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Identification of the conserved, conformation-dependent cytokeratin epitope recognized by monoclonal antibody (lu-5)

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Summary. The epitope recognized by the murine monoclonal antibody (mAB lu-5) recently described as a formaldehyde-resistant, "pan-epithelial marker" of great value in tumour diagnosis is located on the surface of cytokeratin filaments. It has been preserved during vertebrate evolution from amphibia to man. As this epitope is not reactive after SDS-polyacrylamide gel electrophoresis (SDS-PAGE), the epitope-bearing protein has been identified by a dot-blot antibody binding assay, using purified proteins in which the epitope is reconstituted. We show that the epitope is present in most cytokeratin polypeptides of both the acidic (type I) and basic (type II) subfamily but does not occur in other cytoskeletal proteins. The location of this widespread epitope is discussed with respect to homologies of amino acid sequences of cytokeratins and their conformations.

Key words: Cytokeratin – Intermediate Filament Proteins – Conformation-dependent epitopes – Immunocytochemistry – Tumor diagnosis

Introduction

Epithelial cells are characterized by the presence of intermediate-size filaments (IFs) formed by cytokeratins, i.e. a large multigene family of proteins related to the α-keratins of epidermis and its appendages (Franke et al. 1978a, b; 1979a; 1982; Sun and Green 1978; Sun et al. 1979; Moll et al. 1982). Since the expression of cytokeratins is maintained in tumours of epithelial origin, including carcinomas, both in situ and in cell culture, the use of cytokeratin antibodies for distinguishing between cells and tumours of epithelial origins from those of non-epithelial origin has been proposed (Franke et al. 1978a, b; 1979b). Today, the use of cytokeratin antibodies for this purpose is now well established in histology and pathology (Bannasch et al. 1980; Battifora et al. 1980; Schlegel et al. 1980; Gabbiani et al. 1981; Moll et al. 1982; for reviews see Osborn and Weber 1983; Ramaekers et al. 1983b; Miettinen et al. 1984; Spagnolo et al. 1985; Corson 1986).

In this context, however, it is important to consider that different combinations of the cytokeratin polypeptide family are expressed in different epithelia and epithelium-derived tumours (Franke et al. 1981b, 1982; Fuchs and Green 1981). For example, in human tissues a total of 19 epithelial cytokeratin and 8 hair (i.e. trichocyte)-specific α-keratin polypeptides have been identified (Moll et al. 1982; Cooper et al. 1985; Heid et al. 1986). Consequently, different groups of epithelia and carcinomas can be distinguished on the basis of their specific cytokeratin polypeptide patterns which they express (Franke et al. 1981b; Moll et al. 1982; Tseng et al. 1982). Moreover, in contrast to other IFs, cytokeratin filaments represent obligatory heteropolymers formed by tetramers containing two chains of each members of an acidic (type I) and a basic (type II) subfamily (Fuchs et al. 1981, 1985; Schiller et al. 1982; Tseng et al. 1982; Crewther et al. 1983; Franke et al. 1983; Woods 1983; Quinlan et al. 1984; Sun et al. 1984; Hatzfeld and Franke 1985; for review see Steinert et al. 1985b).

These principles of cytokeratin expression and IF formation have to be kept in mind in using cytokeratin antibodies for the immunohistochemical detection and classification of epithelial and epithelium-derived cells. Obviously, cytokeratin antibodies which react only with one of the various

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cytokeratin polypeptides will be of value in the specific classification of a positive cell, but cases of negative reaction will not allow an epithelial or carcinomatous cell to be excluded (e.g., Debus et al. 1982, 1984; Lane 1982; Ramaekers et al. 1983a; Gown and Vogel 1984; Roop et al. 1984; Bartek et al. 1985; Lane et al. 1985; Knapp et al. 1986; Van Muijen et al. 1986). The same argument holds for antibodies which react with epitopes common only to a certain group of cytokeratin polypeptides (Tseng et al. 1982; Holthöfer et al. 1983; Makin et al. 1984). In contrast, antisera or monoclonal antibodies which bind to at least one cytokeratin epitope present in all of the diverse cytokeratin combinations will permit a definitive conclusion based on the presence or absence of cytokeratins. Only few monoclonal cytokeratin antibodies with such a broad reactivity have been described so far. Antibody K_G 8.13 reacts with practically all polypeptides of the basic (type II) subfamily as well as with the acidic (type I) cytokeratin no. 18 and stains diverse human and bovine epithelia and carcinomas (Gigi et al. 1982) but does not react with cytokeratins of many other species. Moreover, its epitope is inactivated by formaldehyde fixation. The cytokeratin reactivity pattern of another apparently broad reacting cytokeratin antibody, termed KL1, has not been sufficiently characterized but from the data published it is clear that it does not detect all kinds of cytokeratin IFs, e.g. those of the basal cell layers of certain stratified epithelia (Viac et al. 1983).

Recently, the epitope of a monoclonal antibody, termed lu-5, has been shown to be present in all human epithelia and carcinomas and to be resistant to formaldehyde fixation (von Overbeck et al. 1985). Unfortunately, however, in immunoblot analyses this antibody does not reproducibly bind to denatured polypeptides separated on SDS-PAGE, so that its reactivity with individual cytokeratins could not be determined. Because of the potential of this antibody in histology and pathology we have characterized it in greater detail and have determined the cytokeratin nature of its epitope by an immuno-dot blot assay, using chemically purified cytokeratin polypeptides refolded in vitro.

Material and methods

Conditions for the growth of cultured human cell lines PLC (from hepatocellular carcinoma), MCF-7 (from breast carcinoma), A-431 (from epidermoid carcinoma of the vulva) and HeLa (from cervical adenocarcinoma), "SV80" (SV40-transformed fibroblasts) and U333 CG/343 MG (from glioma) have been described (Franke et al. 1979c; Gigi et al. 1982; Moll et al. 1982; 1986; Quinlan et al. 1983). Sources and growth conditions for diverse bovine cell lines (for refs. see Schmid et al. 1983), rat hepatoma MH_1C_1 cells (Franke et al. 1981a), rat kangaroo line PtK_2 (from kidney epithelium; cf. Franke et al. 1978a, b), and the *Xenopus laevis* kidney epithelial cell line A_6 (XLKE; Franke et al. 1979c) have also been given elsewhere. For immunofluorescence microscopy cells were grown on cover slips.

Small samples from various tissues of human, cow, rat, chicken and amphibia (*Xenopus laevis, Pleurodeles waltli*) were snapfrozen in isopentane precooled in liquid nitrogen immediately upon dissection, and approximately 5 µm thick sections were prepared (Franke et al. 1979a, 1980, 1981b; Schmid et al. 1979; Benavente et al. 1985).

The production and immunological characterization of monoclonal antibody lu-5 (IgG1) has been previously described in detail (von Overbeck et al. 1985). For comparison, other murine monoclonal antibodies (PKK1; from Labsystems Oy, Helsinki, Finland; cf. Holthöfer et al. 1983; K_G 8.13, Gigi et al. 1982; CK-2, Debus et al. 1982, 1984; available from Boehringer, Mannheim, FRG) and guinea pig sera against cytokeratins (cf. Franke et al. 1978a, b; 1979a) as well as antibodies to desmoplakins (Cowin et al. 1985) were used. Secondary antibodies used for indirect immunofluorescence microscopy were from Medac or Dianova (Hamburg, FRG). Secondary antibodies used for dot blot assays were goat antibodies against total murine immunoglobulins coupled to peroxidase (for details see Hazan et al. 1986).

Vimentin was purified from bovine lens tissue and human fibroblast cell cultures as described (Geisler and Weber 1980, 1981; Söllner et al. 1985; Quinlan et al. 1986). Smooth muscle desmin from chicken gizzard or human myometrium were obtained using a similar procedure (cf. Quinlan et al. 1986). Alternatively, purified IF proteins, including glial filament protein and neurofilament polypeptide NF-L obtained by combined ion exchange chromatography and high performance liquid chromatography (HPLC), were obtained from Progen Biotechnics (Heidelberg, FRG). Human cytokeratin polypeptides 1, 4–6, 5 and 6, 8, 9, 14, 18 and 19 as well as rat liver cytokeratins A (equivalent to human cytokeratin no. 8; cf. Franke et al. 1981) and D (equivalent to human cytokeratin no. 18) were purified by combinations of DEAE ion exchange chromatography and reverse phase HPLC as described (Hatzfeld and Franke 1985; Achtstätter et al. 1986; Quinlan et al. 1986). Alternatively, human cytokeratins nos. 8, 18 and 19 (from MCF-7 cells) as well as rat and mouse cytokeratins A (8) and D (18) were prepared by DEAE chromatography alone (cf. Achtstätter et al. 1986; Hazan et al. 1986). The purity of these preparations was monitored by SDS-PAGE and two-dimensional gel electrophoresis and has been documented elsewhere (Hatzfeld and Franke 1985; Achtstätter et al. 1986; Quinlan et al. 1986).

In some experiments, polypeptides purified by excision of bands after preparative SDS-PAGE of cytoskeletal proteins and elution as described (Hatzfeld and Franke 1985) were used. Similarly, the hair-specific basic (Hb) and acidic (Ha) α-keratin polypeptides were separated as two groups by gel electrophoresis (Heid et al. 1986), eluted, and processed for dot blot assays.

In order to carry out dot blot assays of antibody binding purified individual cytokeratins or control proteins were dissolved in 9 M urea, 10 mM Tris-HCl (pH 7.5) and allowed to partly refold for ca. 15 min in a final concentration of 4 M urea (same buffer; protein was determined according to Bradford 1976). They were applied in approximately 30 µl drops (ca. 1 µg protein) to nitrocellulose paper sheets (S&S Membrane Filters, Schleicher and Schuell, Dassel, FRG) mounted on three layers of filter paper, using a special multiple drop application apparatus. Protein amounts blotted on the paper were moni-

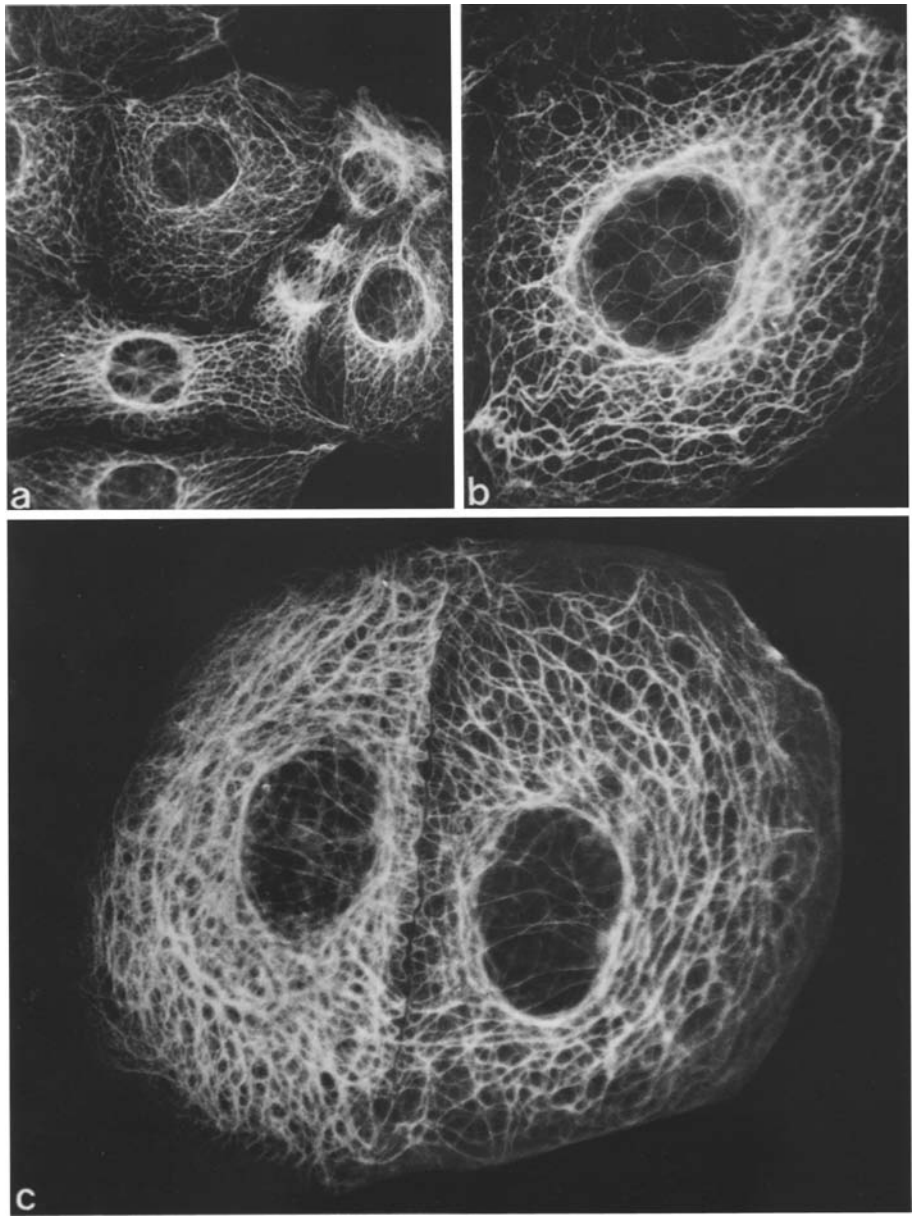

Fig. 1 a–c. Immunofluorescence microscopy of cultured human hepatocellular carcinoma cells (*line PLC*) showing fibrillar arrays decorated with monoclonal murine antibody lu-5.
a Survey micrograph, showing intense fibrillar reaction in cells of various sizes and cell cycle stages (note the two postmitotic daughter cells in the right half). Identical arrays are observed in such cells with proven cytokeratin antibodies (460 ×).
b Higher magnification, showing details of the meshwork arrays of cytokeratin fibrils which also run over and under the nucleus (1000 ×).
c Lu-5-decorated cytokeratin fibrils in two adjacent cells terminating at distinct sites along the cell-to-cell boundary (center) which correspond to desmosomes, as demonstrable by double-label staining with guinea pig antibodies to desmoplakin (1000 ×)

tored by staining with Ponceau-S (cf. Hazan et al. 1986). After soaking and air-drying the paper sheets were incubated for 2 h at room temperature, first with phosphate-buffered saline (PBS) containing 0.05% Tween-20, and then with antibody lu-5 (final concentration of antibody 0.8 μg/ml). After 2 h incubation the nitrocellulose paper sheets were washed three times, 30 min each, in PBS containing 0.1% Triton X-100, then PBS containing 0.5% Triton X-100 and finally in PBS containing 0.64 M NaCl. Further processing, including reaction with several peroxidase-coupled secondary antibodies, washing steps and the enzyme reaction, were as recently described (Hazan et al. 1986).

Single- or double-label indirect immunofluorescence microscopy of frozen tissue sections or cultured cells grown on cover slips was carried out as described before (Franke et al. 1979a, b, 1980, 1984; Hazan et al. 1986).

Results

When antibody lu-5 was examined by immunofluorescence microscopy of cultured cells from various tissues of origin, it decorated typical cytokeratin fibril arrays in all epithelium-derived cell lines tested (Fig. 1a–c presents the example of human PLC cells), irrespective of the species. Positive fibril staining of this type was found in the human carcinoma cell lines PLC, HeLa, MCF-7 and A-431, the bovine epithelial cell lines MDBK, BMGE-H and BMGE+H, the rat hepatoma line MH_1C_1, the rat kangaroo kidney epithelial line PtK_2 and

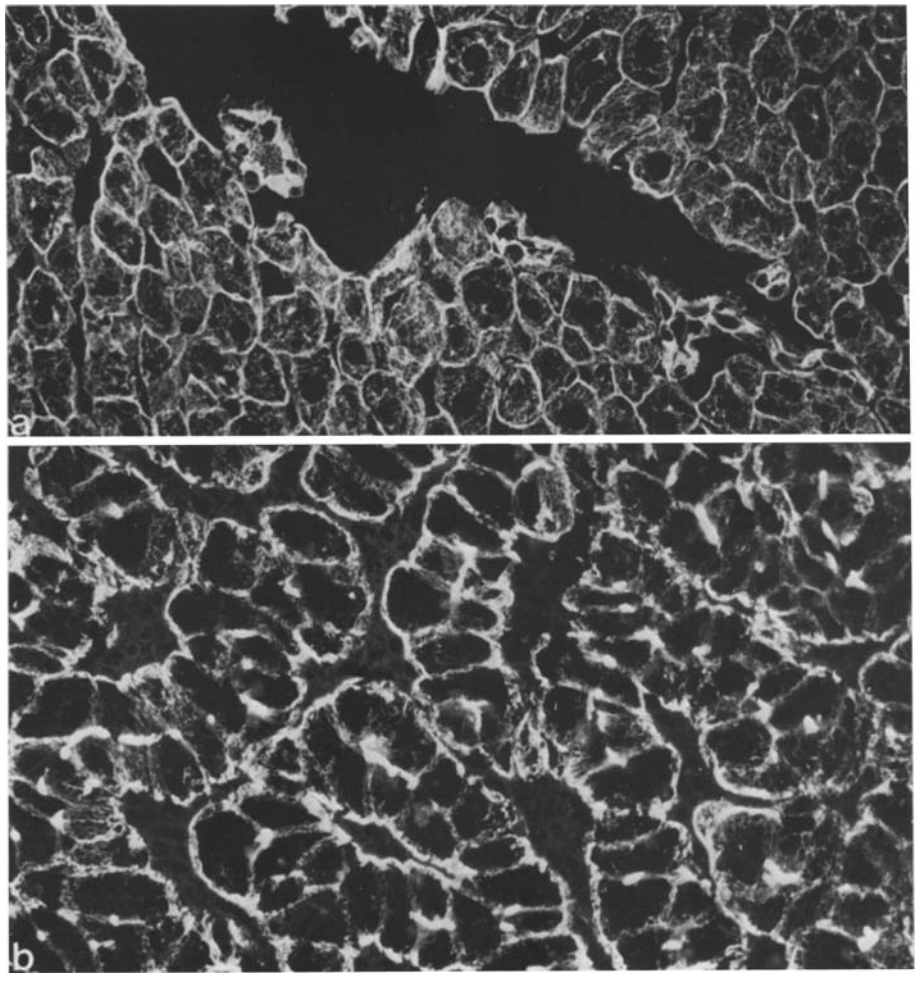

Fig. 2a, b. Immunofluorescence microscopy of antibody lu-5 on frozen sections of liver tissue of rat (**a**) and the toad, *Xenopus laevis* (**b**), showing strong fibrillar staining in hepatocytes (**a, b**) and bile duct epithelium (**a**) whereas mesenchymal components are unstained. Note, in (**b**), the polar concentration of lu-5-stained fibrils around bile canaliculi and near sinusoidal surfaces (400 ×)

the cell kidney epithelial line A6 from the toad, *Xenopus laevis* (XLKE cells). In all these cells the fibrillar decoration pattern coincided with that obtained with other proven cytokeratin antibodies, as shown by parallel staining of the cultures or double label immunofluorescence microscopy (not shown). Double labelling of murine lu-5 and guinea pig antibodies to desmoplakin (cf. Franke et al. 1983) showed that many of the lu-5-positive fibrils terminated at desmosomes (not shown). No significant reaction was observed with a variety of non-epithelial cells containing other IF proteins such as vimentin and glial filament protein in human, bovine, rat and chicken fibroblasts or human glioma cells.

Using immunofluorescence microscopy we examined antibody lu-5 on frozen sections of simple and stratified epithelia from species as diverse as man, cow, rat, chick, a snake and two amphibian species (*Xenopus laevis* and *Pleurodeles waltli*) and in all samples observed strong and epithelium-specific immunostaining. As examples, we show the intense lu-5 staining on liver tissues of rat (Fig. 2a) and *Xenopus* (Fig. 2b) as well as on intestinal tissue of chicken (Fig. 3). At higher magnification, the lu-5-decorated structures could be resolved into fibrillar meshworks indistinguishable from those obtained with proven cytokeratin antibodies used in parallel (Figs. 2 and 3; for comparison see, e.g., Franke et al. 1979, 1981; Schmid et al. 1979; for simple epithelium-type cytokeratins of *Xenopus* see Franz et al. 1983).

Although the "hard", cysteine-rich α-keratin polypeptides of wool, hair and nail show, in their central α-helical "rod" portions (for nomenclature see Weber and Geisler 1984; Steinert et al. 1985a), significant amino acid sequence homologies to epithelial cytokeratins (Geisler and Weber 1982; Crewther et al. 1983; Hanukoglu and Fuchs 1983; Dowling et al. 1986) reports of immunological cross-reactions between the cytokeratin filaments of epithelial cells and those of trichocytes, i.e. the hair-forming cells, are limited (e.g., Weber et al. 1980; Lane et al. 1985; Heid et al. 1986). As shown

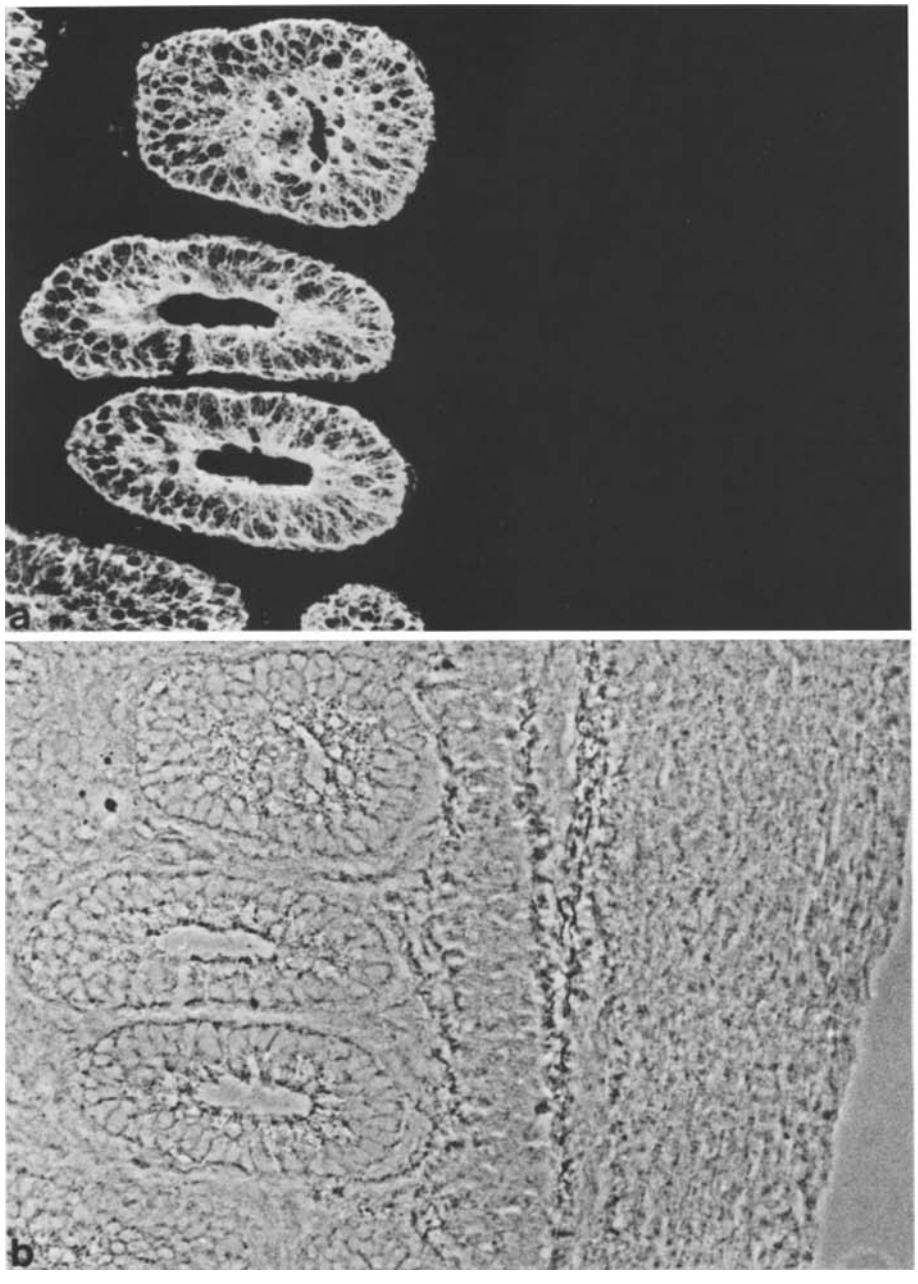

Fig. 3a, b. Immunofluorescence microscopy of antibody lu-5 on frozen section of chicken intestine shown in epifluorescence (**a**) and phase contrast (**b**) optics. Note the specificity of reaction in the epithelial cells (left half) and the absence of reaction in cells of the *lamina propria* and the *muscularis* layers (*right half*) (400 ×)

in Fig. 4a–c the epitope recognized by antibody lu-5 is present not only in human fetal epidermis (right hand side of Fig. 4a and b) and outer root sheath epithelium of hair follicles (left part of Fig. 4a–c) but also in the living trichocytes and the maturing cells of the forming hair shaft (Fig. 4). Similar results were obtained with hair follicles of cow (Fig. 5) and rodents (not shown).

Since negative or inconsistent results were obtained when antibody lu-5 was tested by immunoblotting of denatured polypeptides separated by SDS-PAGE we have chosen a different way to examine its specificity. To this end, purified proteins were allowed to refold, at least in part, and applied in a dot-blot fashion to nitrocellulose paper sheets (see Materials and methods). When these proteins were incubated with antibody lu-5, specific binding to various cytokeratin polypeptides was observed. Figure 6 presents examples of positive reactions with human cytokeratin nos. 1, 5 and 6, 8, 18 and 19 as well as with rat cytokeratin polypeptides A (equivalent to human cytokeratin no. 8) and D (equivalent to no. 18) but not with vimentin, desmin and bovine serum albumin. In another set of

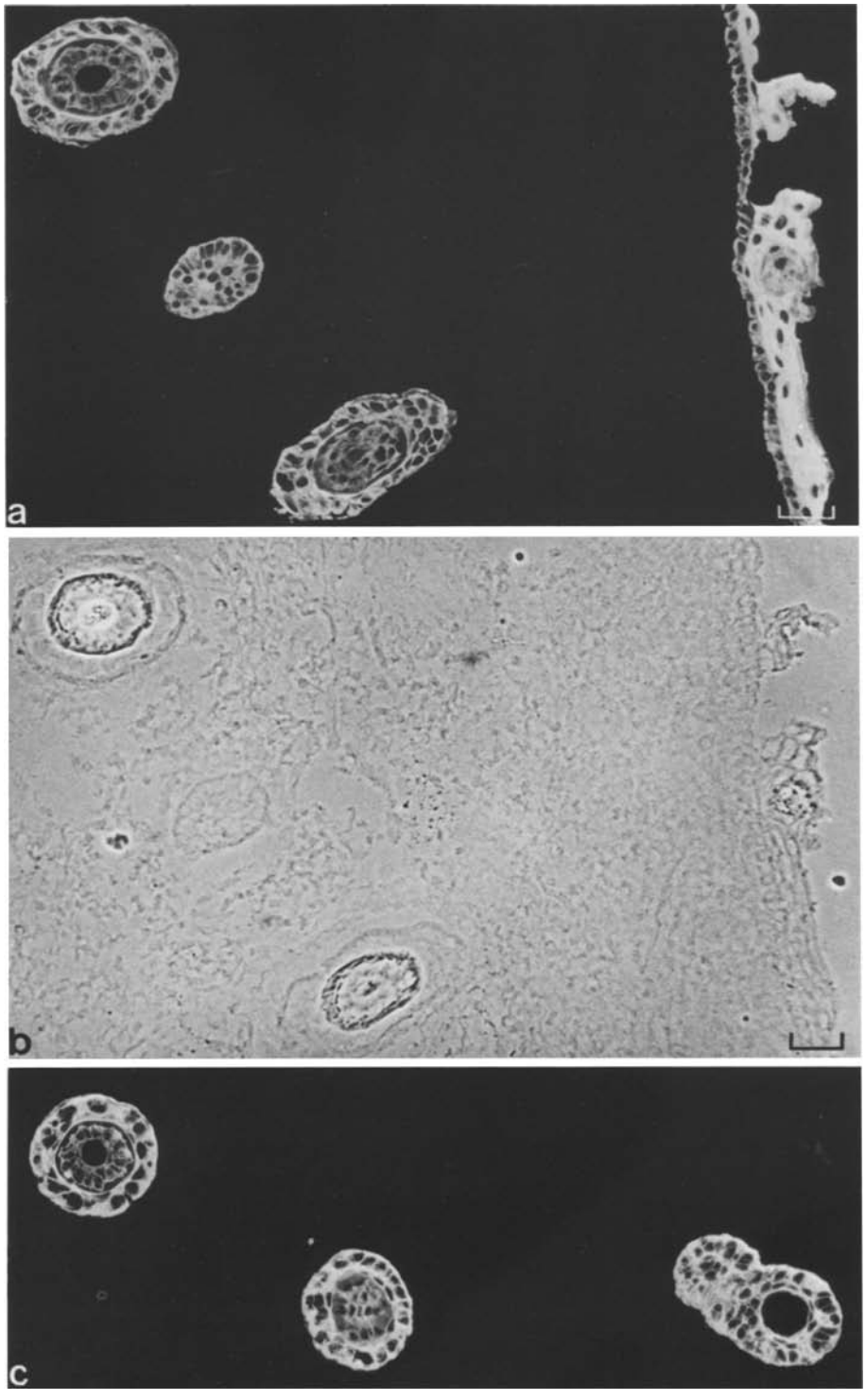

Fig. 4a–c. Immunofluorescence microscopy of frozen sections through human fetal epidermis (week 21), showing the specific reaction of antibody lu-5 with early fetal epidermis (**a**, at the right margin) and with hair follicles in which outer and inner root sheath epithelia as well as the internal column of trichocytes forming the hair shaft (the same field is shown in epifluorescence, **a**, and phase contrast, **b**, optics) are positive. When cross-sections through different levels of the hair follicle are compared, the innermost portion of the maturing shaft appears negative in more distal regions (**c** shows three different situations) (210 ×)

such dot blot assays a positive reaction with human epidermal cytokeratin no. 14 was also seen. The reactivity with the pooled acidic (Ha; type I) and basic (Hb; type II) α-keratins from human hair follicles (for preparation see Heid et al. 1986) was comparatively weaker but still significant. These results indicate that lu-5 reacts with an epitope present in various cytokeratin polypeptides of both subfamilies.

Discussion

The results of the present and the preceeding study (von Overbeck et al. 1985) indicate that monoclon-

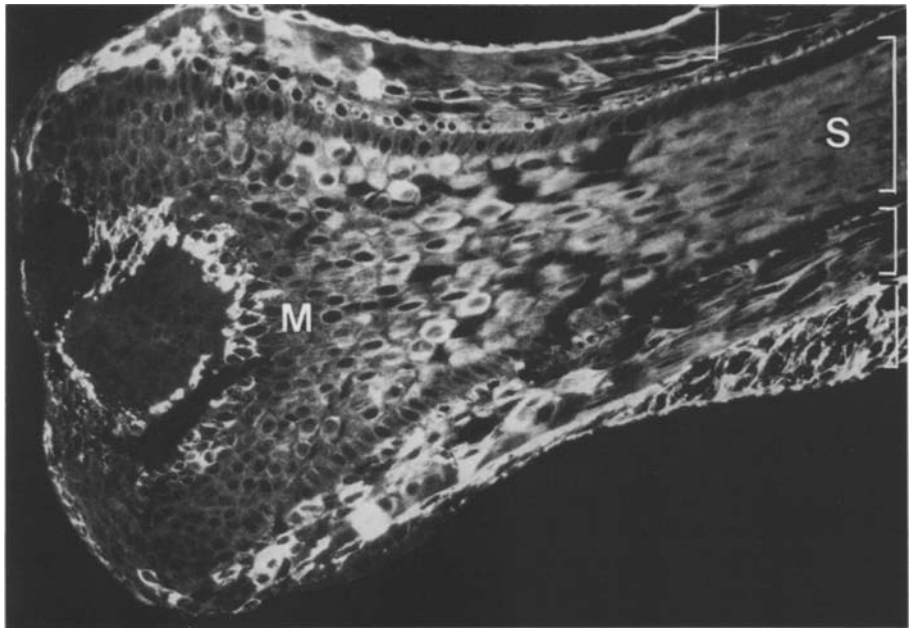

Fig. 5. Immunofluorescence microscopy of frozen sections of bovine epidermis, showing a near-longitudinal section through a hair follicle after reaction with antibody lu-5. Note positive reaction in outer root sheath epithelium (denoted by the upper and lower brackets), inner root sheath (second bracket from bottom) and forming shaft (*S*, demarcated by the long central bracket) as well as in trichocytes of the matrix (*M*) (280 ×)

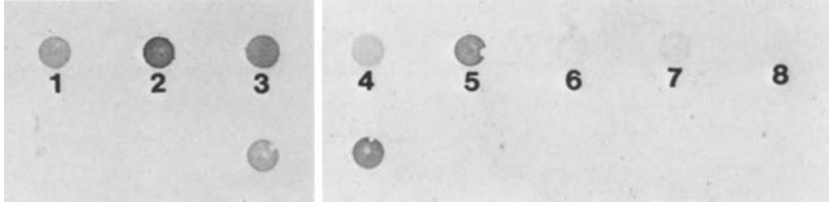

Fig. 6. Reaction of antibody lu-5 with purified cytokeratin polypeptides demonstrated in an immunological dot-blot assay. Drops (30 µl) of purified and solubilized proteins (~1 µg) were applied as dots to nitrocellulose paper and reacted with antibody lu-5, followed by visualization of the reaction by immunoperoxidase reaction. The dots in the upper row contain: dot 1, human cytokeratins no. 1 (from epidermis); dot 2, cytokeratins nos. 5 and 6 (mixture as isolated from human oral mucosa); dot 3, cytokeratin no. 8 (from MCF-7 cells); dot 4, cytokeratin no. 18 (from MCF-7 cells); dot 5, cytokeratin no. 19 (from MCF-7 cells); dot 6, vimentin (from human fibroblasts); dot 7, desmin (from human myometrium) and dot 8, bovine serum albumin. The two dots in the lower row contain rat liver cytokeratin polypeptides A (equivalent to human cytokeratin no. 8; below dot 3) and D (equivalent to human cytokeratin no. 18; below dot 4). Note specificity of reaction for cytokeratins as well as some differences of intensity among the various cytokeratins (e.g., the reaction with cytokeratin no. 18 appears to be less intensive than those with cytokeratins nos. 8 and 19)

al antibody lu-5 is a cytokeratin-specific antibody which reacts with native IFs in both tissue sections and whole mount preparations of isolated cells or cells grown in vitro but differs in several properties from other cytokeratin antibodies so far described.

Lu-5 recognizes an epitope which is present in a wide range, perhaps all, cytokeratins but does not occur in other IF proteins. This is in contrast to the antibody, IFA, described by Pruss et al. (1981) which binds to an epitope common to all types of IF proteins although it does not efficiently react, in immunoblot assays using SDS-PAGE-separated polypeptides, with certain cytokeratins of the acidic (type I) subfamily (see, for example, Cooper et al. 1984). On the other hand, the occurrence of the lu-5 epitope is more widespread among the different cytokeratins than the epitopes of the most broadly reacting cytokeratin antibodies so far reported, i.e. antibody K_G 8.13 which reacts with practically all basic (type II) cytokeratins as well as with the acidic (type I) cytokeratin no. 18 (Gigi et al. 1982), and AE3 which reacts with most cytokeratins of the acidic (type I) subfamily (Tseng et al. 1982; Cooper et al. 1984).

The lu-5 epitope is not only widespread among different cytokeratins of the same species but has been well conserved during evolution from amphibia to man. In contrast, other broad range monoclonal cytokeratin antibodies such as K_G 8.13 do not recognize the cytokeratins of certain species (Gigi et al. 1982). Whether the epitope of lu-5 is also present in the ca. 10 nm diameter filaments

of certain invertebrates, as it has been shown for antibody IFA (Bartnik et al. 1985, 1986) and the vimentin-positive antibody Ah6/5/9 (Walter and Biesmann 1984) remains to be seen.

The lu-5 epitope is present in both epithelial and trichocyte-specific cytokeratin polypeptides, which has been noted for some cytokeratin antisera (Weber et al. 1980; Heid et al. 1986) but only for few monoclonal antibodies (e.g., Lane et al. 1985).

The epitope recognized by lu-5 appears to be dependent on a certain configuration of the individual cytokeratin polypeptide. This configuration is present in both the IF structure and in the refolded isolated cytokeratin polypeptide but it is not effectively and reproducibly formed in the SDS-treated, PAGE-separated polypeptides blotted on nitrocellulose paper. Therefore, lu-5 may be regarded as a conformation-dependent cytokeratin antibody which, however, does not require the formation of the heterotypic subunit with a complementary cytokeratin. Clearly, this type of conformation-dependence is different from that recently described by Hazan et al. (1986) which presents an epitope that is recognized upon the unfolding of the cytokeratin structure but is masked in the normal intracellular IFs.

Although the lu-5 epitope is sensitive to changes in conformation, it is remarkably resistant to treatment with formaldehyde. In this property it also differs from the epitopes of most other cytokeratin antibodies (for antibody KL1, however, see Viac et al. 1983).

It is presently not clear whether the lu-5 epitope is "continuous", i.e. defined by an uninterrupted amino acid sequence, or represents a configuration formed by contributions from two or more regions of the molecule which are not immediately adjacent ("discontinous" epitopes; for references and a recent discussion see Barlow et al. 1986). If antibody lu-5 binds to a certain continuous sequence identical – or at least very similar – in different cytokeratin polypeptides of both subfamilies as well as in the same cytokeratin over a wide range of species, from *Xenopus laevis* to man, the remarkable conservation of the lu-5 epitope suggests that it is located in the α-helical rod domain (for nomenclature see Geisler and Weber 1982; Weber and Geisler 1984; Steinert et al. 1985) as sequence identities and homologies between the cytokeratin subfamilies and taxonomically distant species are, by and large, restricted to this region (Crewther et al. 1983; Hanukoglu and Fuchs 1983; Marchuk et al. 1984; Fuchs et al. 1985; Hoffmann et al. 1985; Jonas et al. 1985; Steinert et al. 1985a, b; Bader et al. 1986; Franz and Franke 1986; Dowling et al. 1986; Leube et al. 1986; Magin et al. 1986; Oshima et al. 1986). Since the members of the basic and the acidic cytokeratin subfamilies share less than 30% amino acid sequence homology in the rod domain (Hanukoglu and Fuchs 1983; Steinert et al. 1985; Franz and Franke 1986) there are only a few possibilities for an uninterrupted epitope specific for cytokeratins that do not also occur in the other IF proteins. Examples are located toward the end of coil 1a and around the center of coil 1b. However, none of the sequence intercepts selected in this respect shows a striking homology. Therefore, we consider it more likely that lu-5 recognizes a cytokeratin-specific configuration formed by discontinuous positions. We are currently using deletion mutant proteins produced in *E. coli* to identify the lu-5 epitope.

Our immuno-dot-blot results obtained with purified cytoskeletal proteins explain both important properties of antibody lu-5, i.e. the epithelial specificity and the broad tissue and species cross-reactivity. Obviously, for histological classifications of tissues and for the diagnosis of epithelium-derived tumors, antibody lu-5 provides the most general cytokeratin probe presently available.

References

Achtstätter T, Hatzfeld M, Quinlan RA, Parmelee DC, Franke WW (1986) Separation of cytokeratin polypeptides by gel electrophoretic and chromatographic techniques and their identification by immunoblotting. Meth Enzymol 134:355–371

Bader BL, Magin TM, Hatzfeld M, Franke WW (1986) Amino acid sequence and gene organization of cytokeratin no. 19, an exceptional tail-less intermediate filament protein. EMBO J 5:1865–1875

Bannasch P, Zerban H, Schmid E, Franke WW (1980) Liver tumors distinguished by immunofluorescence microscopy with antibodies to proteins of intermediate-sized filaments. Proc Natl Acad Sci USA 77:4948–4952

Barlow DJ, Edwards MS, Thornton JM (1986) Continuous and discontinuous protein antigenic determinants. Nature 322:747–748

Bartek J, Durban EM, Hallowes RC, Taylor-Papadimitriou J (1985) A subclass of luminal epithelial cells in the human mammary gland, defined by antibodies to cytokeratins. J Cell Sci 75:17–33

Bartnik E, Osborn M, Weber K (1985) IFs in non-neuronal cells of invertebrates: isolation and biochemical characterization of keratin-like intermediate filaments from the esophageal epithelium of the mollusc *Helix pomatia*. J Cell Biol 101:427–440

Bartnik E, Osborn M, Weber K (1986) Intermediate filaments in muscle and epithelial cells of nematodes. J Cell Biol 102:2033–2041

Battifora H, Sun TT, Rao S, Bahu R (1980) Antikeratin antibodies in tumor diagnosis: thymoma versus lymphoma. Hum Pathol 11:635–641

Benavente R, Krohne G, Franke WW (1985) Cell type-specific

expression of nuclear lamina proteins during development of *Xenopus laevis*. Cell 41:177–190

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cooper D, Schermer A, Pruss R, Sun T-T (1984) The use of aIF, AE1, and AE3 monoclonal antibodies for the identification and classification of mammalian epithelial keratins. Differentiation 28:30–35

Cooper D, Schermer A, Sun T-T (1985) Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: Strategies, applications, and limitations. Lab Invest 52:243–256

Corson JM (1986) Keratin protein immunohistochemistry in surgical pathology practice. In: Sommers SC, Rosen PP, Fechner RE (eds) Pathology Annual, vol 21, part 2. Appleton-Century-Crofts, Norwalk, Connecticut, pp 49–81

Cowin P, Kapprell H-P, Franke WW (1985) The complement of desmosomal plaque proteins in different cell types. J Cell Biol 101:1442–1454

Cowin P, Kapprell H-P, Franke WW, Tamkun J, Hynes RO (1986) Plakoglobin: A protein common to different kinds of intercellular adhering junctions. Cell 46:1063–1073

Crewther WG, Dowling LM, Steinert PM, Parry DAD (1983) Structure of intermediate filaments. Int J Biol Macromol 5:267–274

Debus E, Weber K, Osborn M (1982) Monoclonal cytokeratin antibodies that distinguish simple from stratified squamous epithelia: Characterization of human tissues. EMBO J 1:1641–1647

Debus E, Moll R, Franke WW, Weber K, Osborn M (1984) Immunohistochemical distinction of human carcinomas by cytokeratin typing with monoclonal antibodies. Am J Pathol 114:121–130

Dowling LM, Crewther WG, Inglis AS (1986) The primary structure of component 8c-1, a subunit protein of intermediate filaments in wool keratin. Biochem J 236:695–703

Franke WW, Schmid E, Osborn M, Weber K (1978a) Different intermediate-sized filaments distinguished by immunofluorescence microscopy. Proc Natl Acad Sci USA 75:5034–5038

Franke WW, Weber K, Osborn M, Schmid E, Freudenstein C (1978b) Antibody to prekeratin. Decoration of tonofilament-like arrays in various cells of epithelial character. Exp Cell Res 116:429–445

Franke WW, Appelhans B, Schmid E, Freudenstein C, Osborn M, Weber K (1979a) Identification and characterization of epithelial cells in mammalian tissues by immunofluorescence microscopy using antibodies to prekeratin. Differentiation 15:7–25

Franke WW, Schmid E, Osborn M (1979b) HeLa cells contain intermediate-sized filaments of the prekeratin type. Exp Cell Res 118:95–109

Franke WW, Schmid E, Winter S, Osborn M, Weber K (1979c) Widespread occurrence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates. Exp Cell Res 123:25–46

Franke WW, Schmid E, Freudenstein C, Appelhans B, Osborn M, Weber K, Keenan TW (1980) Intermediate-sized filaments of the prekeratin type in myoepithelial cells. J Cell Biol 84:633–654

Franke WW, Mayer D, Schmid E, Denk H, Borenfreund E (1981a) Differences of expression of cytoskeletal proteins in cultured rat hepatocytes and hepatoma cells. Exp Cell Res 134:345–365

Franke WW, Schiller DL, Moll R, Winter S, Schmid E, Engelbrecht I, Denk H (1981b) Diversity of cytokeratins. Differentiation specific expression of cytokeratin polypeptides in epithelial cells and tissues. J Mol Biol 153:933–959

Franke WW, Schmid E, Schiller DL, Winter S, Jarasch E-D, Moll R, Denk H, Jackson B, Illmensee K (1982) Differentiation-related patterns of expression of proteins of intermediate-sized filaments in tissues and cultured cells. Cold Spring Harbor Symp Quant Biol 46:431–453

Franke WW, Schiller DL, Hatzfeld M, Winter S (1983) Protein complexes of intermediate-sized filaments: Melting of cytokeratin complexes in urea reveals different polypeptide separation characteristics. Proc Natl Acad Sci USA 80:7113–7117

Franke WW, Schmid E, Mittnacht S, Grund C, Jorcano JL (1984) Integration of different keratins into the same filament system after microinjection of mRNA for epidermal keratins into kidney epithelial cells. Cell 36:813–825

Franz JK, Gall L, Williams MA, Picheral B, Franke WW (1983) Intermediate-size filaments in a germ cell: expression of cytokeratins in oocytes and eggs of the frog *Xenopus*. Proc Natl Acad Sci USA 80:6254–6258

Franz JK, Franke WW (1986) Cloning of cDNA and amino acid sequence of a cytokeratin expressed in oocytes of *Xenopus laevis*. Proc Natl Acad Sci USA 83:6475–6479

Fuchs EV, Coppock SM, Green FH, Cleveland DW (1981) Two distinct classes of keratin genes and their evolutionary significance. Cell 27:75–84

Fuchs E, Green H (1981) Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. Cell 25:617–625

Fuchs E, Hanukoglu I, Marchuk D, Grace MP, Kim KH (1985) The nature and significance of differential keratin gene expression. In: Wang E, Fischman D, Liem RKH, Sun T-T (eds) Intermediate Filaments, vol 455. The New York Academy of Sciences, New York, pp 436–450

Gabbiani G, Kapanci Y, Barazzone P, Franke WW (1981) Immunochemical identification of intermediate-sized filaments of in human neoplastic cells: A diagnostic aid for the surgical pathologist. Am J Pathol 104:206–216

Geisler N, Weber K (1980) Purification of smooth-muscle desmin and a protein-chemical comparison of desmins from chicken gizzard and hog stomach. Eur J Biochem 111:425–433

Geisler N, Weber K (1981) Isolation of polymerization-competent vimentin from porcine eye lens tissue. FEBS Lett 125:253–256

Geisler N, Weber K (1982) The amino acid sequence of chicken muscle desmin provides a common structural model for intermediate filament proteins. EMBO J 1:1649–1656

Gigi O, Geiger B, Eshhar Z, Moll R, Schmid E, Winter S, Schiller DL, Franke WW (1982) Detection of a cytokeratin determinant common to diverse epithelial cells by a broadly cross-reacting monoclonal antibody. EMBO J 1:1429–1437

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

Hanukoglu I, Fuchs E (1983) The cDNA sequence of a type II cytoskeletal keratin reveals constant and variable structural domains among keratins. Cell 33:915–924

Hatzfeld M, Franke WW (1985) Pair formation and promiscuity of cytokeratins: Formation in vitro of heterotypic complexes and intermediate-sized filaments by homologous and heterologous recombinations of purified polypeptides. J Cell Biol 101:1826–1841

Hazan R, Denk H, Franke WW, Lackinger E, Schiller DL (1986) Change of cytokeratin organization during development of mallory bodies as revealed by a monoclonal antibody. Lab Invest 54:543–553

Heid HW, Werner W, Franke WW (1986) The complement of native α-keratin polypeptides of hair-forming cells: A subset of eight polypeptides that differ from epithelial cytokeratins. Differentiation 31:141–153

Hoffmann W, Frank JK, Franke WW (1985) Amino acid sequence microheterogeneities of basic (type II) cytokeratins of *Xenopus laevis* epidermis and evolutionary conservativity of helical and non-helical domains. J Mol Biol 184:713–724

Holthöfer H, Miettinen A, Paasivuo R, Lehto V-P, Linder E, Alfthan O, Virtanen I (1983) Cellular origin and differentiation of renal carcinomas. Lab Invest 49:317–326

Jonas E, Sargent TD, Dawid IB (1985) Epidermal keratin gene expressed in embryos of *Xenopus laevis*. Proc Natl Acad Sci USA 82:5413–5417

Knapp AC, Franke WW, Heid H, Hatzfeld M, Jorcano JL, Moll R (1986) Cytokeratin no. 9, an epidermal type I keratin characteristic of a special program of keratinocyte differentiation displaying body site specificity. J Cell Biol 103:657–667

Lane EB (1982) Monoclonal antibodies provide specific intramolecular markers for the study of epithelial tonofilament organization. J Cell Biol 92:665–673

Lane EB, Bártek J, Purkis PE, Leigh IM (1985) Keratin antigens in differentiating skin. In: Wang E, Fischman D, Liem RKH, Sun T-T (eds) Intermediate Filaments, vol 455. The New York Academy of Sciences, New York, pp 241–258

Leube RE, Bosch FX, Romano V, Zimbelmann R, Höfler H, Franke WW (1986) Cytokeratin expression in simple epithelia. III. Detection of mRNAs encoding human cytokeratins nos. 8 and 18 in normal and tumor cells by hybridization with cDNA sequences in vitro and in situ. Differentiation 33:69–85

Magin TM, Jorcano JL, Franke WW (1986) Cytokeratin expression in simple epithelia. II. cDNA cloning and sequence characteristics of bovine cytokeratin A (no. 8). Differentiation 30:254–264

Makin CA, Bobrow LG, Bodmer WF (1984) Monoclonal antibody to cytokeratin for use in routine histopathology. J Clin Pathol 37:975–983

Marchuk D, McCrohon S, Fuchs E (1984) Complete sequence of a gene encoding a human type I keratin: Sequences homologous to enhancer elements in the regulatory region of the gene. Cell 39:491–498

Miettinen M, Lehto V-P, Virtanen I (1984) Antibodies to intermediate filament proteins in the diagnosis and classification of human tumors. Ultrastruct Pathol 7:83

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

Moll R, Cowin P, Kapprell H-P, Franke WW (1986) Desmosomal proteins: New Markers for identification and classification of tumors. Lab Invest 54:4–25

Osborn M, Weber K (1983) Tumor diagnosis by intermediate filament typing: A novel tool for surgical pathology. Lab Invest 48:372–394

Oshima RG, Millan JL, Ceceña G (1986) Comparison of mouse and human keratin 18: A component of intermediate filaments expressed prior to implantation. Differentiation 33:61–68

Overbeck von J, Stähli C, Gudat F, Carmann H, Lautenschlager C, Dürmüller U, Takacs B, Miggiano V, Staehelin Th, Heitz PhU (1985) Immunohistochemical characterization of an anti-epithelial monoclonal antibody (mAB lu-5) Virchows Arch A (Pathol Anat) 407:1–12

Pruss RM, Mirsky R, Ruff MC, Thorpe R, Dowding AJ, Anderton BH (1981) All classes of intermediate filaments share a common antigenic determinant defined by a monoclonal antibody. Cell 27:419–428

Quinlan RA, Franke WW (1983) Molecular interactions in intermediate-sized filaments revealed by chemical crosslinking. Heteropolymers of vimentin and glial filament protein in cultured human glioma cells. Eur J Biochem 132:477–484

Quinlan RA, Cohlberg JA, Schiller DL, Hatzfeld M, Franke WW (1984) Heterotypic tetramer (A_2D_2) complexes of non-epidermal keratins isolated from cytoskeletons of rat hepatocytes and hepatoma cells. J Mol Biol 178:365–388

Quinlan RA, Hatzfeld M, Franke WW, Lustig A, Schulthess T, Engel J (1986) Characterization of dimer subunits of intermediate filament proteins. J Mol Biol 192:337–349

Ramaekers F, Huysmans A, Moesker O, Kant A, Jap P, Herman C, Vooijs P (1983a) Monoclonal antibody to keratin filaments, specific for glandular epithelia and their tumors. Lab Invest 49:353–361

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

Roop DR, Cheng CK, Titterington L, Meyers CA, Stanley JR, Steinert PM, Yuspa SH (1984) Synthetic peptides corresponding to keratin subunits elicit highly specific antibodies. J Biol Chem 259:8037–8040

Schiller DL, Franke WW, Geiger B (1982) A subfamily of relatively large and basic cytokeratin polypeptides as defined by peptide mapping is represented by one or several polypeptides in epithelial cells. EMBO J 1:761–769

Schlegel R, Banks-Schlegel S, McLeod JA, Pinkus GS (1980) Immunoperoxidase localization of keratin in human neoplasms. Am J Pathol 101:41–50

Schmid E, Tapscott S, Bennett GS, Croop J, Fellini SA, Holtzer H, Franke WW (1979) Differential location of different types of intermediate-sized filaments in various tissues of the chick embryo. Differentiation 15:27–40

Schmid E, Schiller DL, Grund C, Stadler J, Franke WW (1983) Tissue type specific expression in a cultured epithelial cell line from bovine mammary gland. J Cell Biol 96:37–50

Söllner P, Quinlan RA, Franke WW (1985) Identification of a distinct soluble subunit of an intermediate filament protein: Tetrameric vimentin from living cells. Proc Natl Acad Sci USA 82:7929–7933

Spagnolo DV, Michie SA, Crabtree GS, Warnke RA, Rouse RV (1985) Monoclonal anti-keratin (AE1) reactivity in routinely processed tissue from 166 human neoplasms. AM J Clin Pathol 84:697–704

Steinert PM, Parry DAD, Idler WW, Johnson LD, Steven AC, Roop DR (1985a) Amino acid sequences of mouse and human epidermal type II keratins of M_r 67,000 provide a systematic basis for the structural and functional diversity of the end domains of keratin intermediate filament subunits. J Biol Chem 260:7142–7149

Steinert PM, Steven AC, Roop DR (1985b) The molecular biology of intermediate filaments. Cell 42:411–419

Sun T-T, Green H (1978) Immunofluorescent staining of keratin fibers in cultured cells. Cell 14:469–476

Sun T-T, Shih CH, Green H (1979) Keratin cytoskeletons in epithelial cells of internal organs. Proc Natl Acad Sci USA 76:2813–2817

Sun T-T, Eichner R, Nelson WG, Tseng SCG, Weiss RA, Jarvinen M, Woodcock-Mitchell J (1983) Keratin classes: molecular markers for different types of epithelial differentiation. J Invest Dermatol 81:109s–115s

Sun T-T, Eichner R, Schermer A, Cooper D, Nelson WG, Weiss RA (1984) Classification, expression, and possible mech-

anisms of evolution of mammalian epithelial keratins: a unifying model. In: Levine AJ, Vande Woude GF, Topp WC, Watson JD (eds) Cancer Cells 1, The transformed phenotype. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 169–176

Tseng SCG, Jarvinen MJ, Nelson WG, Huang J-W, Woodcock-Mitchell J, Sun T-T (1982) Correlation of specific keratins with different types of epithelial differentiation: monoclonal antibody studies. Cell 30:361–372

Van Muijen GNP, Ruiter DJ, Franke WW, Achtstätter T, Haasnoot WHB, Ponec M, Warnaar SO (1986) Cell type heterogeneity of cytokeratin expression in complex epithelia and carcinomas demonstrated by monoclonal antibodies specific for cytokeratins nos. 4 and 13. Exp Cell Res 162:97–113

Viac J, Reano A, Brochier J, Staquet M-J, Thivolet J (1983) Reactivity pattern of a monoclonal antikeratin antibody (KL1). J Invest Dermatol 81:351–354

Walter MF, Biessmann H (1984) Intermediate-sized filaments in Drosophila tissue culture cells. J Cell Biol 99:1468–1477

Weber K, Osborn M, Franke WW (1980) Antibodies against merokeratin from sheep wool decorate cytokeratin filaments in non-keratinizing epithelial cells. Eur J Cell Biol 23:110–114

Weber K, Geisler N (1984) Intermediate filaments from wool α-keratin to neurofilaments: a structural overview. In: Levine AJ, Vande Woude GF, Topp WC, Watson JD (eds) Cancer Cells 1, The transformed phenotype. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 153–159

Woods EF (1983) The number of polypeptide chains in the rod domain of bovine epidermal keratin. Biochem Int 7:769–774

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