure 1R).

K18 (RCK 106). The budding tips of early gestational age fetuses expressed the keratin18 antibody in the basal cell compartment in fewer than half of the cases. In most cases, the centrally located cells stained. Immunoreactivity and the number of cells staining in the luminal cell compartment of acini and periuretheral acini was higher than in the basal cell compartment(Figure 1S). In fetuses older than 22 weeks, centrally located cells in acini usually stained. Cells in the other compartments showed increased immunoreactivity, both in the number of cells staining and the staining intensity compared to fetuses of less than 22 weeks gestation (Figure 1T).

K19 (RCK 108). The keratin19 antibody stained a few budding tips and luminizing acini in fetuses of gestational age less than 22 weeks. Immunoreactivity was moderate. The periuretheral acini showed much higher levels of immunoreactivity with practically all luminal cells staining and a considerable number of basal cells were also immunoreactive. In fetuses older than 22 weeks, staining levels were higher in both compartments (Figure 1U).

Vimentin. Up to and including 21 weeks, this intermediate filament, characteristic of mesenchymal cells, was always present in most of the basal epithelial cells of the budding

tips and luminizing acini (Figure 1V). The periuretheral acini were entirely unstained (Figure 1W). In the fetuses with gestational age of more than 22 weeks, budding tip expression was approximately the same as in those before the gestational age of 22 weeks, while levels of expression in luminizing acini were very low.

Discussion

Keratin profiling studies of the prostate have both practical and basic implications. Profiling basal cells is useful in the differential diagnosis between benign prostate epithelium and prostate carcinoma (13). Other studies, such as this one, use keratin antibodies to clear up questions concerning cell lineage in the prostate (4-12).

Using a broad panel of keratin antibodies, we examined how the keratin profile of prostate epithelium changes during human development. We were particularly interested in which keratins appear first in the prostate and how the keratin profile parallels organ development and maturation. This information could be helpful in identifying subsets of cells which have different functions, such as primordial epithelial cells, *i.e.* stem cells, from which basal and luminal cell types develop.

In the early gestational age fetuses, we observed, that keratin5 is not present in all cells in all budding tips using a specific keratin5 marker,. With prostate maturation, keratin5 expression increased, with partial restriction to the basal cell compartment. This was also the case for the simple keratins 7, 18 and 19. Keratin 8 however was universally present in all cells at the earliest gestational ages, showing only minor compartmentalization with fetal age.

Based on our observations, we propose the following two models for epithelial lineage in the prostate (see Figure 2). We suggest that the prostate stem cell has a simple keratin phenotype consisting of keratin8 only. Very early in gestation, this cell can transform into a basal type cell. These cells acquire keratin5 and also other keratins such as 13, which are variably expressed, along with simple keratins. Basal cells that are the progenitor cells of luminal cells will, on transformation into luminal cells, therefore lose only the basal cell keratins. Another possibility is that the stem cell containing keratin8 could be the progenitor cell for the luminal cell on transformation it acquires expression of keratins 18 and 19. Some support for this idea may be further derived from the fact that tissue cultures from *p63* knockout mice show the development of a glandular prostate in which basal type cells are not found, indicating that the luminal cells must arise from a cell with a simple keratin phenotype (15).

These data must be considered in the light of previous studies that show partly contradictory results. Hudson *et al.* (7), in adult studies, speculated that basal cells containing only keratins 5 and 14 are true stem cells, while cells which also contain keratins 8 and 18 are differentiating into luminal cells. Some authors call these latter cells the intermediate type cells in the Isaacs and Coffey model (13).

The Wang study (10), examining embryonic prostates, suggests a different model in which stem cells contain the full complement of epithelial markers, both basal and luminal, namely keratins 5, 14, 8, 18 and 19 in this model, when stem cells transform into basal cells they lose keratins 8, 18 and 19; if they transform into luminal cells they lose, keratins 5 and 14, and eventually also 19.

Our observations do not support the Wang report. Firstly, we observed high levels of keratin8 in the projected pluripotent stem cells in the earliest phases of human development, while expression of other simple keratins such as keratins 7, 18 and 19 increased proportionally to the gestational age and did not decrease as the complement of candidate pluripotent stem cells in the basal cell compartment decreases as Wang *et al.* showed in their paper. Wang *et al.* explain their observations by the fact that the prostate epithelium develops from the urogenital sinus, which contains keratin5. This explanation seems logical and we favour it, but our observations do not support it. To answer this question one could investigate whether there is a basal cell in the adult prostate that contains keratin8 alone, without keratin5, using double staining techniques in combination with keratin antibodies recognizing one keratin subtype.

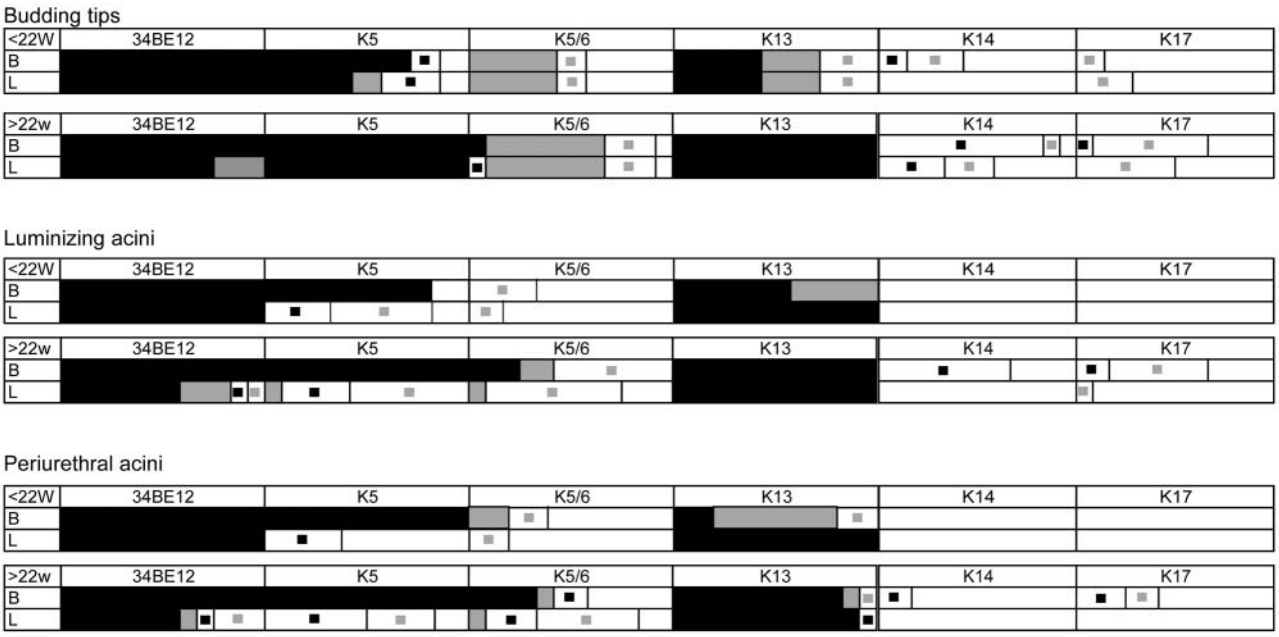
A problem with the Wang study is the choice of basal cell markers. Using 34βE12 our results are identical to those of the Wang study (10, 12). However, using specific antibodies for keratin5 we observed that not all cells stained in the budding tips and that the number of cells staining, increased with gestational age. This suggests that the status of keratin5 may be incorrectly estimated on the basis of 34βE12 staining.

However improbable it may seem, the non basal type keratins 1 and 10 have been reported in the prostate (5), also detected by 34βE12.

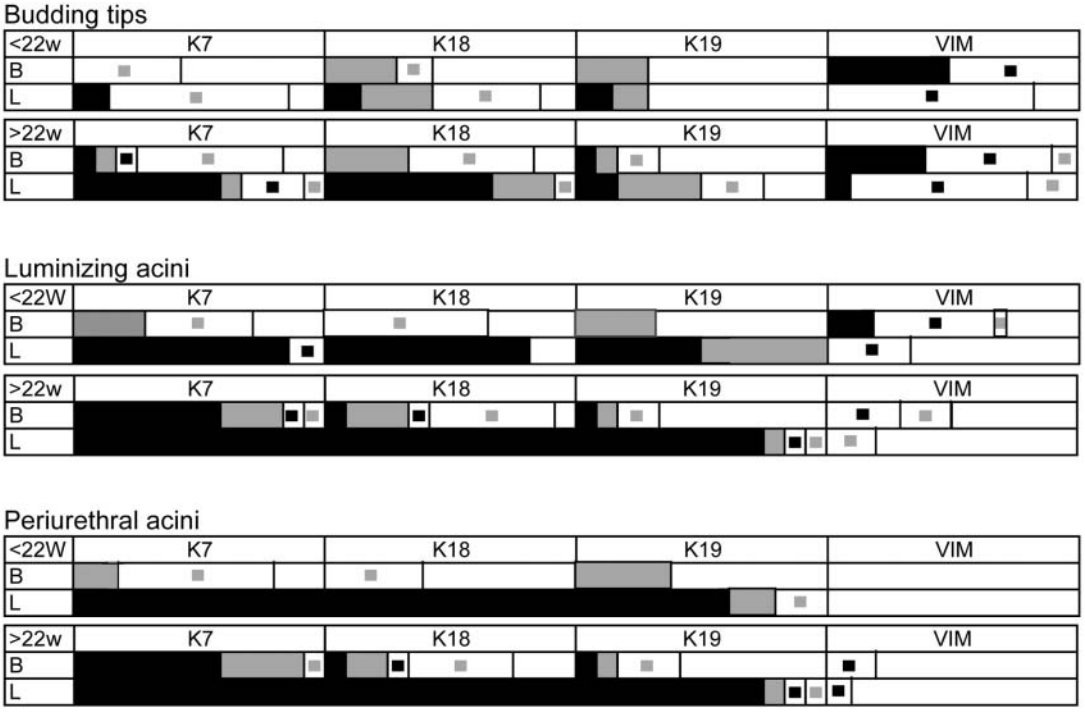
To date, this is the first study separately investigating keratin5 in the developing prostate. As mentioned earlier, other papers base their results on antibodies that recognize keratin5 along with other keratins. To evaluate keratin5 staining, we also used an antibody that detects keratins 5 and 6 in combination with a keratin6 antibody, again finding lower levels of keratin5 early in gestation. Particularly in the budding tips many of the keratin5 negative cells stained only for keratin8 and vimentin. Furthermore, keratin8 expression was ubiquitous in the fetuses of early gestational age. It therefore seems possible that the earliest developing primitive epithelial cells are, in the majority, (stem)cells which contain keratin8, with keratin5 appearing shortly thereafter.

Table II. *A schematic representation of the immunostaining results of the fetal prostates. The percentage of shading in each box indicates the number of cases staining for the respective antibody. The shading in the box indicates the most predominant staining pattern noted in these cases according to the following code:*

A



B



B, basal cells; L, luminal cells; K, keratine subtype; Vim, vimentin; w, weeks.

■, Intense staining in more than 50% of cells; ▣, intense staining in fewer than 50% of cells; ▩, weak staining in more than 50% of cells; ▣, weak staining in fewer than 50% of cells; □, no staining.

In summary, contradictory to previous studies, our study suggests that the keratin profile in the developing human prostate epithelium moves from a simple pattern to a more complex one. This observation has implications for our concept of epithelial cell lineage in the developing human prostate.

References

1 Owens DW and Lane EB: The quest for the function of simple epithelial keratins. BioEssays *25*: 748-758, 2003.

2 Ramaekers F, Smedts F and Vooijs GP: Keratins as Differentiation Markers in Tumor Biology and Surgical Pathology. Current Perspecitives in Molecular and Cellular Oncology. (ed.) Spandidos D, London; JAI Press: 285-318, 1992.

3 Sherwood ER, Berg LA, Mitchell NJ, McNeal JE, Kozlowski JM and Lee C: Differential cytokeratin expression in normal, hyperplastic and malignant epithelial cells from human prostate. J Urol *143*: 167-171, 1990.

4 Verhagen APM, Ramaekers FCS, Aalders TW, Schaafsma HE, Debruyne FMJ and Schalken JA: Co-localization of basal and luminal cell-type cytokeratins in human prostate cancer. Cancer Res *52*: 6182-6187, 1992.

5 Nagle RB, Brawer MK, Kittelson J and Clark V: Phenotypic relationships of prostatic intraepithelial neoplasia to invasive prostatic carcinoma. Am J Path *138*: 119-128, 1991.

6 Xue Y, Smedts F, Umbas R, Aalders TW, Debruyne FMJ, de la Rosette JJMCH and Schalken JA: Changes in keratin expression during the development of benign prostatic hyperplasia. Eur Urol *32*: 332-338, 1997.

7 Hudson DL, Guy AT, Fry P, O'Hare MJ, Watt FM and Masters JRW: Epithelial cell differentiation pathways in the human prostate: Identification of intermediate phenotypes by keratin expression. J Histochem Cytochem *49*: 271-278, 2001.

8 Leenders van JHL, Dijkman HBPM, Hulsbergen-van de Kaa CA, Ruiter DJ and Schalken JA: Demonstration of intermediate cells during human prostate epithelial differentiation *in situ* and *in vitro* using triple staining confocal scanning microscopy. Lab Invest *80*: 1251-1258, 2000.

9 Pheel DM, Sellers RG and McNeal JE: Keratin 19 in the adult human prostate: tissue and cell culture studies. Cell Tissue Res *285*: 171-176, 1996.

10 Wang Y, Hayward S, Cao M, Thayer K and Cunha G: Cell differentiation lineage in the prostate. Differentiation *68*: 270-279, 2001.

11 Wernert N, Seitz G and Achtstätter T: Immunohistochemical investigation of different cytokeratins and vimentin in the prostate from the fetal period up to adulthood and in prostate carcinoma. Path Res Pract *182*: 617-626, 1987.

12 Xue Y, Smedts F, Debruyne FMJ, Rosette JJMCH and Schalken JA: Indentification of intermediate cell types by keratin phenotyping in the developing human prostate. Prostate *34*: 292-301, 1998.

13 Zhou M, Aydin H, Kanane H and Epstein JI: How often does alpha-methylacyl-CoA-racemase contribute to resolving an atypical diagnosis on prostate needle biopsy beyond that provided by basal cell markers? Am J Surg Path *28*: 239-243. 2004.

14 Isaacs JT and Coffey DS: Etiology and disease process of benign prostatic hyperplasia. Prostate *2 (suppl)*: 33-50, 1989.

15 Kurita T, Medina RT, Mills AA and Cunha GR: Role of P63 and basal cells in the prostate. Development *131*: 4955-4964, 2001.

Received October 10, 2007
Revised November 26, 2007
Accepted December 17, 2007