

The Catalog of Human Cytokeratins: Patterns of Expression in Normal Epithelia, Tumors and Cultured Cells

Review

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Introduction

A large proportion of the cytoplasm of vertebrate cells, normal or transformed, is represented by components of the cytoskeleton, including actin-containing microfilaments, tubulin-containing microtubules and filaments of intermediate size, with diameters of 7–11 nm. Although such structures have a widespread occurrence in diverse cell types, examples have been reported in which they are formed in different cell types from different proteins of a multigene family of proteins, or from different subunit polypeptides of a class of related proteins. For example, differentiation specificity of expression of different actins has been described in different cell types of mammals (Vandekerckhove and Weber, 1979). By far the most striking differentiation specificity of composition has been observed for the intermediate-sized filaments. Although all filaments of this category are morphologically identical in different cell types, are insoluble in solutions of a broad range of low or high salt concentrations and non-ionic detergents and seem to share some common assembly properties (Steinert et al., 1981b) and antigenic determinants (Pruss et al., 1981), immunological and biochemical criteria allow us to distinguish at least five different types of intermediate filaments (Bennett et al., 1978; Franke et al., 1978a, 1981f; Hynes and Destree, 1978; Lazarides, 1980; Anderton, 1981; Holtzer et al., 1981; Osborn et al., 1981). First, filaments containing keratin-like proteins ("cytokeratins") are characteristic of epithelial cells. Second, vimentin filaments occur in mesenchymally derived cells, in astrocytes, in Sertoli cells, in vascular smooth muscle cells and in many cultured cell lines. Third, desmin filaments are typical of most types of myogenic cells. Fourth, neurofilaments are typical of neuronal cells. Fifth, glial filaments are typical of astrocytes. During cell transformation and tumor development this cell type specificity of intermediate filaments is largely conserved (Franke et al., 1978a, 1978b, 1979a; Hynes and Destree, 1978; Sun and Green, 1978a; Sun et al., 1979; Bannasch et al.,

1980; Battifora et al., 1980; Schlegel et al., 1980a; Altmannsberger et al., 1981; Gabbiani et al., 1981; Denk et al., 1982), and classification of tumors by their specific type of intermediate filaments has recently become very valuable in clinical histodiagnosis (see, for example, Schlegel et al., 1980a; Gabbiani et al., 1981; Ramaekers et al., 1981).

The intermediate filaments of the vimentin, desmin or glial types all consist usually of only one type of subunit protein (desmin and vimentin can occur in the same filament in BHK cells and vascular smooth muscle cells; Steinert et al., 1981a; Quinlan and Franke, 1982). In contrast with these, the cytokeratin filaments, which are composed of proteins related to, but not identical with, epidermal α keratins, are a complex family of many different polypeptides. These cytokeratins, which show biochemical and immunological relationships of various degrees, are expressed, in different epithelia, in different combinations of polypeptides ranging in their isoelectric pH values from 5 to 8 and in their apparent molecular weights from 40,000 to 68,000 (Doran et al., 1980; Winter et al., 1980; Fuchs and Green, 1980, 1981; Franke et al., 1981a, 1981b, 1981c; Milstone and McGuire, 1981; Wu and Rheinwald, 1981). A given epithelium or epithelial cell can therefore be characterized by the specific pattern of its cytokeratin components.

Human Cytokeratin Polypeptides and Their Tissue Distribution

Cytoskeletal preparations from epithelial tissues extracted in high salt buffer and Triton X-100 are highly enriched in intermediate-sized filaments containing proteins that react specifically with antibodies to authentic epidermal α keratin (see, for example, Sun and Green, 1977; Fuchs and Green, 1978, 1980, 1981; Franke et al., 1978b, 1980, 1981a, 1981b, 1981c; Wu and Rheinwald, 1981) and that are recovered in filaments reconstituted in vitro from denatured monomers (Tezuka and Freedberg, 1972; Lee and Baden, 1976; Steinert et al., 1976, 1981a; Sun and Green, 1978b; Gipson and Anderson, 1980; Milstone, 1981; Franke et al., 1981b, 1981c; Renner et al., 1981). When such preparations are made from different human tissues and examined by two-dimensional gel electrophoresis, with the aid of isoelectric focusing as well as nonequilibrium pH gradient electrophoresis for better resolution of basic polypeptides, complex patterns of cytokeratin polypeptides are found. The distinct cytokeratin polypeptides that we have so far identified in various human tissues are schematically summarized and arranged according to their specific coordinates on two-dimensional gel electrophoresis in Figure 1, and the corresponding tissue distribution is shown in Table 1A. Typically, the cytokeratin polypeptides appear in series of isoelectric variants; all but the most basic spot usually represent phosphorylated

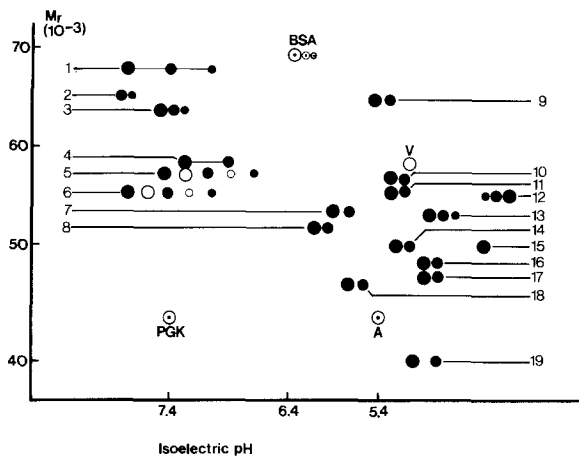


Figure 1. Schematic Chart of Human Cytokeratin Polypeptides
Cytoskeletal proteins from various human epithelia, carcinomas and cultured epithelial cells were separated by two-dimensional gel electrophoresis. Cytokeratin polypeptides detected and identified by antibody binding in immunoblot experiments, and in most cases also by peptide mapping, are arranged according to their mobilities in these gel electrophoretic systems. Abscissa: isoelectric pH values for molecules denatured in 9.5 M urea (data combined from isoelectric focusing and nonequilibrium pH gradient electrophoresis). Ordinate: relative molecular weight (M_r) as determined by SDS-polyacrylamide gel electrophoresis. Cytokeratin polypeptides are designated by Arabic numerals. Horizontal series of spots: different isoelectric variants of the same polypeptide. Large dots: the specific major variant, usually the most basic, nonphosphorylated one. Small dots: minor variants. Open circles: variants of the specific polypeptide not always found in analyses from various cell types. PGK: 3-phosphoglycerokinase. BSA: bovine serum albumin. A: rabbit α actin. V: human vimentin.

Two-dimensional gel electrophoresis was performed with nonequilibrium pH gradient electrophoresis (O'Farrell et al., 1977; ampholine range, pH 2–11; lysis buffer containing 0.25% SDS) or isoelectric focusing (O'Farrell, 1975; ampholine range, pH 4–7) in the first dimension. For second dimension electrophoresis on 12% SDS-polyacrylamide gels (Laemmli, 1970), a modified electrode buffer and an acrylamide to *N,N'*-methylene bisacrylamide ratio of 30:0.15 was used (Thomas and Kornberg, 1975). Gels were stained with Coomassie brilliant blue or, mostly for analysis of protein present in microdissected samples, by a silver staining method (Ansorge, 1982). Gels containing ^{35}S -methionine-labeled proteins were processed for autoradiography.

Usually, cytoskeletal material was dissolved directly in lysis buffer (O'Farrell et al., 1977). Cytoskeletons of cultured cells and, for control experiments, total epithelial tissue were lysed in boiling SDS-containing sample buffer (Laemmli, 1970), and the solubilized protein was acetone-precipitated, dried and taken up in lysis buffer (O'Farrell et al., 1977), as described by Franke et al. (1981b).

Polypeptides separated by one- or two-dimensional gel electrophoresis were transferred electrophoretically onto nitrocellulose paper and characterized by antibodies to cytokeratins as described previously (Towbin et al., 1979; Franke et al., 1981c; Moll et al., 1982a, 1982b, 1982c; Gigi et al., manuscript submitted).

modifications, as shown in epidermis and hepatocytes from diverse species as well as in HeLa cells (Sun and Green, 1978b; Gilmartin et al., 1980; Franke et al., 1981c; Schiller et al., 1982; Steinert et al., 1982).

The different human cytokeratin polypeptides, as identified by binding of keratin antibodies in immuno-

blotting experiments (Franke et al., 1981c; Moll et al., 1982a, 1982b, 1982c; O. Gigi, Z. Eshhar, B. Geiger, R. Moll, E. Schmid, S. Winter, D. L. Schiller and W. W. Franke, manuscript submitted) and by tryptic peptide mapping (see, for example, Schiller et al., 1982), can be divided according to their migration on two-dimensional gel electrophoresis into certain subgroups:

—Relatively large and slightly basic polypeptides are typical of many stratified epithelia. Components 1 and 2 have been described in keratinizing epidermis from various body sites (Baden and Lee, 1978; Fuchs and Green, 1980; Franke et al., 1981c; Moll et al., 1982b) and in epithelium of anal canal (Figure 2f) and exocervix (Franke et al., 1981c). Component 3 occurs in the cornea of man (Gigi et al., manuscript submitted) and shows relationships to components 1, 2 and 5 by tryptic peptide map (data not shown; for data on bovine cornea see Franke et al., 1981c; Schiller et al., 1982). Components 4–6 are observed, in various proportions, in many nonkeratinizing stratified squamous epithelia of man (for example, tongue mucosa, Figure 2e) as well as in epithelia of trachea and apocrine (Figure 2d) and sweat glands of skin and of mammary gland (Table 1; see also Moll et al., 1982b, 1982c). Of these, polypeptides 5 and 6 also occur in epidermis and hair follicles (see Figure 2f; Fuchs and Green, 1980; Moll et al., 1982b) and are closely related to each other, as judged from peptide maps (Schiller et al., 1982).

—Cytokeratin polypeptides of intermediate size and electrical charge (components 7 and 8) are found in diverse simple epithelia, in trachea (Moll et al., 1982c), in transitional epithelium (urothelium) of bladder (Figure 2c), in several complex glands (Figure 2d; Moll et al., 1982c) and in HeLa cells (Franke et al., 1981c, 1982; Bravo et al., 1982). Cytokeratin 8 is the only representative of this subgroup in several simple epithelia of man and various animals ("cytokeratin A"; Figures 2a and 2b; see Franke et al., 1981a, 1981b, 1981c, 1981f; Denk et al., 1982; Moll et al., 1982a; Schiller et al., 1982), and it is also detected in early embryonic epithelia (Jackson et al., 1980, 1981; Franke et al., 1981f).

—Components 9–11 are relatively large or intermediate-sized and acidic keratin polypeptides that are only found in epidermis (Fuchs and Green, 1980; Bowden and Cunliffe, 1981; Franke et al., 1981c; Moll et al., 1982b). Of these, polypeptide 9 is prominent in foot sole epidermis (component "63K" of Fuchs and Green, 1980; see also Moll et al., 1982b), whereas polypeptides 10 and 11 (Figure 2f) seem to be expressed in epidermis of most body locations; polypeptides 10 and 11 show very similar tryptic peptide maps (data not shown).

—Polypeptide 12 has so far only been observed in human cornea (Gigi et al., manuscript submitted; two

similarly acidic polypeptides are present in bovine cornea; Franke et al., 1981c) and its relationship to other cytokeratins is not yet clear.

—Polypeptide 13 is a characteristic major component of several noncornified stratified squamous epithelia (for example, tongue mucosa; Figure 2e; and anal canal epithelium; Figure 2f; see Franke et al., 1981c) and of tracheal epithelium (Moll et al., 1982c), and probably corresponds to the similarly acidic cytokeratin with relative molecular weight of 47,000 found in bovine esophagus and tongue (Franke et al., 1981c; Milstone and McGuire, 1981).

—Components 14–17 are small and acidic keratins occurring, in different combinations, in epidermis and cultured keratinocytes (Sun and Green, 1978b; Fuchs and Green, 1978, 1980; Baden and Lee, 1978), hair follicles (Moll et al., 1982b) and many noncornified stratified epithelia (see, for example, Figures 2e and 2f; Franke et al., 1981c; Moll et al., 1982c), as well as in trachea and some glands (see, for example, Figure 2d; see Moll et al., 1982c). Of these, cytokeratins 14, 16 and 17 are closely related, as recognized by peptide maps (Moll et al., 1982b).

—Cytokeratin 18 shows a tissue distribution similar

to that of polypeptide 8 (Figures 2a–2d; see Table 1A) and corresponds to “cytokeratin D” originally described in some simple epithelia of human (Denk et al., 1982), bovine (Schiller et al., 1982) and murine (Jackson et al., 1980, 1981; Franke et al., 1981a, 1981b, 1981c) origin.

—Cytokeratin 19 is found in a broad range of epithelial tissues (see, for example, Figures 2b–2d). It appears as a major component in many simple epithelia, and usually as a minor component in diverse stratified epithelia as well as in certain cultured keratinocytes (Fuchs and Green, 1981) and squamous cell carcinoma cells (Wu and Rheinwald, 1981), but is not detected in hepatocytes (Figure 2a) and in epidermis or related tissues (Figure 2f; Moll et al., 1982b). A similar small and acidic cytokeratin has been described in rat intestinal brush border (Franke et al., 1981b).

The significance of these different cytokeratin polypeptides is also apparent when whole tissue samples are directly denatured in boiling lysis buffer, and acetone powder made therefrom is used for electrophoresis (see Franke et al., 1981b, 1981c).

In determining the distribution of the different cyto-

Table 1 (overleaf)

Individual cytokeratins are defined by number, relative molecular weight and isoelectric pH value (major isoelectric variants only), as shown in Figure 1. Only primary tumors are listed, since in no case did the corresponding metastases show qualitative changes of cytokeratin pattern. +: component always observed in substantial amounts. (+): component present in minor or variable amounts. Distribution of polypeptides 7, 8, 18 and 19 is accentuated by vertical rules. Polypeptides 1–8 have been shown by tryptic peptide mapping to be closely related (Schiller et al., 1982). Specimens of human normal and tumor tissue were obtained, in most cases, as biopsies taken during surgical removal of tumors, and were immediately frozen in isopentane chilled in liquid N₂. In a few cases (for example, cornea and tongue) tissue samples were taken from corpses 2–3 hr after death. All tissues and tumors used in this study were monitored histologically by stained sections. Tumors were classified according to the standards of the World Health Organization (1967–1981). Epithelial cells from small intestine and trachea were isolated mechanically by gentle scraping after incubation with EDTA-containing buffer A (see Franke et al., 1981b). Epithelia of the tongue and anal canal were obtained as thin, superficial tangential slices, with the use of a fine scalpel. Outer root sheaths of plucked human hair follicles were prepared as described by Moll et al. (1982b). From all other normal tissues and from most tumors, pure epithelial and carcinoma tissue was prepared under microscopic control by microdissection from 20 μm thick frozen sections as described by Moll et al. (1982a, 1982b, 1982c). Monolayer cultures of cell lines were grown as described or recommended by the following: HeLa cells and KB cells, Franke et al. (1979a, 1979c); Henle-407 cells (ATCC-CCL 6, “intestine-407”); MCF-7 cells derived from pleural effusion of a patient with metastatic mammary gland adenocarcinoma (see Soule et al., 1973; Lippman et al., 1976); A-431 cells, Fabricant et al. (1977); HT-29 cells, Fogh and Trempe (1975). Cell monolayers were labeled with ³⁵S-methionine as described by Franke et al. (1981c).

For the preparation of cytoskeletons highly enriched in intermediate-sized filaments, isolated small intestine and tracheal epithelial cells, as well as tongue and anal canal epithelia, were lysed and extracted with low and high salt buffers and Triton X-100 as described by Franke et al. (1981c). Outer root sheaths of hair follicles and microdissected tissue regions were extracted as described by Moll et al. (1982a, 1982b, 1982c). Cytoskeletons from monolayer cell cultures were prepared as described by Franke et al. (1981c).

^a Characteristics for epidermal keratins are in general agreement with relative molecular weight values estimated from SDS-polyacrylamide gel electrophoresis by Fuchs and Green (1980), except that their band “46K” corresponds to component 16 (relative molecular weight ~48,000) in our gel systems.

^b See also Moll et al. (1982b).

^c See also Gigi et al. (manuscript submitted).

^d See also Franke et al. (1981c).

^e See also Moll et al. (1982c).

^f The presence of cytokeratin in cultured human amnion epithelial cells has also been shown by Virtanen et al. (1981); detailed gel electrophoretic characterization will be presented elsewhere (R. Moll, S. Regauer, B. Ochs, I. Treiss, M. Cremer, E. Schmid and W. W. Franke, manuscript in preparation).

^g See also Schiller et al. (1982).

^h See also Denk et al. (1982).

ⁱ *n*: number of cases examined. Type 1 adenocarcinoma of colon was well differentiated; type 2, moderately differentiated. Of the cultured cell lines examined, HeLa, KB and Henle-407 cells also contained vimentin filaments.

^j See also Moll et al. (1982a).

^k See also Franke et al. (1982).

^l For relationship to HeLa cells, see text.

Table 1. Distribution of Major Cytokeratin Polypeptides in Different Human Epithelia, Carcinomas and Cultured Cells

No.	1	2	3	4	5	6
Molecular Weight ($\times 10^{-3}$)	68	65.5	63	59	58	56
Isoelectric pH	7.8	7.8	7.5	7.3	7.4	7.8
A. Normal Epithelia						
Epidermis (various locations) ^a	+	(+)			+	
Foot sole epidermis ^a	+	(+)			+	+
Anal canal epithelium	+	(+)			+	+
Hair follicle (outer root sheath) ^b					+	+
Sebaceous gland ^b				+	+	+
Cornea ^c			+		+	
Portio uteri (exocervix) ^d	+	(+)		+	+	+
Tongue epithelium ^d				+	+	+
Epiglottis epithelium ^e				+	+	+
Esophagus epithelium ^d				+	+	(+)
Apocrine gland from axilla (acini)				+	+	
Eccrine sweat gland (total) ^b					+	+
Mammary gland ducts ^e					+	
Tracheal epithelium ^e					+	(+)
Amnion epithelium ^f					+	+
Transitional epithelium of bladder					(+)	
Gallbladder epithelium ^e						
Small intestine (mucosa) ^g						
Colon (mucosa)						
Hepatocytes ^h						
B. Tumorsⁱ						
Hepatocellular carcinoma ($n = 5$) ^h						
Adenocarcinoma of colon (type 1, $n = 6$)						
Adenocarcinoma of colon (type 2, $n = 1$) ^e						
Adenocarcinoma of stomach ($n = 2$) ^e						
Adenocarcinoma of esophagus ($n = 3$) ^e						
Adenocarcinoma of pancreas ($n = 1$) ^e						
Ductal (adeno-) carcinoma of breast (type 1, $n = 4$) ^e						
Basal cell epithelioma ($n = 12$) ^b					+	(+)
Squamous cell carcinoma of skin ($n = 2$) ^e					+	+
Squamous cell carcinoma of tongue ($n = 2$)					+	+
Ductal carcinoma of breast (type 2, $n = 3$) ^e						+
Undifferentiated carcinoma of bronchus (large-cell type, $n = 3$) ^e						+
Solid carcinoma of maxillary sinus					+	
Adamantinoma ($n = 2$) ^b				+	+	
Squamous cell carcinoma of epiglottis ($n = 1$) ^e				+	+	+
Squamous cell carcinoma of esophagus ($n = 2$) ^e				(+)		+
Squamous cell carcinoma of rectal-anal region ($n = 4$)				(+)	+	+
Cloacogenic carcinoma ($n = 1$) ^j	+				+	(+)
C. Cultured Cell Lines						
A-431 (epidermoid carcinoma of vulva)					+	(+)
HeLa (cervical adenocarcinoma) ^d						
Henle-407 (embryonal intestine) ^{kl}						
KB (epidermoid carcinoma of mouth) ^{kl}						
MCF-7 (breast adenocarcinoma) ^k						
HT-29 (colon adenocarcinoma)						

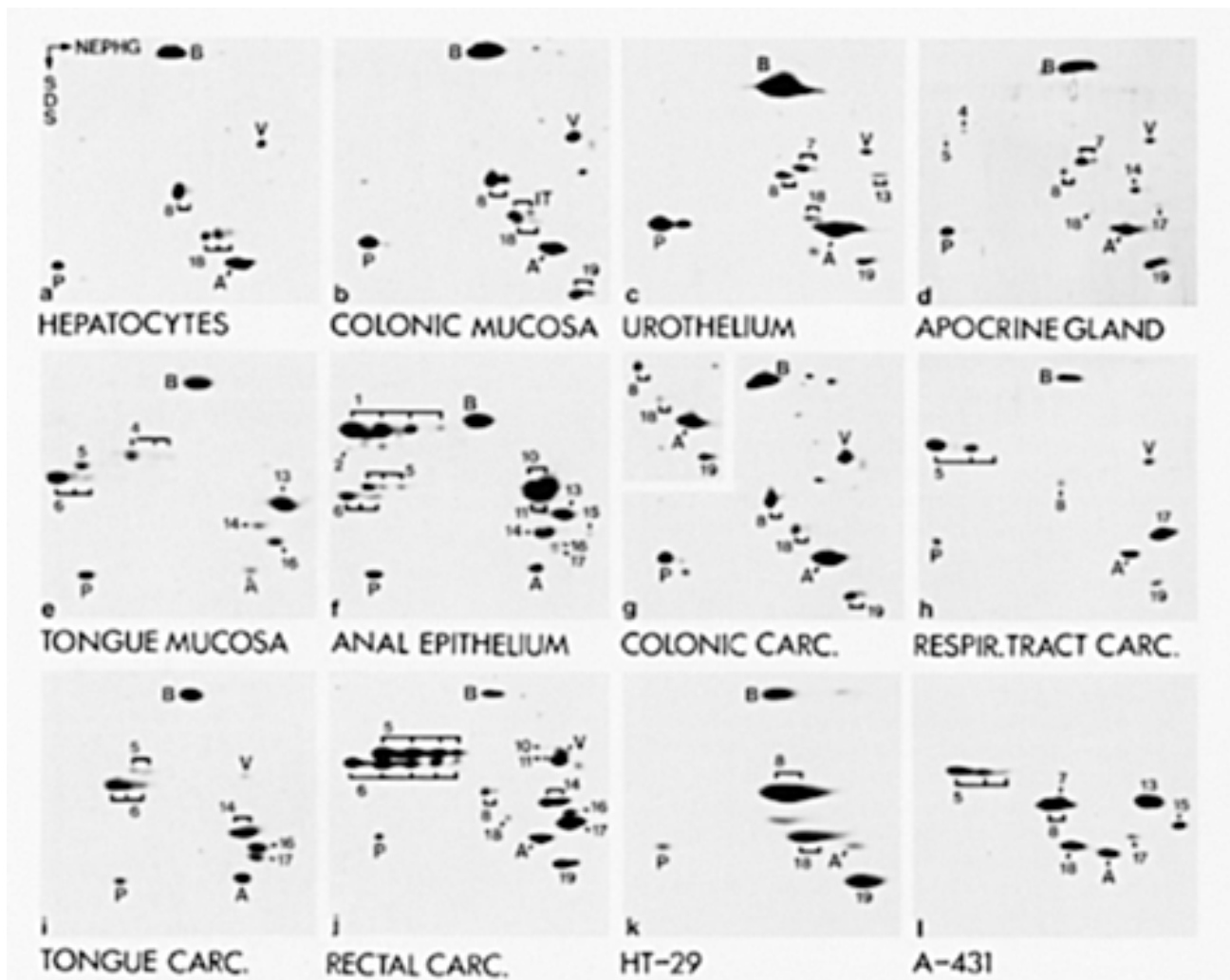


Figure 2. Diversity of Cytokeratin Polypeptide Patterns in Some Human Epithelia, Carcinomas and Cultured Cells

Two-dimensional gel electrophoresis of cytoskeletal proteins was performed with nonequilibrium pH gradient (NEPHG) electrophoresis in the first dimension (horizontal arrow; basic polypeptides to the left, acidic ones to the right). Vertical arrow (SDS): direction of second dimension. (a-d, g, h and j) Silver staining of proteins of microdissected epithelia; (e, f, i and k) Coomassie-blue-stained gels; (l) autoradiograph. Marker polypeptides (see Figure 1) were coelectrophoresed. P: 3-phosphoglycerokinase (relative molecular weight, 43,000; isoelectric at pH 7.4). B: bovine serum albumin (relative molecular weight, 68,000; major variant isoelectric at pH 6.35). A: α actin (relative molecular weight, 42,000; isoelectric at pH 5.4). V: vimentin, present in mesenchymal cells of lamina propria or tumor stroma. IT: component observed in variable amounts in intestinal mucosa, including colon, that is positive in reactions with cytokeratin antibodies but may represent a degradation product (see Franke et al., 1981b).

(a) Hepatocytes of liver (microdissected lobules devoid of bile ducts). (b) Colonic mucosa (microdissected basal regions from crypts). (c) Transitional epithelium (urothelium) of urinary bladder (component 5 is detected only if very high concentrations of proteins are loaded on the gel; data not shown). (d) Acini of apocrine glands of axillary skin. (e) Tongue mucosa. (f) Total anal canal epithelium, including parts of anal glands. (g) Well differentiated adenocarcinoma (CARC.) of colon (same tumor as shown in Figure 3); primary tumor; (inset) lymph node metastasis from the same patient, displaying the same cytokeratin polypeptide pattern. (h) Solid carcinoma of respiratory (RESPIR.) tract epithelium (sinus maxillaris); lymph node metastasis. (i) Squamous cell carcinoma (CARC.) of tongue; primary tumor. (j) Squamous cell carcinoma (CARC.) of anorectal region, primary tumor. (k) Cultured HT-29 cells. (l) Cultured A-431 cells (autoradiograph of ^{35}S -methionine-labeled cytoskeletons; components 6, 14 and 16 are present in minor amounts and can be detected only upon longer exposure of autoradiographs or by immunoblotting).

keratin polypeptides in epithelia of complex organs (Table 1A) it is important to examine defined cell types, such as those obtained by microdissection from frozen tissue sections (for procedures see Moll et al., 1982a, 1982b, 1982c). It is apparent that the patterns of cytokeratin polypeptides expressed in different epithelia present certain principles. Basic cytokeratins are lacking in bladder epithelium and in simple epithelia

of liver, gallbladder and intestine, which instead produce relatively large proportions of polypeptides 8 and 18, and also partly polypeptides 7 and 19 (see, for example, Figures 2a-2c; Moll et al., 1982c). Tracheal epithelium and complex glands express polypeptides 7, 8, 18 and 19 but also synthesize basic cytokeratins in various amounts and patterns (see, for example, Figure 2d; see also Moll et al., 1982c). In

stratified squamous epithelia other than epidermis, one finds basic cytokeratins and usually also minor amounts of polypeptide 19. Cells of epidermis and pilosebaceous tract produce various patterns of basic cytokeratins but do not express significant amounts of cytokeratins 7, 8, 18 and 19 (Moll et al., 1982b; see Fuchs and Green, 1980).

Cytokeratin Polypeptides of Human Tumors

Carcinomas can be positively identified, with immunofluorescence microscopy on frozen sections of biopsies, by their reaction with antibodies to both epidermal and hepatic cytokeratins (Figures 3a and 3b), as well as with antibodies to certain desmosomal-plaque proteins (see Franke et al., 1981d). In all cases examined, positive reaction for cytokeratins and desmosomal-plaque proteins has been found to be correlated with absence of antibody reaction to vimentin (see, for example, Figure 3c) and desmin (data not shown; Altmannsberger et al., 1981; Gabbiani et al., 1981; Franke et al., 1981f; Osborn et al., 1981; Ramaekers et al., 1981). In most carcinomas, vimentin antibodies specifically stain the stromal components between the carcinoma cells (see, for example, Figure 3c), in agreement with the exclusively mesenchymal origin of stromal tissue. In certain critical cases, especially with metastases, such immunofluorescent staining for vimentin can also be performed, in serial sections, directly in conjunction with the microdissection procedure to monitor and minimize stromal tissue contributions to the carcinoma samples analyzed (for details see Moll et al., 1982a, 1982c). Metastatic material that we have examined has been mostly from lymph node metastases, since normal lymph node tissue does not contain cytokeratins and contributes only vimentin as the sole endogenous intermediate-filament protein.

Adenocarcinomas of the Digestive Tract and Associated Glands

We have compared cytokeratin polypeptide patterns of normal epithelia with those of benign and malignant primary tumors, as well as with those from carcinoma metastases, by two-dimensional gel electrophoresis (Table 1B). The pattern of cytokeratin polypeptides from normal human colonic mucosa (Figure 2b), for example, is identical with the patterns of the most common type of primary adenocarcinoma of colon (Figure 2g; type 1; same tumor as shown by immunofluorescence in Figure 3) and of the corresponding lymph node metastases (Figure 2g, inset). This has been found in a total of six well differentiated colonic adenocarcinomas examined that all contained, in primary tumors as in metastases, the same major cytokeratins (8, 18 and 19). Only in one case of colonic adenocarcinoma, which showed a less differentiated morphology, has a very small amount of cytokeratin

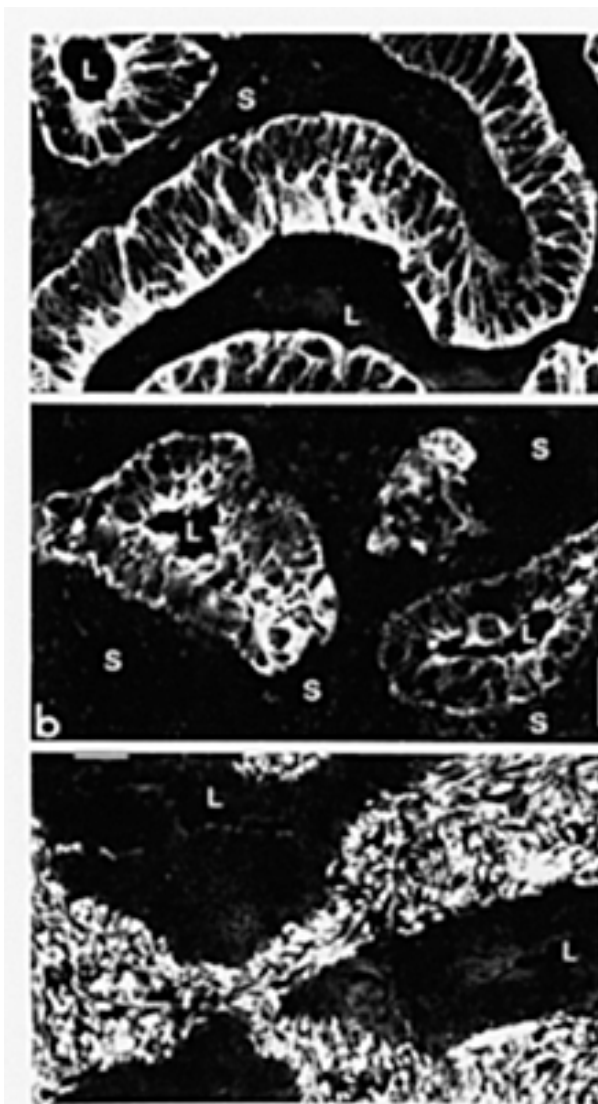


Figure 3. Identification of Carcinoma Cells by Epithelial and Mesenchymal Markers with Immunofluorescence Microscopy

Guinea pig antibodies against various cytokeratins and vimentin (Franke et al., 1978b, 1979c, 1980, 1981a, 1981b, 1981c; Moll et al., 1982b) were used on frozen sections of primary tumor (a) and lymph node metastasis (b, c) of an adenocarcinoma of the colon with tubular differentiation. (a and b) Antibodies to prekeratin stain cytokeratin filaments in tumor cells arranged in tubular structures (lumen; L) in the primary tumor (a) as well as in the lymph node metastasis (b), whereas stromal (S) cells are negative. (c) Antibodies to vimentin stain mesenchymal cells of stroma (lymph node metastasis), whereas the carcinoma cell tubules remain unstained. Each bar = 40 μ m.

17 been detected in addition to cytokeratins 8, 18 and 19, again in both primary tumor and metastasis (Table 1B; for details see Moll et al., 1982c; the exact cell type of derivation of this tumor is not clear). Similar cytokeratin polypeptide patterns are found in adenocarcinomas of the stomach, pancreas and the lower part of the esophagus (Table 1B; Moll et al., 1982c). An even more simplified pattern is characteristic of hepatocellular carcinomas, in which only polypeptides

8 and 18 have been detected (Denk et al., 1982), identical with the major cytokeratins A and D of hepatocytes of man, mouse and rat (Franke et al., 1981a; Denk et al., 1982; Schiller et al., 1982). In none of these adenocarcinomas derived from the gastrointestinal tract, pancreas and liver have we found any of the cytokeratins (1–6) more basic than bovine serum albumin. A similar simple cytokeratin pattern, showing only polypeptides 7, 8, 18 and 19, has been found in four cases of a certain type of ductal carcinoma of mammary gland containing some tubular structures (type 1; Table 1B). Here the tumor reveals a more simple cytokeratin pattern than the ducts of normal mammary gland, which, however, are complex in cell type composition (Moll et al., 1982c; see Franke et al., 1980; Asch et al., 1981; Krepler et al., 1981).

Basal Cell Epitheliomas and Squamous Cell Carcinomas Lacking Cytokeratins 7, 8, 18 and 19

Tumors (primary tumors) of this group show a cytokeratin pattern profoundly different from that of the adenocarcinomas described above (Table 1B). For example, basal cell epitheliomas (Moll et al., 1982b; see also Kubilus et al., 1980) and squamous cell carcinomas of the skin (Moll et al., 1982c; see also Bretkreutz et al., 1981) as well as squamous cell carcinomas of the tongue (Figure 2i) are characterized by the presence of basic cytokeratins, predominantly polypeptides 5 and 6 in various proportions, and a specific set of acidic cytokeratin polypeptides (14–17) occurring in various combinations. In contrast, cytokeratins 7, 18 and 19 are not detected in these tumors in appreciable amounts, and cytokeratin 8 has been found, in minor amounts, only in some basal cell epitheliomas (Moll et al., 1982b). Comparison with normal tissue shows that the cytokeratin polypeptide pattern of the basal cell epitheliomas is very similar to that obtained with epithelia of the microdissected pilosebaceous tract, but considerably different from that of whole epidermis, including samples of microdissected interfollicular epidermis and laser-dissected basal cell layers (Moll et al., 1982b). Striking similarity but not identity of cytokeratin patterns is also noted between tongue mucosa and lingual squamous carcinomas (Figures 2e and 2i). The major difference between this tumor and samples of total tongue mucosal tissue is the absence of cytokeratins 4 and 13 in tumor cytoskeletons.

Carcinomas Containing a Basic Cytokeratin in Addition to Cytokeratins 7, 8, 18 and 19

One type of ductal carcinomas of mammary gland and carcinomas derived from bronchiolar epithelium or other parts of the respiratory tract contain not only cytokeratins 7, 8, 18 and 19 but also a neutral-to-basic cytokeratin, usually polypeptide 5 or 6 (an example is shown in Figure 2h; Table 1B). Again, essentially identical patterns have been found in primary

tumors and metastases. Comparison of the tumor cytokeratins with those present in the corresponding tissue of origin (Moll et al., 1982c) has revealed a high degree of similarity, although some differences can also be noted, perhaps due to the derivation of the tumor from specific cell types present in the complex tissue.

Tumors with Very Complex Cytokeratin Patterns Showing Various Combinations of Basic and Acidic Cytokeratins

Cytokeratin patterns considerably more complex than those of both the adenocarcinomas of the alimentary tract and the epidermal tumors are found in primary tumors and metastases of certain types of squamous cell carcinomas (an example of an anorectal squamous cell carcinoma is shown in Figure 2j) and in adamantinomas (Moll et al., 1982b). Several cytokeratins of these carcinomas (most characteristically the basic components 4–6) are also found in the corresponding normal epithelia. However, some carcinoma cytokeratins have not been observed, in significant amounts, in the normal tissue (for example, components 8 and 18 of rectal and epiglottis carcinomas and components 14, 16 and 17 of esophagus squamous cell carcinomas; see Tables 1A and 1B). Again, component 13, frequently a major cytoskeletal polypeptide in normal tissues, is not found in such carcinomas.

Certain relatively rare tumors expressing an unusual wealth of cytokeratin polypeptides in both primary tumors and metastases have also been found. For example, a solid, noncornified cloacogenic carcinoma apparently derived from the rectal–anal transition zone (“basaloid carcinoma,” “transitional cell carcinoma”; Moll et al., 1982a, 1982c) contains, in the primary tumor as well as in the lymph node metastasis, as many as 12 of the 19 known human cytokeratin polypeptides, including components 7, 8 and 19 as well as the basic cytokeratins 5 and 6, and even component 1, which is typical of keratinizing layers of epidermis. Such an example, in particular the lymph node metastasis, indicates that a high complexity of cytokeratin composition can occur in the same cell or cell type and does not necessarily reflect cell type heterogeneity in the specific sample of tissue.

Cytokeratin Polypeptides of Cultured Human Cells

We have previously described that HeLa cells, a line derived from cervical adenocarcinoma, contain a pattern of cytokeratin polypeptides different from that of all tissues so far examined (Franke et al., 1979a, 1981c). Likewise, cytokeratin patterns of cultures of human keratinocytes and other cells from multistratified epithelia and squamous cell carcinomas (Fuchs and Green, 1978, 1981; Sun and Green, 1978b; Doran et al., 1980; Wu and Rheinwald, 1981) have

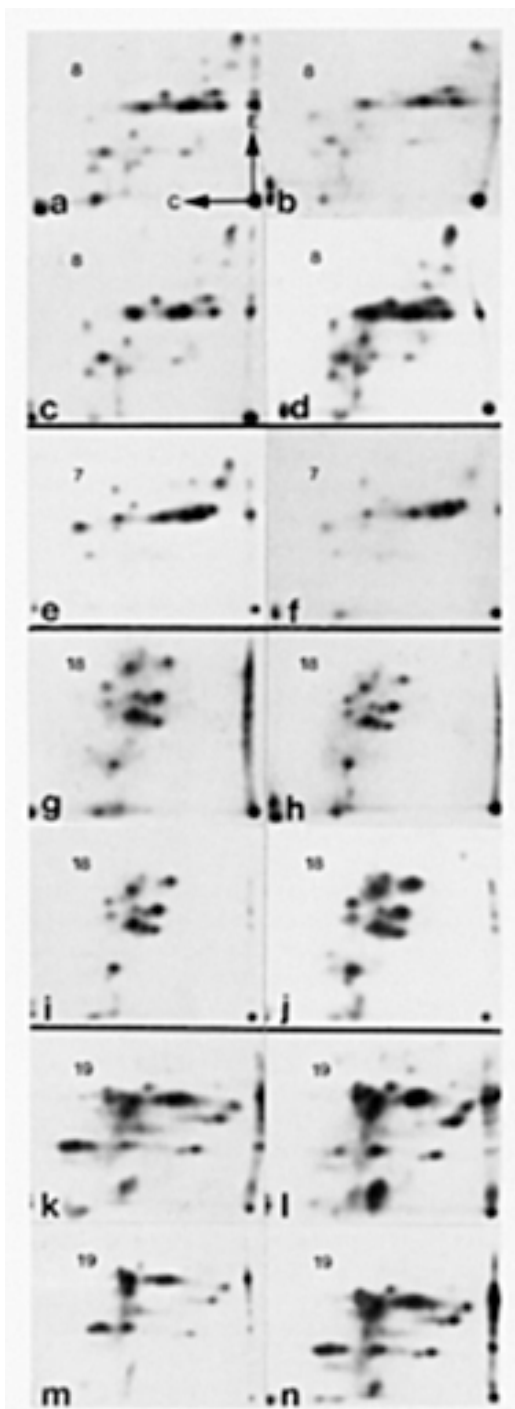


Figure 4. Identification of Some Human Cytokeratin Polypeptides by Tryptic Peptide Mapping

Individual polypeptides separated by two-dimensional gel electrophoresis were excised, radioiodinated and digested by trypsin, and the resulting peptides were analyzed by thin-layer electrophoresis and chromatography (Elder et al., 1977; see Schiller et al., 1982). Autoradiographs of tryptic peptide maps of the following cytokeratin polypeptides from diverse cells are compared. (a) Polypeptide 8 from small intestine; (b) polypeptide 8 from HeLa cells; (c) polypeptide 8 from A-431 cells; (d) polypeptide 8 from bronchial carcinoma (large cell type); (e) polypeptide 7 from Henle-407 cells; (f) polypeptide 7 from bronchial carcinoma; (g) polypeptide 18 from small intestine; (h)

been described, but could not be decisively correlated with the cytokeratins present in the corresponding tissue. A more systematic examination of the cytokeratin polypeptides of different human cell cultures of epithelial origin (Franke et al., 1982; Table 1C) has revealed that different human epithelium-derived cell lines produce tonofilaments containing different sets of cytokeratin polypeptides. The most complex pattern of cytokeratin polypeptides, including the expression of basic cytokeratins, has been found in the cell line A-431, derived from an epidermoid carcinoma of vulva that is characterized by spinous cell-like growth patterns and high density of receptors for epidermal growth factor (Fabricant et al., 1977; Schlessinger and Geiger, 1981). Clonal sublines of this cell continue to express as many as ten different cytokeratin polypeptides (Figure 2i; Table 1C). Coelectrophoresis with cytokeratins from various human tissues has demonstrated the identity of each of these with a specific cytokeratin polypeptide present in one or another normal epithelium (Franke et al., 1982). Most other cell lines express only three or four cytokeratin polypeptides, which in all cases examined include components 8 and 18. Some cell lines (for example, MCF-7 and HT-29) produce the small and acidic cytokeratin 19, which recently has also been identified in some other human cell cultures (Fuchs and Green, 1981; Wu and Rheinwald, 1981). Again, coelectrophoresis with cytokeratins from normal tissues and tumors (Franke et al., 1982) and comparison of tryptic peptide maps (Figure 4) has shown that the various cytokeratin polypeptides from these cultured cells are identical to specific cytokeratins expressed in normal tissues and in tumors. Whereas for some of the cell cultures the cytokeratin polypeptide patterns appear to be related to that of the tissue of origin (compare, for example, HT-29 and MCF-7 with intestinal mucosa and ductal breast carcinoma of type 1, respectively; Table 1; Figure 2b, 2g and 2k), correlations with identified patterns of specific normal cell types are not yet possible in other cell lines, such as HeLa and A-431.

Unexpected cytokeratin patterns have been found in certain human cell lines that show no relationship to the assumed tissue of origin but that contain a set of cytokeratins identical with that of HeLa cells. Examples include (Table 1C; for details see Franke et al., 1982) KB cells and the Henle-Deinhardt cell line 407, which show a cytokeratin pattern practically identical with that of several sublines and clones of

polypeptide 18 from HeLa cells; (i) polypeptide 18 from Henle-407 cells; (j) polypeptide 18 from MCF-7 cells; (k) polypeptide 19 from small intestine; (l) polypeptide 19 from MCF-7 cells; (m) polypeptide 19 from cloacogenic carcinoma; (n) polypeptide 19 from bronchial carcinoma. Note the identical maps for corresponding cytokeratins from different cells, for polypeptide 8 (a-d), 7 (e and f), 18 (g-j) and 19 (k-n).

HeLa cells (Franke et al., 1982; see also Franke et al., 1981c; Bravo et al., 1982). All four cytokeratin polypeptides of KB and Henle-407 cells were identical by comigration and tryptic peptide maps with the four HeLa cytokeratins (see, for example, Figures 4h and 4i; see also Franke et al., 1982). Cell lines showing the HeLa-type cytokeratin polypeptide pattern are known to be positive for chromosomal HeLa markers (The American Type Culture Collection, 1979, catalog of strains II).

Peptide map comparisons of cytokeratins (for some examples see Figure 4) also have yielded identical results for the cytokeratin polypeptides excised after two-dimensional gel electrophoresis from normal tissue and tumor cells. Moreover, comparison of such peptide maps with those obtained from the corresponding polypeptides of other species (data not shown) have revealed close relatedness across species (rat, mouse, cow, man; Schiller et al., 1982), indicating that the diverse cytokeratins have been rather stably conserved during vertebrate evolution.

Conclusions and Perspectives

Cytokeratins are a multigene family of polypeptides expressed in different sets during different routes of epithelial differentiation. As many as ten different α -keratin polypeptides have been described in human epidermis and hair follicles (Baden and Lee, 1978; Fuchs and Green, 1978, 1979, 1980, 1981; Bowden and Cunliffe, 1981; Moll et al., 1982b; for earlier studies see also Skerrow, 1977), and most of them have been shown to be products of distinct mRNAs (Fuchs and Green, 1979). Similar results have been reported in guinea pig (Gibbs and Freedberg, 1982) and cow (Drochmans et al., 1978; Steinert et al., 1980; Franke et al., 1981c; Schiller et al., 1982), and in the latter species it has been demonstrated that all nine cytokeratin polypeptides detected in snout epidermis are products of specific mRNAs (Schiller et al., 1982). A number of nonepidermal cytokeratin polypeptides have recently been recognized in various organisms, including a conspicuous small and acidic cytokeratin (19) that is widespread in various species and cell types (Franke et al., 1981b, 1981c; Fuchs and Green, 1981; Wu and Rheinwald, 1981). We can currently distinguish at least 19 different human cytokeratin polypeptides (Figure 1; Table 1). Most of these can also be found when whole cells or tissue samples are directly denatured in boiling lysis buffer, and many of them have also been identified as products of translation *in vitro*, with poly(A)⁺ mRNA fractions from various tissues and tumors as well as HeLa, MCF-7 and A-431 cells (J. Jorcano and W. W. Franke, unpublished data). We therefore consider the majority of these cytokeratin polypeptides as genuine products of translation and not as fragments proteolytically derived from precursor molecules. Although a number

of epithelia still have not been examined in this respect, we suppose that the total number of human polypeptides of this family will not increase much over 20. Similarly complex patterns of cytokeratin polypeptides have been found in bovine and murine cells (Winter et al., 1980; Franke et al., 1981f; Schiller et al., 1982; for murine epidermal keratins see also Schweizer and Winter, 1982).

The finding that the diverse cytokeratins are expressed in specific sets of polypeptides that are characteristic of certain cell types allows us to distinguish and classify epithelial cells with respect to their specific cytokeratin pattern. For example, the nonstratified epithelia of the digestive tract and its associated glands and ducts (liver, pancreas, gallbladder) are characterized by a very simple cytokeratin composition, containing only two to four major polypeptides. These invariably include components 8 and 18, which also appear to be the first cytokeratins expressed during early embryogenesis (Jackson et al., 1980, 1981), but neutral-to-basic cytokeratins are not found in these cells. Tracheal epithelium and several complex glands contain both basic cytokeratins and polypeptides 7, 8, 18 and 19. The other extreme is represented by the epidermis and several other stratified squamous epithelia, which lack the cytokeratins typical of the simple epithelia of the digestive tract but instead express a wealth of other acidic and basic cytokeratins (Table 1). Some of these cytokeratin polypeptides found in multilayered epithelia may be, as has been shown in detail in epidermis (Fuchs and Green, 1980; Banks-Schlegel, 1982), tissue-specific products of differentiation exclusive to upper strata. Another problem of assignment of cytokeratin patterns to specific types of cells comes from the cell heterogeneity of many epithelial tissues. For example, mammary gland epithelium consists of at least three different epithelial cell types (myoepithelial, ductal and secretory cells) that may contain different cytokeratin polypeptides, since they have already been shown to exhibit different antigenic determinants in their cytokeratin filaments (Franke et al., 1980; Asch et al., 1981; Krepler et al., 1981). Even more complex is skin, in which so far no less than seven different cell types have been distinguished by their cytokeratin patterns, including diverse types of interfollicular epidermal cells, cells of hair follicle root sheath and cells of sebaceous and sweat glands (Table 1; Moll et al., 1982b). Clearly, more analyses of cytoskeletal proteins from microdissected cell groups are needed to define true cell type patterns of cytokeratins.

Biochemical analysis of cytoskeletons of human carcinomas shows that tumors derived from different types of epithelia also display different cytokeratin polypeptide patterns that are characteristic of certain groups of tumors. The patterns of cytokeratins of a given type of tumor appear to be identical in primary tumors and metastases, independent from the specific

location and size. The complexity of cytokeratin patterns also shows great differences in different tumors. In general, epithelium-derived tumors appear to maintain the expression of many of the cytokeratin polypeptides typical of the specific nontransformed cells. In certain cases, such as adenocarcinomas of the digestive tract and hepatocellular carcinomas, identical cytokeratin patterns are found in the carcinoma and the putative cell type of origin. Many other types of tumors, especially those derived from stratified epithelia and other complex epithelia, display a far-reaching similarity of cytokeratin patterns with those of normal tissue, allowing a reasonably good correlation. The observation in several tumors that some cytokeratin polypeptides found in the normal tissues are not detected in the specific tumors could be explained by the absence of expression of cytokeratins that are typical for upper strata in multilayered epithelia, as indicated by our data (for example, the absence of component 1 or 13) in tumors of the skin, tongue, epiglottis and esophagus. Alternatively, such differences may reflect selection, during cell transformation and tumor development, of a cell type that is not a quantitatively predominant one in the total tissue used for comparison. Cell type heterogeneity in epithelial tissues and selection might also provide a plausible explanation for cases in which a certain cytokeratin polypeptide is predominant in a tumor but not in the whole tissue from which this tumor has emerged. Our finding that the cytokeratin pattern of basal cell epitheliomas is related to the epithelium of the pilosebaceous tract, and probably also its precursor cells, but not to that of interfollicular epidermis, including dissected basal cell layer (Moll et al., 1982b), is a good example for this. Future analyses will have to show whether it is possible to relate the cytokeratin patterns of specific tumors to specific cell types. However, the alternative explanation, that the nutritional state of a tumor influences the specific expression of cytokeratin polypeptides, cannot be dismissed at the moment, in view of demonstrations of effects of vitamin A on cytokeratin expression in cultured human keratinocytes (Fuchs and Green, 1981) and of hormonal effects on cytoskeletal composition in cultured bovine mammary gland cells (Schmid et al., 1982). It will also be important to examine, in certain tumors such as "keratinizing" squamous cell carcinomas, the possibility of regional heterogeneities of the state of differentiation within one and the same tumor.

The observation of diversity of expression of cytokeratins in different epithelium-derived tumors, together with the general tendency toward maintenance of cytokeratin polypeptide patterns during malignant growth and metastasis, should serve as a basis for a novel approach to tumor characterization, with cytokeratins as differentiation markers. Although gel electrophoresis of cytoskeletal proteins from small biopsy samples is feasible, with silver staining techniques,

the strategy for histodiagnosis that is obviously more practical would be the use of monoclonal antibodies specific to certain cytokeratin polypeptides. Different reactivities of cytokeratin antibodies with the specific cytokeratins of different epithelia have been noted by several authors with conventional antisera (Franke et al., 1978a, 1980, 1981a, 1981c; Sun et al., 1979; Schlegel et al., 1980a, 1980b; Wu and Rheinwald, 1981; Asch et al., 1981) or monoclonal antibodies (Lane and Klymkowsky, 1981; Lane, 1982; Gigi et al., manuscript submitted). Such characterization of tumors with respect to their cytokeratin polypeptide patterns should contribute to an improved tumor diagnosis and classification, especially in cases of undifferentiated metastases.

As different epithelial tissues and tumors can be distinguished and characterized by their specific cytokeratin polypeptide patterns, so different cell culture lines from epithelia or carcinomas also display different patterns of expression of cytokeratin polypeptides. We have previously shown (Franke et al., 1981e) that cultured rat hepatocytes and hepatoma cells show a tendency to continue expression of the hepatocyte-type cytokeratins, although minor differences have been noted in some lines. Loss of larger (and basic) α keratins with maintenance of expression of smaller cytokeratins has been noted in cultures of human and murine keratinocytes (Fuchs and Green, 1978, 1979, 1980, 1981; Sun and Green, 1978b; Steinert and Yuspa, 1978; Franke et al., 1979b; Doran et al., 1980; Wu and Rheinwald, 1981). In some cell lines we have found rather simple cytokeratin patterns related to those of the tissue of origin (MCF-7; HT-29), whereas some carcinoma cell lines (for example, A-431) show clonally stable cytokeratin patterns of a bewildering complexity, including the basic cytokeratin 5. Whether it will be possible to identify, by the use of the cytokeratin polypeptide patterns as marker molecules, the specific cell type from which a given cultured cell line has actually been derived has to await more detailed comparisons. Complex patterns of cells such as A-431 also demonstrate that as many as ten different cytokeratin polypeptides can be synthesized in the same cell. It would also be important to know whether these different polypeptides are located in the same type of tonofilament, or whether compositionally different types of filaments exist in these cells.

As tissues and tumors, cultured epithelial and carcinoma cells may also be characterized by their cytoskeletal composition (presence of vimentin and specific cytokeratin pattern), and this should also be a valuable nonmorphological criterion for identifying cells. For example, HeLa cells have a very distinct and uncommon cytokeratin pattern that appears to be very stable. Since contamination of cell cultures with HeLa cells is a worldwide tantalizing problem, the presence of HeLa-cell-type cytokeratin polypeptides 7, 8, 17

and 18 provides an additional marker to the chromosomal markers used so far.

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