

Invited Review

Cytokeratin expression patterns in normal and malignant urothelium: a review of the biological and diagnostic implications

J. Southgate¹, P. Harnden² and L.K. Trejdosiewicz¹

¹ICRF Cancer Medicine Research Unit, Research School of Medicine and

²Department of Pathology, St James's University Hospital, Leeds, UK

Summary. The cytokeratins are the intermediate filament proteins characteristic of epithelial cells. In human cells, some 20 different cytokeratin isotypes have been identified. Epithelial cells express between two and ten cytokeratin isotypes and the consequent profile which reflects both epithelial type and differentiation status may be useful in tumour diagnosis. The transitional epithelium or urothelium of the urinary tract shows alterations in the expression and configuration of cytokeratin isotypes related to stratification and differentiation. In transitional cell carcinoma, changes in cytokeratin profile may provide information of potential diagnostic and prognostic significance. The intensification of immunolabelling with some CK8 and CK18 antibodies may underly an active role in tumour invasion and foci of CK17-positive cells may represent proliferating populations. Loss of CK13 is a marker of grade and stage and *de novo* expression of CK14 is indicative of squamous differentiation and an unfavourable prognosis. However, perhaps the most important recent finding is the demonstration that a normal CK20 expression pattern is predictive of tumour non-recurrence and can be used to make an objective differential diagnosis between transitional cell papilloma and carcinoma. This review will consider cytokeratin expression in urothelium and discuss the application of cytokeratin typing to the diagnosis and prognosis of patients with TCC.

Key words: Urothelium, Bladder cancer, Cytokeratins, Transitional cell carcinoma

Introduction

The 1980s saw an explosion of interest in the cytokeratins, centred on research into their basic

biological function, but which found practical applications in histopathology. In the past decade, progress has been steady, rather than spectacular. Nevertheless, new findings have emerged and it is now timely to re-examine the cytokeratins in the context of normal and malignant urothelium.

Cytokeratin isotypes

The cytokeratins are a complex group of water-insoluble polypeptides, ranging in size from 40,000-68,000 Mr. Cytokeratins form the 10nm intermediate filament (IF) cytoskeleton in the majority of cells of epithelial and mesothelial derivation. In human cells, 20 cytokeratin isotypes have been identified. The different isotypes have been ranked from largest (CK1: 68,000 Mr) through to smallest (CK19: 40,000 Mr), with the most recently identified 46,000 Mr cytokeratin designated CK20 (Moll et al., 1990). With the exception of kidney podocytes and lens epithelium, each type of epithelial cell expresses a characteristic combination of two to ten cytokeratin isotypes (Moll et al., 1982). Thus, normal epithelial cells *in vivo* or *in vitro* can be identified with respect to their cytokeratin isotype profile.

The characteristic cytokeratin profiles of the different normal epithelial cell types tend to be retained following malignant transformation and this feature may be exploited in tumour diagnosis (reviewed Miettinen, 1993a; Lane and Alexander, 1990). However, some caution is needed, as the cytokeratin profiles of epithelial cells are fundamentally a reflection of functional differentiation, rather than tissue of origin. Thus, meta-plastic cells express cytokeratin profiles characteristic of their morphology, and dedifferentiation can result in convergence of cytokeratin expression profiles.

Cytokeratin isotypes are classified as members of either the small, acidic (Type I) or larger, basic (Type II) cytokeratin subfamilies. It is thought that the two cytokeratin subfamilies arose through gene duplication and subsequent divergence of the CK8 and CK18

Offprint requests to: Dr. J. Southgate, ICRF Cancer Medicine Research Unit, St. James's University Hospital, Leeds LS9 7TF, UK. Fax: +44(113)-242-9886. e-mail: j.southgate@leeds.ac.uk

ancestral genes (Blumenberg, 1988). The type II cytokeratin genes are clustered on chromosome 12 with a cluster of type I cytokeratin genes on chromosome 17. The exception is CK18 which is located close to CK8 within the type II gene cluster (Waseem et al., 1990). Two commonly used monoclonal antibodies, AE1 and AE3, originally described by Woodcock-Mitchell et al. (1982), can distinguish the two types antigenically: AE1 reacts with most Type I and AE3 reacts with all Type II cytokeratins.

Tonofilament organisation

The cytokeratins have a central highly-conserved α -helical domain, flanked by non-helical head and tail domains. They are obligate heteropolymers and assemble to form coiled-coil dimers of Type I and Type II polypeptides which further associate in antiparallel fashion to form the basic tetrameric subunit which underpins the structural organisation of cytokeratin filaments (reviewed Coulombe, 1993). In the cytoplasm, cytokeratin filaments associate into thick fibrils or tonofilaments which are anchored at desmosomes, resulting in an ordered array of filaments throughout the epithelium. There is some pairing of particular Type I and Type II polypeptides with frequent co-expression and co-localisation of the cytokeratin isotype pairs in tissues. CK8 and CK18 are the most closely integrated pair in terms of coexpression; this probably arises through common transcriptional regulation of the genes (reviewed by Oshima et al., 1996).

Analysis of cytokeratin expression in cells and tissues

The original classifications of tissue cytokeratin expression profiles were performed by 2D gel electrophoresis. However, it was the development of monoclonal antibodies specific to single cytokeratin isotypes which furthered the study of cytokeratin expression within tissues, particularly in terms of differentiation-associated expression patterns. A number of cytokeratin isotype-specific monoclonal antibodies are now available which are reactive on routine paraffin wax-embedded tissues, although antigen retrieval techniques may be required to restore immunoreactivity following tissue processing (reviewed by Lane and Alexander, 1990; Miettinen, 1993a,b; Hazelbag et al., 1995).

In interpreting antibody labelling patterns, several factors need to be considered:

Cross reactivity

Because of homology in IF subunit structure, monoclonal antibodies raised against one cytokeratin isotype may show cross-reactivity against other cytokeratin isotypes or even other IF types. In some cases, the cross-reactivity may be of a lower affinity and only detectable by immunochemistry when the primary

antigen is not available. Thus, it is important that antibodies have been fully characterised against a broad range of tissue types, using a full range of techniques. An example is the panel of anti-CK20 antibodies described by Moll et al. (1992). By immunoblotting, all eight monoclonal antibodies showed specificity to CK20; however, using immunolabelling techniques, antibody CK20.8 reacted with CK20-negative cells and tissues unless processed by formalin-fixation.

Epitope masking

Due to the tertiary structure of cytokeratin tonofilaments, particular epitopes may be masked or cryptic and hence sterically unavailable for antibody binding. In other cases, epitopes may only become unmasked and hence available for antibody binding in limited circumstances. An example includes the anti-CK19 antibody, LAS86, which reacted with CK19 of all rodent tissues on immunoblots, but which recognised an epitope which was only unmasked *in situ* during terminal urothelial cytodifferentiation (Trejdosiewicz et al., 1988).

Spurious reactivity

The high affinity of Type I and II cytokeratin isotypes for each other (below) can result in recombination of free cytokeratin species on immunoblots and apparent cross-reactivity of an antibody between cytokeratin species. Some cytokeratin polypeptides may show degradation products on immunoblots which can also make interpretation difficult.

Comparison of immunolabelling and immunoblotting data or the use of several antibody clones to different epitopes of the same cytokeratin isotype should help resolve discrepancies. However, in some cases, it may be difficult to reconcile whether differentiation-associated changes in cytokeratin immunolocalisation are due to cytokeratin expression changes or to conformational changes. This is also the case where cytokeratin antibodies show one reactivity pattern on cryosections, but may show apparently altered expression patterns following antigen retrieval of paraffin wax-embedded tissues due to the unmasking of cryptic epitopes.

Cytokeratins as differentiation markers

The cytokeratins may be regarded as differentiation markers insofar as: a) cytokeratin isotypes are expressed by almost all cells committed to an epithelial cell lineage; b) distinct cytokeratin expression profiles are associated with particular epithelial differentiation pathways or "programs" (below); and c) expression of particular cytokeratin isotypes may be associated with a specific maturation stage.

These different aspects, which may be modulated according to the differentiation and/or pathological

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status of a tissue, need to be taken into consideration when interpreting cytokeratin expression profiles. Nevertheless, there are fundamental "rules" underlying cytokeratin isotype expression which relate to the development, differentiation and proliferation of the tissue. Understanding these rules has enabled the cytokeratins to be used as subtle discriminators of epithelial cell type and differentiation state.

The «rules» of cytokeratin expression

The cytokeratins are the first IF type to appear during embryogenesis and all cells at some stage of fetal development are cytokeratin-positive. The first cytokeratin isotypes to be expressed are the CK8+CK18 pair. During embryological epithelial-mesenchymal transformation, cytokeratin expression is lost by certain lineages of cells, which either remain cytokeratin-negative connective tissue derivatives, or later re-express cytokeratins as secondary epithelia. In the post-fetal organism, cytokeratins are restricted in expression to epithelial and mesothelial cells, where they form the structural basis for tonofilaments. However, the developmental pattern of cytokeratin expression probably underlies the ectopic expression of cytokeratins, especially CK8+CK18, by some mesenchymal cell types and tumours (reviewed Miettinen, 1993a).

Post-fetal epithelial tissues are commonly classified according to their structural organisation as non-stratified, pseudostratified and stratified, with further distinction according to cell morphology. By investigating cytokeratin expression as a correlate of epithelial tissue development, morphology and organisation, Cooper et al. (1985) proposed a classification based on three major "epithelial differentiation programs". According to this scheme, "simple" epithelia included all non-stratified epithelial types, whereas the "stratified squamous" category included both cornifying and non-cornifying stratified squamous epithelia. All other stratified and pseudo-stratified epithelia were categorised as "complex".

Expression of the CK8+CK18 pair is retained by all non-stratified ("simple"), ductal and pseudostratified epithelia. CK8+CK18 may be expressed alone by hepatocytes and pancreatic acinar cells, with CK19 and CK20 in gastrointestinal tract epithelia, or with CK19 and CK7 in most other simple epithelia (e.g. pancreatic duct, mesothelium and lung alveoli). CK8+CK18 may also be expressed in some complex epithelia in conjunction with isotypes from both stratified and non-stratified epithelial programs (discussed by Cooper et al., 1985).

The embryological development of stratified epithelia from the "simple" epithelia of the embryonic ectoderm and endoderm is accompanied by loss of CK8+CK18 and *de novo* expression of isotypes associated with stratified epithelia. The so-called "stratification keratins" comprise CK10 to CK17 (Type I) and CK1 to CK6 (Type II). In stratified squamous

epithelia, CK5+CK14/15 are expressed basally, with expression of other cytokeratin isotypes occurring suprabasally and defined by the differentiation type (Cooper et al., 1985). Epidermal-type differentiation is associated with specific expression of cornification isotypes CK1/2+CK10 and corneal-type differentiation is accompanied by specific expression of the CK3+CK12 pair. Oesophageal-type differentiation (as adopted by stratified squamous epithelia of internal organs, e.g. oesophagus and tongue) is accompanied by expression of the CK4+CK13 isotypes. Changes in differentiation type, as seen in squamous metaplasia, can result in expression of the appropriate cytokeratin isotypes (Cooper et al., 1985). In addition, cornifying stratified squamous cells express the CK6+CK16 pair in all normal and pathological hyperproliferative conditions. These observations imply a fundamental role for different cytokeratin polypeptide types in differentiated epithelial tissues.

Cytokeratin expression in urothelium

The urinary bladder, ureter and renal pelvis are lined by transitional epithelium (urothelium) which is specialised to function as a barrier to urine and to accommodate changes in intraluminal volume. Urothelium appears as a multilayered epithelium, although there has been some controversy as to whether it is a true stratified or pseudostratified epithelium (Jost et al., 1989). In essence, three cell zones are apparent: 1) a basal cell layer composed of cells in contact with and orientated perpendicularly to the plane of the basement membrane; 2) the intermediate cell zone composed of a variable number of cell layers depending on the contracted state of the tissue; and 3) a luminal or superficial cell layer composed of late intermediate cells and large, frequently binucleated, "umbrella" cells which are orientated perpendicular to the basement membrane and with their apical edge facing the lumen. The umbrella cells are characterised by a zonula occludens and by the presence of specialised plaques of asymmetric unit membrane (AUM) in the apical membrane and within intracellular fusiform vesicles.

Several groups have studied human urothelial cytokeratins by 2D gel electrophoresis (Moll et al., 1982, 1988; Wu et al., 1982; Achstatter et al., 1985; Rheinwald and O'Connell, 1985). The consensus findings are that normal adult urothelial cells express cytokeratin isotypes characteristic of "simple" epithelia (CK7, CK8, CK18, CK19 and CK20), as well as isotypes associated with stratified epithelia, predominantly CK13. Some of these studies also detected small amounts of CK5, CK4 and CK17 in normal urothelium (Moll et al., 1982, 1988; Achstatter et al., 1985).

With the advent of monoclonal antibodies against specific cytokeratin isotypes, a clearer pattern has emerged (Moll et al., 1988; Schaafsma et al., 1989; Booth et al., 1997). The consensus for normal adult urothelium is that CK7, CK8, CK18 and CK19 are

expressed throughout all urothelial cell layers, CK17 and CK5 are basally expressed, CK13 is present in all but the superficial cell layer and CK20 is associated with umbrella cells. We have also observed a minority subset of CK20-positive cells in the intermediate cell layer of some normal specimens (Harnden et al., 1996). Several studies have used antibody 6B10 to study CK4 expression in normal urothelium. While it is evident that occasional urothelial cells express CK4, there is no consensus as to which urothelial compartment these cells belong (van Muijen et al., 1986; Moll et al., 1988; Schaafsma et al., 1989).

Some regional variations in expression of CK4, CK7, and CK13 have been noted within the urinary tract (Schaafsma et al., 1989). For example, CK7 expression was homogeneous in the renal pelvis and ureter, but heterogeneous within the urinary bladder, including the trigone. It was suggested that such differences may reflect the transition of urothelium into morphologically distinct epithelial types (Schaafsma et al., 1989).

Cytokeratin expression in urothelial neoplasia

Neoplastic transformation of urothelial cells gives rise to transitional cell carcinoma (TCC), the commonest form of bladder cancer in Western societies. TCC has a complex natural history with a non-linear progression pathway in which muscle-invasive disease may develop from carcinoma *in situ* (high risk) or from superficial/papillary disease (lower risk), but with convergence of the molecular genetic pathways (Spruck et al., 1994; Knowles, 1995; Reznikoff et al., 1996). Many patients present with superficial tumours which are either non-invasive (pTa) or invade the lamina propria only (pT1). Whilst recurrence is common (50-70%), the disease can usually be controlled by local treatment. Nevertheless, 10-15% of patients with superficial disease eventually progress to muscle-invasive and metastatic disease (Kroft and Oyasu, 1994). An increase in grade, characterised by a loss of differentiation, and the presence of dysplasia are both determinants of poor prognosis (Harnden and Parkinson, 1996). In addition, many TCC show changes associated with squamous differentiation, which has been associated with poorer prognosis (Tannenbaum et al., 1983).

Changes in keratin expression associated with urothelial neoplasia have formed the focus of a number of studies (Achstatter et al., 1985; Cintonino et al., 1988; Moll et al., 1988; Ramaekers et al., 1988; Schaafsma et al., 1991). CK8/CK18 (see below) and CK7/CK19 appear to be retained by all TCCs, whereas expression patterns of the other cytokeratins can be altered. It has been suggested that urothelial neoplasms show a wide spectrum of cytokeratin expression changes which correlate with tumour type, grade of malignancy and degree of squamous differentiation (Moll et al., 1988). These changes are discussed below for individual cytokeratin isotypes.

CK8 and CK18

Interpretation of CK8 and CK18 expression patterns is complicated by the differences in immunolocalisation patterns found with different antibodies. There appear to be two subsets of antibodies, one of which detects expression throughout the urothelium (e.g. CK8 antibody M20 and CK18 antibodies RCK 106 and CK18-2) and the other, which shows a restricted reactivity with superficial cells (e.g. CK8 antibody LE41 and CK18 antibodies 2C8 and RGE53) (Achstatter et al., 1985; Cintonino et al., 1988; Ramaekers et al., 1988; Schaafsma et al., 1990).

Using homogeneously-labelling antibodies, CK8 and CK18 have been detected uniformly in TCC, independent of grade or stage (Schaafsma et al., 1990). The superficial-reacting subset of antibodies showed a normal superficial immunoreactivity in non-invasive regions of low grade TCC whereas the CK18 antibodies showed a more extensive immunoreactivity on G3 TCC (Ramaekers et al., 1988; Schaafsma et al., 1990). Interestingly, there was an increased immunoreactivity noted with the superficial-reactive antibodies LE41 and 2C8 in invasive regions of TCC, particularly in tumour cells bordering the stroma (Schaafsma et al., 1990).

The differential labelling with different CK8 and CK18 antibodies may reflect antigenic epitope masking phenomena, which can arise as a consequence of conformational changes of tonofilament organisation, or may reflect structural processing or other post-translational modifications of the polypeptides, such as phosphorylation (Ku et al., 1996). It has been hypothesised that there might be a parallel in the organisation of the cytoskeleton of superficial urothelial cells and invasive TCC cells: umbrella cells would require a flexible cytoskeleton for surface area change during bladder accommodation whereas cytoskeletal flexibility would facilitate stromal invasion in malignant cells (Schaafsma et al., 1990).

CK13

Two studies have shown that CK13 expression is reduced or lost in TCC in a grade- and stage-dependent manner (Moll et al., 1988; Schaafsma et al., 1990). In both studies, the normal pattern of CK13 expression was retained in most G1 and G2 tumours, with a reduction in positivity restricted to focal areas of basal and parabasal cells in superficial G3 tumours and loss from muscle-invasive areas of G3 invasive tumours.

CK14

Although CK14 is not expressed in normal urothelium (Achstatter et al., 1985; Moll et al., 1988; Schaafsma et al., 1990), a number of studies have demonstrated CK14 expression in TCC (Moll et al., 1988; Schaafsma et al., 1990). Using a CK14-specific monoclonal antibody, LL002, Schaafsma et al. (1990)

demonstrated an association between CK14 expression and TCC progression. However, no clear relationship was found relating expression of CK14 to the clinicopathological data. In a more recent study, we have shown that CK14 expression was associated with squamous differentiation of TCC and suggested that it might precede development of an overtly squamous phenotype (Harnden and Southgate, 1997).

CK17

Only one CK17-specific antibody has been described. This antibody, E3, shows a CK17-specific immunoreactivity by immunoblotting and immunohistochemistry on cryopreserved and paraffin wax-embedded tissues (Trojanovsky et al., 1989) and has been used for all studies. CK17 is expressed by the basal cells of most specimens of normal urothelium (Trojanovsky et al., 1989; Schaafsma et al., 1991; Guelstein et al., 1993). In the latter study, CK17 expression was examined in a panel of primary TCC consisting of 14 <pT2 tumours graded 1, 1/2, or 2 and 14 pT3 tumours graded 2/3 or 3. In G1 and G1/G2 tumours, two CK17 localisation patterns were observed: a) confined to basal cells or b) labelling of basal cells with decreasing reactivity in suprabasal cell layers. The two patterns were not necessarily exclusive and heterogeneity was observed within some tumours. In the majority of G2 and G2/G3 tumours, CK17 was uniformly and homogeneously expressed throughout all layers. The expression of CK17 was reduced in anaplastic G3 TCC to occasional foci of positive cells; these foci were basally-located in TCC with a squamous component (Guelstein et al., 1993). The association of CK17 with basal cells, the increased expression in TCC and the induction of CK17 in squamous hyperplasia led Guelstein et al. (1993) to suggest that CK17 may mark a proliferatively-active basal cell population.

CK20

CK20 shows a highly-restricted tissue distribution, being confined to urothelium, gastrointestinal epithelium and Merkel cells of the epidermis (Moll et al., 1990). In normal urothelium, expression of CK20 is restricted to the superficial umbrella cells and to very occasional intermediate cells (Moll et al., 1990; Harnden et al., 1996). Moll studied a series of non-staged TCC including 24 low and high grade primary tumours, 21 low and high grade metastases and seven (5 primary and 2 metastatic) showing squamous differentiation. CK20 expression was retained by the majority of pure TCC, retaining the normal superficial localisation pattern in some well-differentiated papillary tumours or showing a heterogeneous or uniformly-positive reaction throughout all cell layers. Squamous differentiation resulted in a reduction of CK20 expression (Moll et al., 1992).

We have examined CK20 expression in a retrospective series of 29 grade 1 and 24 grade 2 non-

invasive superficial (pTa) TCC. CK20 expression was retained by 65.5% of tumours and either showed a predominantly superficial localisation or was expressed throughout all urothelial cell layers. The immunolocalisation pattern was predictive of tumour recurrence and retention of a normal CK20 labelling pattern was invariably associated with tumour non-recurrence over a 5 year period (Harnden et al., 1995). There was some indication that an abnormal CK20 labelling pattern in flat mucosa adjacent to the tumour was associated with dysplastic change and might be an objective marker of dysplasia, which was confirmed in a subsequent study (Harnden et al., 1996). A recent prospective study of patients presenting with superficial non-invasive TCC has confirmed and reinforced the significance of CK20 as a prognostic marker (Harnden et al., 1999).

The role of the cytokeratins

The close correlation between cytokeratin isotype expression patterns and epithelial differentiation programs suggests that cytokeratins are crucial to epithelial tissue structure and/or function. However, it has been difficult to establish what the precise contributions of particular individual or combinations of cytokeratin isotypes are to epithelial cell phenotype and behaviour. A direct relationship between cytokeratins and pathogenesis has been shown in a number of congenital skin diseases, in which explicit disease phenotypes have been related directly to mutations in specific keratin genes (reviewed Corden and McLean, 1996). No such association has been shown for any bladder disease, although a role for urothelial cytokeratins is perhaps implied in urinary bladder voiding and accommodation. This may be due to functional redundancy between the different urothelial cytokeratin isotypes.

Although changes in cytokeratin expression or immunolocalisation have been associated with disease conditions and hence have found application as diagnostic and prognostic markers, there is limited evidence that changes in cytokeratin expression/conformation are directly involved in disease processes themselves. Thus, the changes in CK20 immunolocalisation which indicate a higher risk of TCC recurrence (Harnden et al., 1995, 1999), probably reflect a secondary change related to dysregulation of the urothelial differentiation programme, rather than indicating that CK20 is directly involved. Nevertheless, cytokeratins can be early and sensitive disease markers, as suggested by the *de novo* expression of cytokeratin isotypes associated with squamous differentiation which may precede any overt change in histology (Gijbels et al., 1992; Harnden and Southgate, 1997).

There are several pieces of circumstantial evidence to suggest that CK8 and CK18 may have a role in tumour cell invasion of the stroma. There is the observation that in TCC, the intensity of CK8/CK18 immunolabelling increases at the leading edge of stromal

invasion (Schaafsma et al., 1990). In an *in vitro* model of human TCC cell invasion of bladder stroma, we have shown that the highly invasive EJ cell line showed extracellular deposition of CK8 and CK18 (Booth et al., 1997). Both the altered immunoreactivity and the secretion of cytokeratins suggest changes in cytokeratin configuration and solubility, although this has yet to be established. Nevertheless, CK8 expressed on the external surface of carcinoma cells can act as a plasminogen-binding factor to promote local activation of the plasminogen cascade (Hembrough et al., 1995, 1996a,b). Other direct evidence comes from transfection studies in which a dominant negative mutant of CK18 was found to decrease the invasive ability of a CK8/CK18 positive cell line, whereas transfection of CK8 and CK18 into a non-epithelial cell line led to an increase in invasive ability (Hendrix et al., 1996). Thus, it would appear that CK8/CK18 may play a critical role in tumour cell invasion, although the exact mechanisms are as yet unidentified.

Applications of cytokeratins in bladder cancer diagnosis

As discussed above, cytokeratins have found considerable application in histopathology as epithelial type markers for identifying the tissue origin of primary and secondary tumours. However, further applications of cytokeratins have been described. The presence of cytokeratin mRNA transcripts has been used to detect the presence of circulating, potentially metastatic carcinoma cells in peripheral blood (Burchill et al., 1995). Whereas detection of CK8 and CK19 transcripts is confounded by the presence of multiple pseudogenes and "leaky" transcription by non-epithelial cells, expression of CK20 mRNA may be useful in detecting carcinoma cells of colonic or urothelial derivation (Burchill et al., 1995).

A study by Klein and colleagues (1998) used a reverse transcriptase-polymerase chain reaction (RT-PCR) technique to detect CK20 expression in exfoliated cells of the urine. Although it was suggested that positivity could be used as a biomarker of carcinoma or other premalignant change, this interpretation has been since questioned (Southgate et al., 1998), as normal superficial cells are known to express CK20 (see above).

Cytokeratins have been used extensively as serological tumour markers in the form of tissue polypeptide antigen (TPA), tissue polypeptide-specific antigen (TPS) and the more recently described CYFRA 21-1 assay, which is considered superior in terms of sensitivity (Schambeck et al., 1997). These markers, which consist of partially-degraded complexes of CK8, CK18 and CK19, are thought to be released from necrotic areas of carcinomas into the serum and in the case of bladder cancer, into the urine. The possibility that cytokeratins, released from tumour cells as part of an active invasion mechanism (above), may also contribute to the presence of circulating cytokeratin

fragments has not been considered. The assays based on immunodetection of the cytokeratin fragments have been proposed to have a place in the routine diagnosis of bladder cancer (Correale et al., 1994; Pariente et al., 1997) and as screening tools for the monitoring of tumour relapse, progression or recurrence (Carbin et al., 1989; Dittadi et al., 1996; Senga et al., 1996; Stieber et al., 1996; Morita et al., 1997).

Summary and conclusions

Normal urothelial cells express characteristic patterns of cytokeratins according to cellular positioning within the stratified layer and relating to differentiated phenotype. Changes in cytokeratin profile in TCC can provide valuable additional information of diagnostic and prognostic significance. An intensification of immunolabelling with some CK8 and CK18 antibodies may be indicative of an active invasive process, although the precise biological mechanism has yet to be defined. Foci of CK17-positive cells may represent proliferating populations and loss of CK13 is associated with increasing grade and stage. *De novo* expression of CK14 may be an early indicator of squamous differentiation and an unfavourable prognosis. However, perhaps the most important recent finding is the demonstration that a normal CK20 expression pattern is predictive of tumour non-recurrence and can be used to distinguish between benign transitional cell papilloma and TCC.

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