Virchows Archiv A Pathol Anat (1992) 421:157–161

Virchows Archiv A
Pathological Anatomy and Histopathology

Keratin profiles in normal/hyperplastic prostates and prostate carcinoma

Hiyoshi Okada [1], Airo Tsubura [2], Akiharu Okamura [3], Hideto Senzaki [2], Yuji Naka [1], Yosuke Komatz [1], and Sotokichi Morii [2]

Departments of [1] Urology, [2] Pathology and [3] Clinical and Laboratory Medicine, Kansai Medical University, Fumizono-cho 1, Moriguchi, Osaka 570, Japan

Received January 17, 1992 / Accepted February 14, 1992

Summary. Immunoreactivities in 25 cases of prostatic adenocarcinoma and 10 normal/hyperplastic prostates were investigated in methacarn-fixed, paraffin-embedded serial sections using a panel of nine anti-keratin monoclonal antibodies (mAbs); 34βE12, CK8.12, 312C8-1, CK4.62, RPN1165, RPN1162, 35βH11, CK5, M20, and one of anti-actin mAb, HHF35. In normal/hyperplastic prostates, RPN1162, 35βH11, CK5 and M20 stained luminal cells without staining basal cells, and 34βE12, CK8.12 and 312C8-1 stained basal cells but not luminal cells. Other mAbs, CK4.62 and RPN1165, stained basal cells as well as luminal cells. All of the mAbs labelling luminal cells stained cancer cells with variable frequencies in a manner unrelated to the grade of tumour differentiation. Of the prostate cancer cases 92% were scored positive with M20, 84% with 35βH11, 80% with CK5, 68% with CK4.62, 60% with RPN1165 and 4% with RPN1162. However, basal cell-specific keratins labelled with 34βE12, CK8.12 and 312C8-1 were totally negative in the cancer cells. HHF35 showed no labelling in normal, hyperplastic or neoplastic epithelial cells of the prostate. Our findings indicate that the major part of the cells of prostatic adenocarcinomas have keratin phenotypes similar to luminal cells but not basal cells, and that no myoepithelial differentiation can be detected in epithelial cell of the prostate. Thus, mAbs for keratins facilitate the identification of epithelial cell phenotypes in normal, benign and malignant conditions of the prostate.

Key words: Prostate cancer – Luminal cell – Basal cell – Keratin – Actin

Introduction

Though prostate cancer is one of the commonest malignancies in men, little is known about the basic biology of cell phenotypes in either the normal prostate or prostate cancers. The prostate gland contains epithelial cells expressing two major phenotypes: luminal and basal cells, separated by basement membrane from the stroma. If appropriate markers can be utilized, characterization of the phenotypes of cells would thus represent a major advantage. Keratin is an epithelial-specific intermediate-sized filament with at least 19 subtypes documented with respect to their molecular weights and biochemical analyses (Moll et al. 1982). The patterns of keratin subtype expression can serve as a valuable marker of epithelial phenotypes in normal, neoplastic and metastatic tissues of the prostate. Of Moll's 19 keratins, numbers 5, 7, 8, 15, 18 and 19 have been identified in normal/hyperplastic prostates by two-dimensional gel electrophoresis (Achtstätter et al. 1985; Sherwood et al. 1990). Although the keratin phenotype of prostatic tissue has been precisely determined by chemical techniques, biochemical analysis cannot discern any cellular differentiation among individual cells in the population. Immunohistological characterization has repeatedly shown that luminal and basal cells can be distinguished by the keratin subtypes that they express (Brawer et al. 1985; Purnell et al. 1987; Guinan et al. 1989; Nagle et al. 1991). Several antibodies against keratins have been produced that can be used to classify prostatic epithelial cells; early studies employed polyclonal antiserum, and more recently monoclonal antibody (mAb) for keratin has been used (Gown and Vogel 1984; Bastwick and Brawer 1987; Grignen et al. 1988; Shah et al. 1988). To date, more precise studies using a panel of single keratin or a subset thereof have not been performed adequately. The profiles of keratin expression appear to show little qualitative alterations in malignancies dependent on where the cells originated (Moll et al. 1983; Ramaekers et al. 1983). The dominant keratin phenotype in prostate carcinoma has not been well defined. Recently, the immunoreactivities of mAbs for keratins were described to be considerably preserved after methacarn fixation and paraffin embedding (Srigley et al. 1990; Tsubura et al. 1991a, c). Such tissue specimens possess great advantages in revealing precise histological architectures and distinct cellular atypia. Therefore, in this study, the keratin profiles of

Correspondence to: S. Morii

normal/hyperplastic and malignant epithelial cells in human prostates were assessed by immunohistochemical procedures using the well-characterized mAbs for keratins and actin in their methacarn-fixed, paraffin-embedded sections.

Materials and methods

Ten normal/hyperplastic prostates and 25 prostate carcinomas manifesting various grades of differentiation (including 2 lung metastases) were investigated. Prostate specimens were obtained from 35 Japanese men admitted to Kansai Medical University Hospital at the time of either needle biopsy ($n=16$), radical prostatectomy ($n=4$) or autopsy (prostate cancer; $n=5$, and normal/hyperplastic prostates; $n=10$). The autopsy materials were harvested within less than 6 h post mortem. Normal/hyperplastic prostate samples were collected from the men aged 1–72 years during autopsy of accident victims. These specimens were fixed in methacarn (60% methanol, 30% chloroform, 10% glacial acetic acid) overnight, embedded routinely in paraffin, and then cut into serial 4-μm sections. Some sections were examined routinely with pathological methods, and others were subjected to immunohistochemistry. Prostate cancers were classified according to WHO standards (Mostofi et al. 1980) and also graded according to the criteria of Gleason (1977).

The mAbs against keratins and actin that we used, their specificities, sources and dilutions in this study are indicated in Table 1. The immunoperoxidase staining technique employed has been described previously in detail (Tsubura et al. 1988). In brief, after deparaffinization, the sections were blocked with 0.3% hydrogen peroxide in ethanol for 20 min followed by incubation of 10% normal horse serum for 30 min, incubated in the primary antibody at 4° C overnight, and then treated with ABC staining kit (Vectastain ABC Kit, Vector Laboratories, Burlingame, Calif., USA). Diaminobenzidine was used for colour development, and the sections were counterstained with haematoxylin. For negative controls, a serial section was incubated in non-immune mouse serum instead of the primary antibody. In neoplastic tissue specimens of all the 25 prostate cancer cases, some normal prostate tissue was found and acted as a built-in positive control for keratin staining. In addition, the myofibroblasts and smooth muscles of the blood vessels in the stroma served as built-in positive controls for actin staining. The cancer cases examined were scored positive when at least 50% of non-necrotizing cancer cells were labelled with the antibody throughout the sections observed. In general, negative sections consisted almost entirely of unlabelled cancer cells.

Results

The staining patterns with nine anti-keratin mAbs and one anti-actin mAb in normal/hyperplastic prostates are summarized in Table 2. The expression of each keratin epitope was generally constant. No differences in the staining patterns were found among the normal/hyperplastic tissue components of various age groups. Basically, three types of staining pattern were seen: namely, basal cell-, luminal cell-, and both cell-labelled types. The mAbs of 34βE12 (keratins 5, 10 and 11), CK8.12 (keratins 13 and 16) and 312C8-1 (keratin 14) preferentially stained basal cells diffusely in the cytoplasm as a peripheral single row, while the luminal cells were unstained (Fig. 1a). All mAbs of CK5 and M20, both recognizing keratin 18, 35βH11 recognizing keratin 8, and RPN1162 defining keratin 7 did stain luminal cells but not basal cells (Fig. 1b). Such keratin staining with these mAbs did not reveal any intermediate forms between basal and luminal cells. Either all luminal cells or all basal cells within a prostate gland showed each staining, constantly though not all glands in the section showed such staining and some glands remained unstained. In ducts consisting of several layers of epithelial cells, luminal-cell-specific keratins were uniformly stained, leaving a single outermost layer unstained, indicating stratification of cells with luminal cell phenotypes. The mAbs, RPN1165 and CK4.62, directed toward epitopes of keratin 19 stained basal cells as well as luminal cells (Fig. 1c). The three above-mentioned types of staining pattern were also observed in hyperplastic prostate, but proliferating basal cells were multilayered and strongly stained with basal cell-specific mAbs. The staining patterns were

Table 2. Positive keratin reactions in parenchymal cells of methacarn-fixed, paraffin-embedded sections of 10 normal prostates

Antibody	Specificities	Normal parenchymal cells
34βE12	K. 5, 10 and 11	Basal cells
CK8.12	K. 13 and 16	Basal cells
312C8-1	K. 14	Basal cells
CK4.62	K. 19	Luminal and basal cells
RPN1165	K. 19	Luminal and basal cells
RPN1162	K. 7	Luminal cells
35βH11	K. 8	Luminal cells
CK5	K. 18	Luminal cells
M20	K. 18	Luminal cells
HHF35	Muscle-specific actin	Negative

Table 1. The monoclonal antibodies used, and their specificities, sources and dilutions

Antibody	Specificities (Moll's keratins number)	Source	Dilution
34βE12	Keratins 5, 10 and 11	Enzo Biochem., New York, N.Y., USA	1:1
CK8.12	Keratins 13 and 16	BioMakor, Rehovot, Israel	1:100
312C8-1	Keratin 14	Supplied by Dr. S.H. Dairkee, Lawrence Berleley Lab., Berkeley, Calif., USA	1:500
CK4.62	Keratin 19	BioMakor	1:500
RPN1165	Keratin 19	Amersham, Buckinghamshire, UK	1:50
RPN1162	Keratin 7	Amersham	1:50
35βH11	Keratin 8	Enzo Biochem.	1:1
CK5	Keratin 18	ICN Immunobiol., Lisle, Ill., USA	1:50
M20	Keratin 18	ICN Immunobiol.	1:40
HHF35	Muscle-specific actin	Enzo Biochem.	1:200

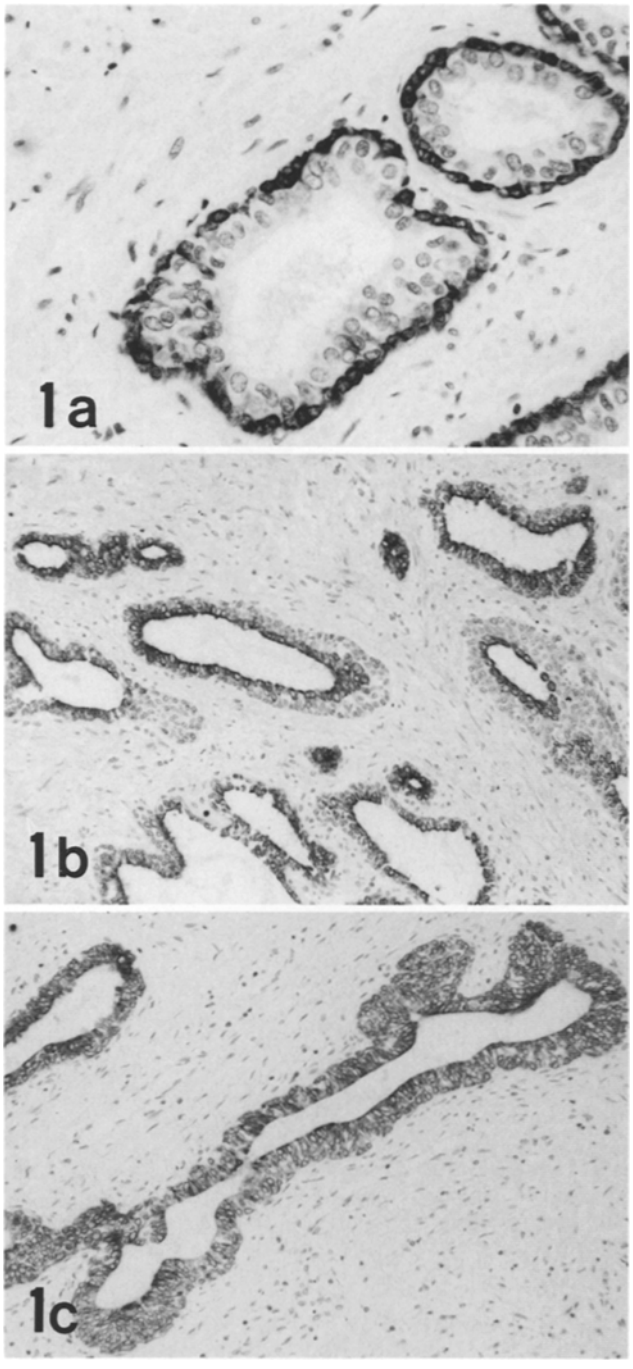

Fig. 1a–c. a Normal prostate gland stained with 312C8-1. The basal cells are homogeneously stained, while the luminal cells remain unstained. ×400. **b** Normal prostate gland stained with M20. The luminal cells are diffusely stained, but the outermost layers of basal cells are completely unstained. ×200. **c** Normal prostate gland stained with RPN1165. Both luminal and basal cells showed positive staining. ×200

not modified with the topographic locations of the whole prostate tissue. No labelling with mAbs for keratins was detected in non-epithelial cells in the prostate. Immunoreactivity with HHF35 was never seen in epithelial cells of the prostate, while it was observed in some myofibroblasts and smooth muscles of the blood vessels within the stroma.

In the 25 cases of prostatic adenocarcinomas, as indicated in Table 3, basal-cell-specific mAbs exhibited no keratin immunoreactivity in any of the cancer cells examined (Fig. 2). Occasionally, positively stained glands were present in areas of cancer tissue, but they were always identifiable as entrapped histologically normal/hyperplastic glands. In brief, the preferential loss of the basal cell layer can be detected in the carcinomas by basal-cell-specific keratin stainings. In contrast, M20 recognizing keratin 18 and labelling luminal cells stained many cancer cells (as shown in Fig. 3) in 23 of 25 prostate cancer cases (92%). Most of the carcinomas were scored positive with luminal-cell-specific mAbs, as indicated in Table 3. Regardless of the degree of tumour differentiation, the positivity was also noted in 84% with 35βH11, and 80% with CK5. Staining intensity seemed to be correlated with tumour differentiation; more differentiated adenocarcinomas stained more strongly than poorly differentiated ones, though the staining was more heterogeneous and more cell-to-cell variation in the intensity was apparent. CK4.62 and RPN1165, both recognizing keratin 19 and labelling both cells, showed positivity in 68% and 60% of the cancer cases, respectively. Positivity with RPN1162 (keratin 7) was noted in 4%. The two metastases revealed identical keratin immunoreactivity to that in the primary lesion. In the present series, all cases were invasive carcinomas and no obvious foci of intraepithelial neoplasia were seen. HHF35 did not label any cancer cells in the present observation. The most valuable finding in this study is that the loss of basal cell phenotype in well-differentiated small acinar adenocarcinoma of Gleason grade 1 or 2 seemed significant when compared with the preservation of basal cells in benign conditions mimicking carcinoma.

Discussion

The prostate is similar to the mammary, sweat and salivary glands in its histological architecture, with a lobuloductal epithelial system composed of luminal and basal cells. The present and previous reports have shown that different antibodies for keratins exhibit characteristic patterns of immunoreactivity which can differentiate between luminal and basal cells in these organs (Srigley et al. 1990; Tsubura et al. 1991b, c). However, results have indicated differences as well as similarities in the immunoreactivities to keratins among different organs. For instance, 312C8-1 is a stable marker of basal cells in the mammary, sweat and salivary gland, (Dairkee et al. 1985) and it also marks non-human basal cells (Tsubura et al. 1989, 1991a). In contrast, however, CK8.12 labelled basal cells in the prostate but luminal cells in the mammary gland (Tsubura et al. 1991c). Furthermore, in contrast to the staining seen in myoepithelial cells of the mammary and salivary glands (Tsukada et al. 1987), no actin staining has been observed in the prostate (Srigley et al. 1990; Tsubura et al. 1991a, c). Our present observations confirmed the absence of reactivity to HHF35 and support the contention that no myoepithelial cell differentiation occurs in the prostate. The same results have been obtained in rodents, with

Table 3. Positivities for keratins and muscle-specific actin in methacarn-fixed, paraffin-embedded sections of 25 prostatic carcinomas

Antibody	Specificities	No. of positive cases/ no. of cases examined	(%)
34βE12	K. 5, 10 and 11	0/25	(0%)
CK8.12	K. 13 and 16	0/25	(0%)
312C8-1	K. 14	0/25	(0%)
CK4.62	K. 19	17/25	(68%)
RPN1165	K. 19	15/25	(60%)
RPN1162	K. 7	1/25	(4%)
35βH11	K. 8	21/25	(84%)
CK5	K. 18	20/25	(80%)
M20	K. 18	23/25	(92%)
HHF35	Muscle-specific actin	0/25	(0%)

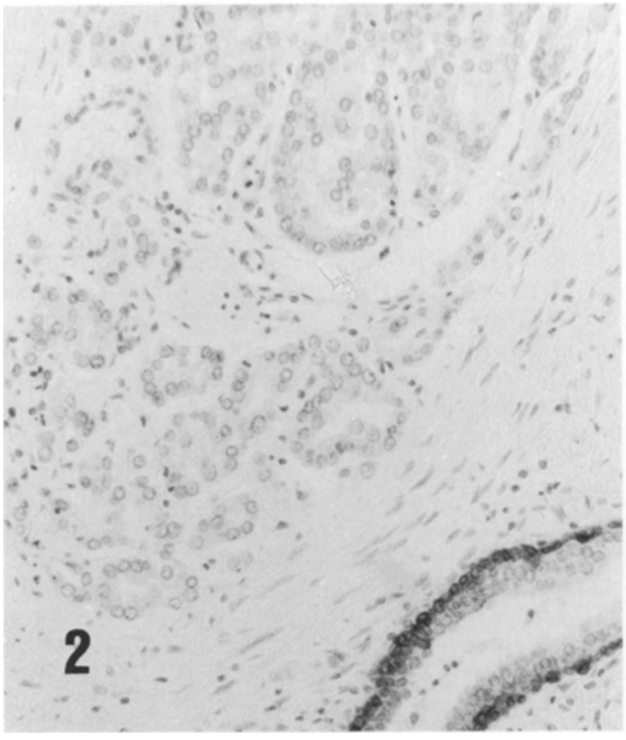

Fig. 2. Prostate adenocarcinoma stained with CK8.12. Cancer cells are completely negative, while the basal cells of remnant prostate gland are clearly positive. ×230

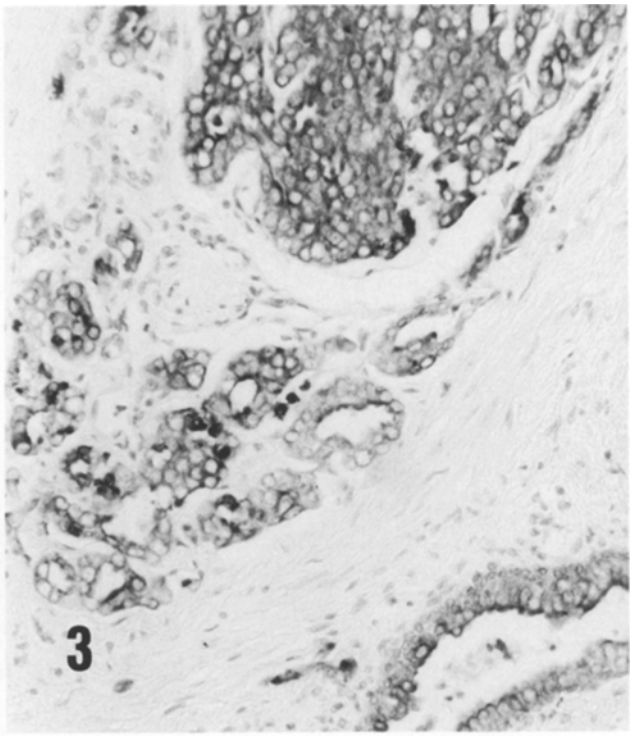

Fig. 3. Prostate adenocarcinoma stained with M20. Heterogeneous positive staining is seen in cancer cells (compare with Fig. 2), in addition to homologous staining in luminal cells of the remnant prostate gland (*right lower corner*). ×230

no such cells being seen in any of the prostatic lobules (Aumüller et al. 1991). The function of basal (not myoepithelial) cells is currently not well known: they probably belong to the proliferative compartment of the prostatic epithelium and normally divide and mature into secretory cells. However, if basal cells are stem cells, why basal cells should manifest different keratins from those of luminal cells is not clear. Our present observations demonstrated keratins 7, 8, 18 in luminal cells and keratin 19 in both basal and luminal cells. Three types of keratin staining pattern were found in epithelial cells of the prostate among various age groups. Though the central and peripheral zones differed in hormone reactivity, analysis of these zones did not reveal quantitative differences in keratin expression immunohistochemically. More attention should be given to the existence of basal cells without myoepithelial differentiation in the prostate.

The selective loss of basal cell layer is repeatedly stressed in the diagnosis of prostatic cancer (Barwick and Mardi 1983; Brawer et al. 1985; Nagle et al. 1987, 1991; Guinan et al. 1989; Hedrich and Epstein 1989; O'Malley et al. 1990). It has also been suggested that prostatic carcinomas arise from malignant clones of luminal cells (Brawer et al. 1985; Nagle et al. 1987, 1991; Sherwood et al. 1990). Our observations with respect to keratin phenotypes support a hypothesis of loss of basal cell phenotype in prostatic carcinomas regardless of the grade of differentiation. Neoplastic transformation of the breast and prostate is also similar in that the majority of breast cancers are of luminal cell origin, and only 1–13% of breast carcinomas have a basal cell phenotype (Dairkee et al. 1988; Tsubura et al. 1991; Senzaki et al. 1992). The present study indicates that the majority of adenocarcinomas (over 80%) expressed keratins 8 and 18, 60–68% expressed keratin 19, and 4% expressed keratin 7. In brief, keratins 18 and 19 persist following malignant transformation, while keratin 7 persists only

partially. The positivities for keratins 8, 18 and 19 differed as defined by their respective mAbs. Antibodies directed toward the same keratin subtype but defining different epitopes reflect differing keratin expression in carcinoma. Our findings indicated that none of the carcinomas had biological properties in common with basal cells.

The identification of basal cells is often helpful in excluding a diagnosis of carcinoma, but the identification of basal cells in routinely H & E stained sections is usually difficult. The present results show distinct patterns of keratin immunoreactivities in cancer cells which may be useful in distinguishing well-differentiated adenocarcinomas from questionable foci sometimes confused with carcinomas.

In conclusion, luminal cell phenotypes are involved in neoplastic transformation of the prostate and basal cell phenotypes do not appear to contribute to cancer cell populations. The majority of carcinomas manifested phenotypes containing keratins 8, 18 and 19 with few expressing keratin 7. Prostate cancers with different keratin compositions may exhibit differing patterns of biological behaviour. Clinical follow-up of patients with differing keratin phenotypes is necessary to settle this issue.

Acknowledgement. This work was supported in part by a Grant-in-aid for Scientific Research (No. 03670764) from the Ministry of Education, Science and Culture of Japan.

References

Achtstätter T, Moll R, Moore B, Franke WW (1985) Cytokeratin polypeptide patterns of different epithelia of the human male urogenital tract: immunofluorescence and gel electrophoretic studies. J Histochem Cytochem 33:415–426

Aumüller G, Gröschel-Stewart U, Altmannsberger M, Mannherz HG, Steinhoff M (1991) Basal cells of H-Dunning tumor are myoepithelial cells. A comparative immunohistochemical and ultrastructural study with male accessory sex glands and mammary gland. Histochemistry 95:341–349

Barwick KW, Mardi JA (1983) An immunohistochemical study of the myoepithelial cell in prostatic hyperplasia and neoplasia. Lab Invest 48:7A

Bostwick DG, Brawer MK (1987) Prostatic intra-epithelial neoplasia and early invasion in prostate cancer. Cancer 59:788–794

Brawer MK, Peehl DM, Stamey TA, Bostwick DG (1985) Keratin immunoreactivity in the benign and neoplastic human prostate. Cancer Res 45:3663–3667

Dairkee SH, Blayney C, Smith HS, Hackett AJ (1985) Monoclonal antibody that defines human myoepithelium. Proc Natl Acad Sci USA 82:7409–7413

Dairkee SH, Puett L, Hackett AJ (1988) Expression of basal and luminal epithelium-specific keratins in normal benign and malignant breast tissue. J Natl Cancer Inst 80:691–695

Gleason DF (1977) Histological grading and clinical staging of prostatic carcinoma. In: Tannenbaum M (ed) Urologic pathology, the prostate. Lea and Febiger, Philadelphia, pp 171–197

Gown AM, Vogel AM (1984) Monoclonal antibodies to human intermediate filament proteins. II. Distribution of filament proteins in normal human tissues. Am J Pathol 114:309–321

Grignon DJ, Ro JY, Ordoñez NG, Ayala AG, Cleary KR (1988) Basal cell hyperplasia, adenoid basal cell tumor, and adenoid cystic carcinoma of the prostate gland: an immunohistochemical study. Hum Pathol 19:1425–1433

Guinan P, Shaw M, Targonski P, Ray V, Rubenstein M (1989) Evaluation of cytokeratin markers to differentiate between benign and malignant prostatic tissue. J Surg Oncol 42:175–180

Hedrick L, Epstein JI (1989) Use of keratin 903 as adjunct in the diagnosis of prostate carcinoma. Am J Pathol 13:389–396

Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24

Moll R, Krepler R, Franke WW (1983) Complex cytokeratin polypeptide patterns observed in certain human carcinomas. Differentiation 23:256–269

Mostofi FK, Sesterhenn I, Sobin LH (1980) Histological typing of the prostate tumors. International histological classification of tumors, no. 22. WHO, Geneva

Nagle RB, Ahmann FR, McDaniel KM, Paquin ML, Clark VA, Celniker A (1987) Cytokeratin characterization of human prostatic carcinoma and its derived cell lines. Cancer Res 47:281–286

Nagle RB, Brawer MK, Kittelson J, Clark V (1991) Phenotypic relationships of prostatic intraepithelial neoplasia to invasive prostatic carcinoma. Am J Pathol 138:119–128

O'Mallery FP, Grignon DJ, Shum DT (1990) Useful of immunoperoxidase staining with high-molecular-weight cytokeratin in differential diagnosis of small-acinar lesions of the prostate gland. Virchows Arch [A] 417:191–196

Purnell DM, Heatfield BM, Anthony RL, Trump BF (1987) Immunohistochemistry of the cytoskeleton of human prostatic epithelium. Evidence for disturbed organization in neoplasia. Am J Pathol 126:384–395

Ramaekers FCS, Puts JJG, Moesker O, Kant A, Huysmans A, Haag D, Jap PHK, Herman CJ, Vooijs GP (1983) Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumors: an overview. Histochem J 15:691–713

Senzaki H, Tsubura A, Shoji T, Okada H, Takahashi H, Morii S (1992) Immunohistochemical detection of keratin, actin and type IV collagen in serial sections of methacarn-fixed breast cancer tissue. Acta Histochem Cytochem 25:31–38

Shah IA, Schlageter M-O, Stinnett P, Lechago J (1991) Cytokeratin immunohistochemistry as a diagnostic tool for distinguishing malignant from benign epithelial lesions of the prostate. Mod Pathol 4:220–224

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

Srigley JR, Dardick I, Warren R, Hartwick J, Klotz L (1990) Basal epithelial cells of human prostate gland are not myoepithelial cells. A comparative immunohistochemical and ultrastructural study with the human salivary gland. Am J Pathol 136:957–966

Tsubura A, Shikata N, Inui T, Morii S, Hatano T, Oikawa T, Matsuzawa A (1988) Immunohistochemical localization of myoepithelial cells and basement membrane in normal, benign and malignant human breast lesions. Virchows Arch [A] 413:133–139

Tsubura A, Inui T, Senzaki H, Morii S, Dairkee SH (1989) Immunolocalization of the human basal epithelial marker monoclonal antibody 312C8-1 in normal tissue and mammary tumours of rodents. Virchows Arch [A] 415:533–538

Tsubura A, Hatano T, Hayama S, Morii S (1991a) Immunophenotypic difference of keratin expression in normal mammary glandular cells from five different species. Acta Anat 140:287–293

Tsubura A, Okada H, Sasaki M, Dairkee SH, Morii S (1991b) Immunohistochemical demonstration of keratin 8 and 14 in benign tumors of the skin appendage. Virchows Arch [A] 418:503–507

Tsubura A, Okada H, Senzaki H, Hatano T, Morii S (1991c) Keratin expression in the normal breast and in breast carcinoma. Histopathology 18:517–522

Tsukada T, McNutt MA, Ross R, Gown AM (1987) HHF35, a muscle actin-specific monoclonal antibody: II. Reactivity in normal, reactive and neoplastic human tissues. Am J Pathol 127:389–402