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# Distribution of TPA and cytokeratins in gastrointestinal carcinomas as revealed by immunohistochemistry

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**Summary.** The presence and distribution of tissue polypeptide antigen (TPA) were assessed in gastrointestinal carcinomas of different origin, morphology and degree of differentiation. Immunocytochemistry was employed, using the PAP technique on formalin-fixed, paraffin-embedded material and compared with the results obtained with antibodies to cytokeratins. Like cytokeratins, TPA was a reliable marker of epithelial differentiation and showed tissue distribution patterns similar to cytokeratins, as revealed by antibodies with broad-range cytokeratin immunoreactivity. In most carcinomas, TPA-specific immunostaining was less intense than in non-neoplastic tissue. No direct relationship between intensity of TPA staining and morphological degree of differentiation and proliferation was found. TPA staining was most pronounced at the periphery of the cells. In stratified epithelium, i.e. oesophageal mucosa, basally located cells exceeded superficial cells in TPA immunoreactivity in contrast to the cytokeratin antibodies which decorated the more superficially placed cell layers. TPA and cytokeratin staining patterns were similar in neoplastic and non-neoplastic gastric, intestinal mucosa, as well as in biliary tract epithelium. Antral and cardial mucoid glands of the stomach as well as gastric carcinomas of the pylorocardial type remained unstained with both types of antibodies. Similar staining with TPA and cytokeratin antibodies was also observed in pancreatic and liver tissue. In this study, hepatocytes were, although weakly, stained by TPA antibodies and an identical staining was found with benign and malignant hepatocellular neoplasms. Ductal and ductular TPA-staining was most conspicuous and so was the immunoreactivity of cholangiocellular carcinomas. A comparison between TPA and cytokeratins was also made by immunoblotting which revealed immunoreactivity of antibodies to TPA with cytokeratin polypeptides of different species (man, mouse) and organs (epidermis, liver), particularly with the cytoker-

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atin component 8 of human liver and the related component A of mouse liver. The significance of this finding is uncertain until the pertinent epitopes have been revealed by monoclonal mapping of the components which exhibit similar molecular weights by SDS polyacrylamide gel electrophoresis.

**Key words:** Gastrointestinal carcinomas – Tissue polypeptide antigen (TPA) – Cytokeratins – Immunohistochemistry

## Introduction

Tissue polypeptide antigen (TPA) was originally purified from diverse carcinomas or HeLa cells and is a serum marker of proliferative activity of carcinomas (Björklund 1980, see for review and further information). Immunohistochemically, TPA is detectable in neoplastic as well as non-neoplastic non-epidermal epithelia. In cancer it is released at higher concentrations into serum and urine (Björklund 1980; Björklund et al. 1973; Menendez-Botet et al. 1978; Lüthgens and Schlegel 1983; Lüthgens and Schlegel 1985; Oehr et al. 1985; Oehr et al. 1984a; Oehr et al. 1984b; Oehr and Vogel 1985; Oehr et al. 1984c; Oehr and Winkler 1984). TPA is also produced by cultured carcinoma cell lines and released into the culture medium. It is not species-specific (Björklund 1980).

Clinical studies have revealed a significant correlation between serum TPA-levels and the presence of carcinoma, the TPA test being more sensitive and reliable in tumour diagnosis than determination of, for example, CEA (Björklund 1980; Lüthgens and Schlegel 1983; Oehr et al. 1985; Vogel and Oehr 1985; Klavins and Cho 1985; Takahashi 1983; Guarino and Taccone 1985). In colonic carcinomas an inverse relationship between serum TPA levels and the degree of tumour differentiation was noted (Björklund 1980). Consequently, elevated TPA serum levels seem to reflect poor prognosis (Björklund 1980; Björklund et al. 1973; Oehr and Winkler 1984). Purified preparations of TPA, as revealed by SDS-polyacrylamide gel electrophoresis, contain several polypeptide components with molecular weights ranging from below 20,000 to 45,000. The major polypeptide fraction, subunit B2, exhibits a molecular weight of 43,000 (Lüning et al. 1980; Wiklund et al. 1981). Amino acid analyses revealed a similar sequence for peptides isolated from TPA-active fractions derived from bronchial carcinomas, pooled carcinomas of different origins or pooled placental tissue (Redelius et al. 1980). Whether this sequence is related to clinical TPA-activity remains to be seen. Several biochemical and immunological features of TPA such as molecular weights of major polypeptides, solubility characteristics, epithelial specificity, lack of species specificity and cellular localization, suggest some sort of relationship between TPA and intermediate filament proteins of the cytokeratin type (Moll et al. 1982; Weber et al. 1984).

In the present study, we compared the immunolocalization of some cytokeratins and TPA in gastrointestinal tumors. We also compared the reactivity of antibodies to preparations of TPA with cytokeratin polypeptides

**Table 1.** Carcinomas investigated immunohistochemically for TPA and cytokeratin content and distribution

| Organ | N | Histological type |
|---|---|---|
| Oesophagus | 1 | Squamous cell carcinoma |
| Stomach | 4 | Adenocarcinoma, intestinal |
| | 1 | Pylorocardial |
| | 1 | Diffuse (signet ring cell carcinoma) |
| Papilla of Vater | 1 | Adenocarcinoma, tubulo-papillary |
| Gall bladder | 4 | Adenocarcinoma, tubular |
| Pancreas | 2 | Adenocarcinoma, tubular |
| Colon | 7 | Adenocarcinoma, tubular |
| Liver | 3 | Hepatocellular carcinoma |
| | 3 | Cholangiocellular carcinoma |
| | 27 | |

after transfer to nitrocellulose paper by blotting. Close similarities were observed between the immunohistochemical images as revealed by TPA and cytokeratin antibodies. TPA antibodies also recognized isolated and denatured cytokeratin polypeptides in immunoblots. The nature of this relationship, however, remains to be clarified.

## Material and methods

*Gastrointestinal tumors.* 27 carcinomas of the gastrointestinal tract were studied (Table 1). In addition, the following specimens (number of specimens within parentheses) were included: tubular adenoma of gastric mucosa (2), normal pancreatic parenchyma (2), colonic tubular adenoma (5), non-specific chronic colitis (2), focal nodular hyperplasia of the liver (1), hepatocellular adenoma (2). The material was obtained either by surgery or at autopsy. It was fixed immediately after removal in 10% (phosphate-buffered, pH 7.4) formaldehyde solution and paraffin-embedded according to conventional procedures. Consecutive sections, 5 μm thick, were stained with haematoxylin-eosin, other routine stains and were immunostained.

*Immunohistochemistry*

For the demonstration of TPA and cytokeratins the peroxidase-anti-peroxidase-(PAP) method (Sternberger 1979) was used with some modifications (Denk et al. 1977). Briefly, the paraffin sections were rehydrated after removal of paraffin by xylene and pretreated with 0.1% protease (type VIII, Sigma Chem. Comp., St. Louis, Mo) for 30 min at room temperature. The following antibodies were used:

*(i)* Affinity-purified antibodies to TPA raised in rabbits (Björklund 1980; Björklund et al. 1982; Nathrath et al. 1984; Nathrath et al. 1985; Vogel and Oehr 1985; Löning et al. 1983; dilution 1:200). In this study sensitivity was enhanced by using 24 h incubation, in some instances, e.g. liver, however, a 15 min incubation was also carried out. Incubation with primary anti-TPA was carried out at 15 min at room temperature by previous authors.

*(ii)* Guinea pig antibodies to mouse liver cytokeratin component D showing a broad range of cytokeratin reactivity (dilution 1:3000; Denk et al. 1981; Franke et al. 1981);

*(iii)* Binding of primary guinea pig antibodies was assessed by subsequent incubation with antibodies to guinea pig IgG (from rabbit; Behring Werke, Marburg, Germany);

*(iv)* Binding of rabbit antibodies was assessed by subsequent incubation with antibodies to rabbit IgG (from pig; Dako, Copenhagen, Denmark) forming a bridge between the rabbit antibody layer and a peroxidase- (rabbit) antiperoxidase-(PAP)-complex (Dako, Copenhagen, Denmark; dilution 1:50). Peroxidase activity was visualized by the 3′,3′-diaminobenzidine (DAB) color reaction (Denk et al. 1977; Sternberger 1979). The sections were then washed, dehydrated and coverslipped.

*Immunoblotting*

The immunoblotting procedure followed the method of Towbin et al. (1979) as described previously (Denk et al. 1981; Franke et al. 1981). Briefly, cytokeratins were purified as high salt-detergent resistant residues from human epidermis, human liver and mouse liver, and separated by 1-dimensional SDS-polyacrylamide gel electrophoresis. The polypeptides were electrophoretically transferred to nitrocellulose paper sheets and their reactivity was assessed by incubation with antibodies to TPA (from rabbit) followed by pig antibodies to rabbit IgG (see immunohistochemistry) and the PAP-reaction (Sternberger 1979). Peroxidase activity was revealed by the DAB-color reaction.

## Results

### *Immunocytochemistry of TPA and cytokeratins in gastrointestinal carcinomas*

*Oesophagus.* With TPA antibodies, normal squamous epithelium of the oesophagus showed cytoplasmic immunostaining which was accentuated at the cell periphery. Staining intensity was most pronounced in the basal cell layers of the stratified epithelium. In the superficial layers a few intensively stained cells were interspersed between cells with poor TPA reactivity (Fig. 1 A). Tumour cells of well differentiated squamous cell carcinomas resembled their non-neoplastic counterparts in basal cell layers in intensity of TPA-specific immunostaining (Fig. 2). Non-epithelial cells remained unstained. Antibodies to cytokeratins revealed similar but not identical immunoreactivity. In contrast to TPA-staining, the superficial epithelial layers exceeded the cells of the basal layers in staining intensity (Fig. 1 B). Carcinoma cells uniformly displayed peripherally accentuated keratin staining identical to that of (superficial) non-neoplastic cells.

*Stomach.* Non-neoplastic mucosa (corpus type) showed cytoplasmic TPA staining in superficial and foveolar epithelium which was more pronounced at the cell periphery. In the corpus region the cells of the gastric glands also reacted with TPA antibodies, and parietal cells exceeded the others in staining intensity. The mucosa of the antral region resembled that of the corpus/fundus-region in its TPA immunoreactivity. However, the mucoid antral glands (like the cardial glands) remained unstained. Epithelial cells lining hyperplastic foveolae displayed peripherally accentuated TPA staining, which was somewhat more intense in superficial proliferated cells. Intestinal metaplasia did not alter pattern and intensity of TPA-specific staining. Goblet cells displayed a small peripheral rim of TPA-positive cytoplasm. Keratin immunoreactivity was identical to TPA staining in neoplastic and non-neoplastic gastric epithelial cells of different types. Tubular adenoma reflected non-neoplastic mucosa (Fig. 3). In tubular adenocarcino-

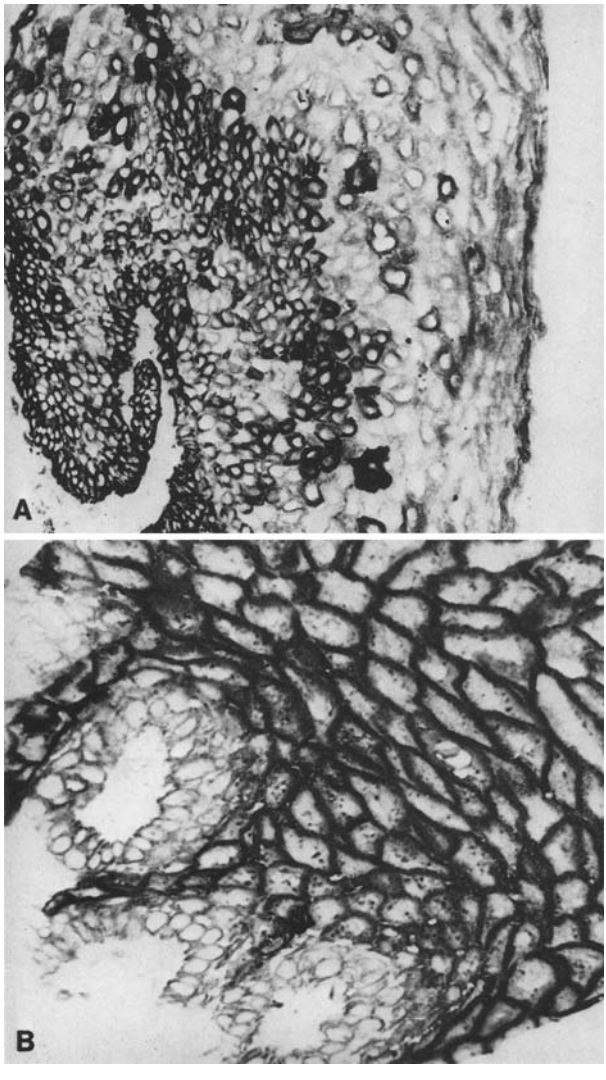


**Fig. 1 A, B. A** Immunocytochemical staining of non-neoplastic stratified squamous epithelium of oesophageal mucosa using TPA antibodies. Note conspicuous staining of basal cell layers. Within the superficial cell layers scattered epithelial cells are TPA-positive. The other cells only showed weak TPA immunoreactivity. **B** Staining of oesophageal epithelium with cytokeratin antibodies reveals pronounced reactivity in superficial cell layers whereas basal cells remain almost unstained. PAP, 100× (**A**), 400× (**B**)

mas the cells lining neoplastic tubules were slightly less reactive than non-neoplastic foveolar cells with respect to TPA and cytokeratin but showed cytoplasmic staining with peripheral and particularly periluminal accentuation. No correlation with mitotic activity and degree of differentiation was found. The signet ring cells of signet ring cell carcinoma also reacted significantly with TPA (Fig. 4) and cytokeratin antibodies with concentration of

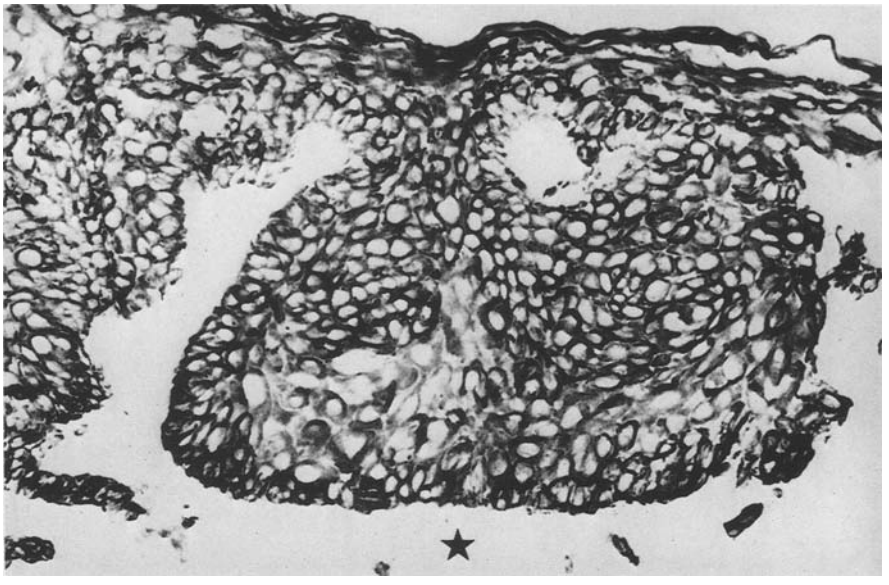

**Fig. 2.** Tumour cells of a well-differentiated squamous cell carcinoma of the oesophagus are intensively stained by TPA antibodies whereas stromal cells (*asterisk*) remain unstained. In contrast to non-neoplastic mucosa, tumour cells show fairly uniform TPA-specific immunoreactivity. PAP, 100×

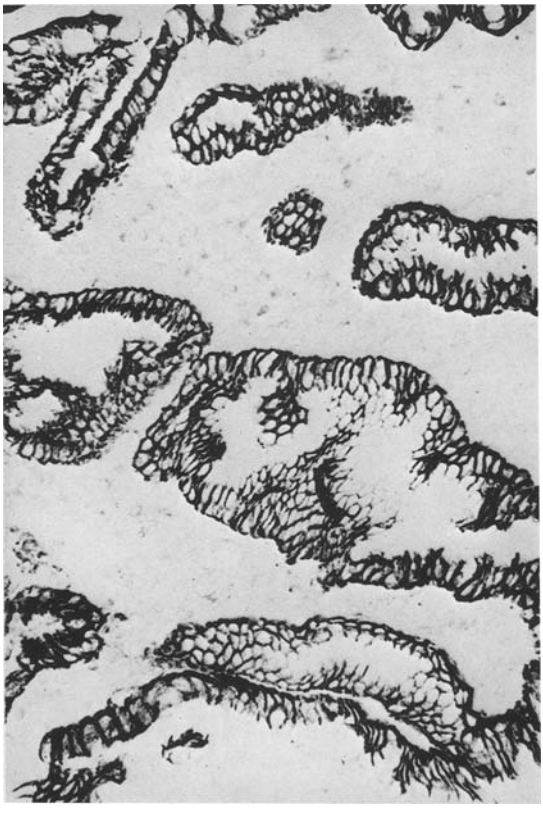

**Fig. 3.** Tubular adenoma of gastric mucosa (intestinal type) showing peripherally accentuated cytoplasmic TPA staining. PAP, 100×

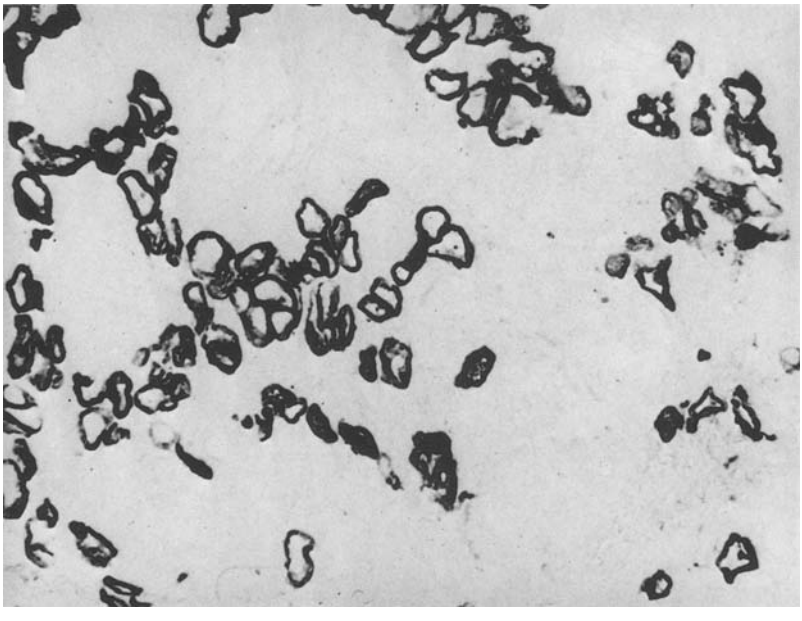

**Fig. 4.** Signet ring cell carcinoma of the stomach (diffuse type) shows pronounced peripheral cytoplasmic TPA staining. Note that individual cells can easily be recognized on basis of their immunoreactivity. PAP, 250 ×

staining at the cell periphery. Carcinomas of the pylorocardial type remained unstained with both types of antibodies suggesting a relationship of this type of carcinoma to mucoid cells of the cardial and antral region.

*Small intestine.* Intestinal cells lining crypts and villi contained TPA with increased staining intensity in peripheral cytoplasmic areas. In goblet cells the immunoreaction was essentially confined to the basal part of the cell. In Paneth cells TPA-related activity was more pronounced at the cell periphery. Brunner's glands were negative (Fig. 5). Cells of a well differentiated tubulo-papillary adenocarcinoma of the papilla of Vater displayed a staining pattern similar to that of non-neoplastic cells; staining, however, was less intense than in non-neoplastic mucosa. Keratin-specific immunoreactivity of neoplastic and non-neoplastic epithelial cells resembled that obtained with TPA antibodies.

*Large intestine.* With TPA antibodies, non-neoplastic epithelial cells of colonic mucosa resembled in staining patterns those of small intestine. The TPA immunoreaction was most pronounced at the cellular periphery and particularly concentrated around the lumina of the crypts. Goblet cells were preferentially stained at the base of the cell. Inflammation did not significantly affect intensity or distribution of the TPA staining. Adenomas (Fig. 6) and adenocarcinomas resembled non-neoplastic mucosa, although staining was somewhat less intense, but without any relationship to differentiation and proliferation. Keratin immunoreactivity showed identical distribution patterns.

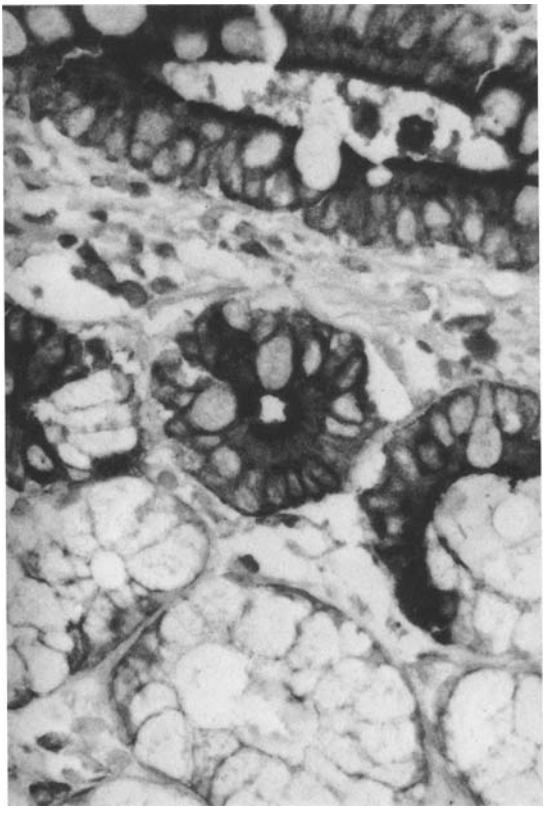

**Fig. 5.** Small intestinal mucosa showing crypt epithelium intensively stained with TPA antibodies but negative Brunner's glands. PAP, 400×

*Gall bladder*. The non-neoplastic epithelial cells of gall bladder mucosa exhibited peripherally accentuated cytoplasmic TPA reactivity which remained unaltered in cholecystitis. Cells of mucoid glands were unstained. Adenocarcinomas of the gall bladder reacted similarly with TPA antibodies. The staining intensity, however, was somewhat less pronounced than in non-neoplastic cells. Immunostaining obtained with cytokeratin antibodies revealed an identical pattern.

*Pancreas*. Epithelial cells of pancreatic ducts were specifically reactive to TPA antibodies with concentration of staining at the cell periphery. Mucoid glands remained unstained. Pancreatic acini did not react significantly with TPA antibodies. Islet cells were negative. Adenocarcinomas of ductal origin resembled normal ductal epithelia in their reactivity with TPA antibodies, although the staining intensity was less pronounced. Ductal staining obtained with cytokeratin antibodies was almost identical to that of TPA antibodies. However, in contrast with TPA antibodies, the antibodies to cytokeratin also specifically decorated acini and islet cells.

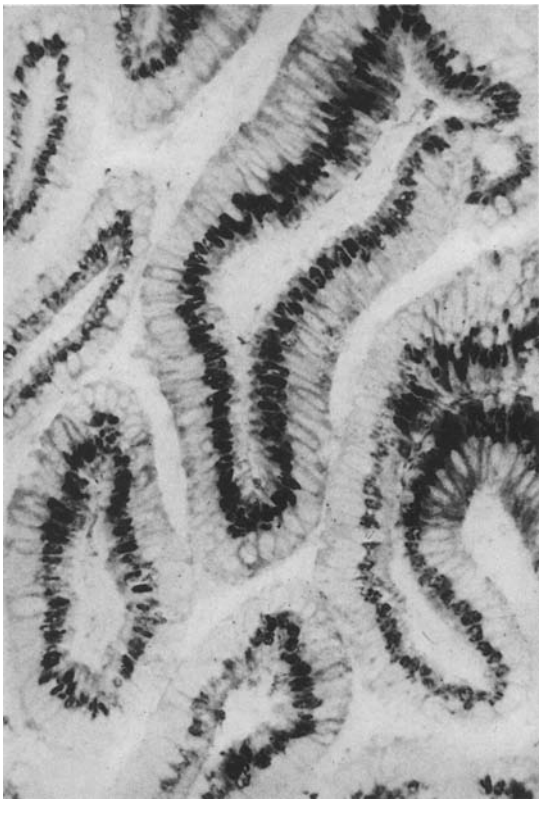

**Fig. 6.** TPA immunoreactivity in a tubulo-villous colonic adenoma is most pronounced at the base of the goblet cells. PAP, 400 ×

*Liver.* The cytoplasm of normal hepatocytes was significantly, although weakly, stained by antibodies to TPA (24 h incubation) with some accentuation of the cell periphery. The cytoplasmic staining showed a suggested network appearance, the nuclei were spared. Following incubation for 15 min (Björklund 1980; Björklund et al. 1982; Nathrath et al. 1984; Nathrath et al. 1985; Vogel and Oehr 1985; Löning et al. 1983) the hepatocytes remained unstained by TPA antibodies. Cytokeratin antibodies revealed an almost identical staining pattern; the intensity of staining, however, was somewhat more pronounced. Bile duct and ductular cells were strongly decorated by TPA as well as cytokeratin antibodies and, again, the cell periphery was accentuated. Immunostaining was most pronounced in specimens incubated with TPA antibodies for 24 h, but significant, although weak, staining was also achieved after antibody application for 15 min. In focal nodular hyperplasia, immunostaining resembled normal liver with respect to TPA and cytokeratin staining. As in normal liver, both types of antibodies reacted with the cytoplasm of hepatocytes only weakly but stained cells of proliferated bile ducts and ductules strongly. Hepatocellular

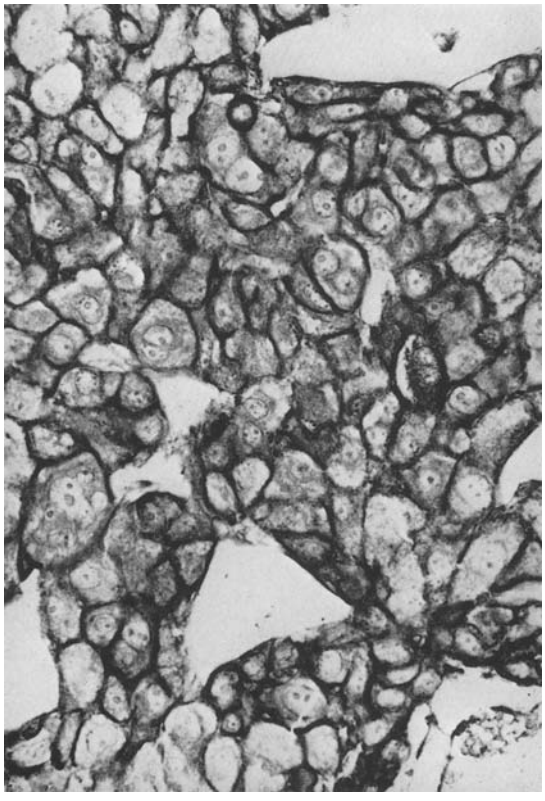

**Fig. 7.** Well differentiated trabecular hepatocellular carcinoma showing cytoplasmic and, particularly, peripherally accentuated TPA-staining, PAP, 400×

adenomas reflected non-neoplastic hepatocytes with respect to TPA and cytokeratin reactivity, but staining intensity was somewhat more pronounced than in non-neoplastic hepatocytes. In hepatocellular carcinomas, irrespective of their morphology and differentiation, the tumour cells showed an increased staining intensity with both TPA and cytokeratin antibodies in comparison to their non-neoplastic counterparts. In two cases, staining was accentuated at the periphery of the cells (Fig. 7). In one less well differentiated tumour, many cells showed a prounced paranuclear staining (Fig. 8). With cytokeratin antibodies, staining was more conspicuous than with TPA antibodies, but the staining patterns were similar.

Cholangiocellular carcinomas were strongly positive for TPA and cytokeratin antibodies. However, tumour areas with cells resembling mucoid metaplasia were less intensely stained and the reaction was more pronounced in the basal cytoplasm close to the nucleus. No difference in staining patterns or intensity existed between neoplastic and non-neoplastic bile duct cells. The degree of differentiation did not significantly affect TPA- and cytokeratin-specific staining.

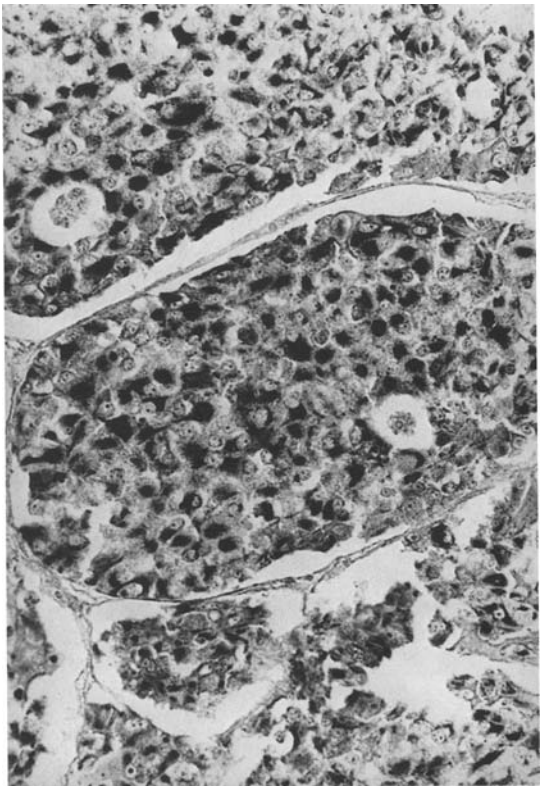

**Fig. 8.** Less well differentiated solid hepatocellular carcinoma consisting of small tumour cells. Note focal concentration of TPA staining in a paranuclear fashion. PAP, 250 ×

*Immunoblotting*

The TPA antibody preparation reacted with cytokeratin polypeptide A ($M_r$ 55,000) and D ($M_r$ 49,000) from mouse liver as well as with minor polypeptides with molecular weights around 53,000 and 40,000 (Fig. 9, lane 3). When tested with cytokeratins isolated from human liver tissue, TPA antibodies reconized polypeptide component 8 ($M_r$ 52,000; Moll et al. 1982), but also showed, although less pronounced, reactivity with polypeptide bands of lower molecular weights including component 18 ($M_r$ 45,000) (Fig. 9, lane 2). When cytokeratin polypeptides, derived from human epidermis, were used as antigens in immunoblotting, TPA antibodies recognized a polypeptide with a molecular weight of 60,000 (Fig. 9, lane 1).

## Discussion

The identification and characterization of cells on the basis of their content of certain antigens, secretion products, and cytoskeletal components is now of considerable interest as an aid to differential diagnosis in tumour patholo-

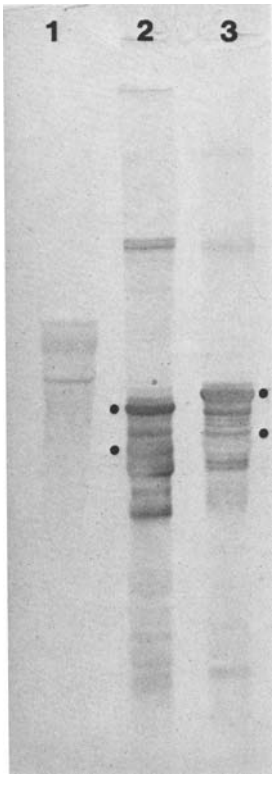


**Fig. 9.** TPA antibodies react with cytokeratin polypeptide components after isolation from human epidermis (lane 1), human liver (lane 2) and mouse liver (lane 3) and electrophoretic transfer to nitrocellulose paper sheets. Most pronounced immunoreactivity is observed with cytokeratin polypeptide component 8 of human liver (*upper dot* in lane 2) but TPA antibodies also react with component 18 (*lower dot* in lane 2) and other (in Coomassie-blue stained gels) minor polypeptide components with lower molecular weights. TPA antibodies show a similar reactivity with mouse liver cytokeratin polypeptides, particularly with component A (upper dot in lane 3). Reactivity with cytokeratin component D (lower dot in lane 3) is less pronounced. TPA antibodies also recognize a cytokeratin polypeptide component ($M_r$ 60,000) derived from human epidermis

gy. The intermediate filament cytoskeleton is a stable structure which is mainly conserved in its chemical composition and antigenicity during neoplastic transformation (Denk et al. 1983; Moll et al. 1982; Osborn and Weber 1983). It contains a large number of interrelated but different polypeptide components with a variety of sets of epitopes. It has been shown that intermediate filament typing is helpful in the diagnosis of tumours when conventional morphological techniques fail to reveal the histogenesis of malignant cells (see Osborn and Weber, 1983; for review). Carcinomas may also be classified by determination of antigens which are typical for epithelial cells; TPA and the epithelial membrane antigen (EMA) are utilized for such purposes (Björklund 1980; Heyderman et al. 1979). TPA has been isolated from carcinoma tissues and established cell lines, such as HeLa cells (Björklund 1980). This antigen is released from tumour cells into the circulation and excreted into the urine. Radioimmunoassay of TPA in serum has become a common precedure in oncology because of its prognostic significance and its reflection of proliferative activity of carcinoma (Björklund et al. 1973; Menendez-Botet et al. 1978; Lüthgens and Schlegel 1983; Oehr et al. 1985; Oehr and Winkler 1984; Vogel and Oehr 1985; Klavins and Cho 1985).

The possible relationship between TPA and cytokeratin was studied by

immunohistochemical and immunochemical techniques. Our results suggest that TPA is related to, but not necessarily identical with, cytokeratins. This issue is not settled because of the complexity of the problem. A relationship between certain components of a particular TPA preparation (Sangtec Medical AB, Sweden) and non-epidermal cytokeratins 8, 18, and 19 of simple epithelial was claimed by Weber et al. (1984). Immunoreactivity with diverse epithelial cells in situ and in vitro and with isolated keratin polypeptides was presented. The purity of the antibody preparations was not described. In this connection the discrepancies in staining patterns for stratified squamous epithelium by TPA antibodies and antibodies to keratin, observed in this study and previously reported by Löning et al. (1983), are interesting. Weber et al. (1984) did not find TPA reactivity in stratified squamous epithelium (of tongue), which indicates differences in immunoreactivities between different TPA antibody preparations.

With regard to staining of hepatocytes and immunoblotting, the results should be considered cautiously. Neither Nathrath et al. (1985) nor Vogel and Oehr (1985) found positive staining of hepatocytes with anti-TPA following 15 min incubation which possibly did not permit certain (contaminating?) antibodies at lower concentrations to react. In the present study a more sensitive technique (24 h incubation) was used, which may have brought to visibility the reactivity of minor antibody components. From the preparative techniques used for TPA, it is conceivable, or likely that intermediate filament proteins co-purify with TPA. If this is true, one would have to be very cautious not to mistake coexistence for identity.

Some of the constituent parts of preparations of TPA, particularly those of low molecular weights around 20,000, could, of course, have resulted from proteolytic degradation. Proteases degrading intermediate filaments have been described by several authors (Schiller and Franke 1983). Whether such mechanisms apply to TPA is not known but they deserve attention in future studies. If one or more cytokeratins were found to resemble TPA, this would not lessen the diagnostic significance of TPA as an indicator of neoplastic growth.

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