Eur J Cancer Clin Oncol, Vol. 18, No. 12, pp. 1251–1257, 1982.
Printed in Great Britain.

0277-5379/82/121251-07$03.00/0

Differential Diagnosis of Human Carcinomas, Sarcomas and their Metastases using Antibodies to Intermediate-sized Filaments*

FRANS RAMAEKERS,† JO PUTS, ARIE KANT, OLOF MOESKER, PAUL JAP and PETER VOOIJS
Department of Pathology and Department of Cytology and Histology, University of Nijmegen, Nijmegen, The Netherlands

Abstract—*Intermediate filaments (IF) are tissue-specific in so far that epithelial, mesenchymal, muscle and neural tissue types can be distinguished by the use of specific antibodies to keratin, vimentin, desmin and neurofilaments or glial filaments respectively. We have examined the possibility of using these sera in the differential diagnosis of human malignant tumors. Using antisera to human skin keratin and bovine lens vimentin we could differentiate between carcinomas (keratin +) and sarcomas (vimentin +). Furthermore, we could show that when cells become malignant and metastasize they retain their original IF and do not develop additional IF systems. We conclude that antibodies to IF proteins are powerful tools in the hands of a pathologist as an additional method to improve identification of tumors and their metastases.*

INTRODUCTION

IT IS now well established that many eukaryotic cells contain a cytoplasmic matrix consisting of ordered arrays of filaments, the cytoskeletal and contractile elements. The most intensively studied structures of this matrix, the microtubules (diameter 25 nm) and microfilaments (diameter 5–7 nm), are apparently composed of similar or identical subunits when comparing different cell types of an organism. In addition to microtubules and microfilaments, filamentous structures with diameters of 7–11 nm have been demonstrated in a number of cell types, including those derived from human tissues. These intermediate-sized filaments (IF) constitute a considerable part of the cytoskeleton of many, if not all, eukaryotic cells [1–6], while the nature of the IF proteins has been shown to correlate with the embryonic origin of the cell. Using biochemical and immunological techniques, the following types of IF can be recognized: (a) IF of the *keratin* type, characteristic of epithelial cells [7–11]; (b) IF of the *vimentin* type, present in cells of mesenchymal origin [12–14]; (c) IF of the *desmin* type, present in muscle cells [15]; (d) IF of the *neurofilament* type, characteristic of nervous tissue [16, 17]; (e) astrocytes contain IF composed of so-called *glial fibrillar acidic protein* [18]. Only 2 exceptions to this rule have thus far been detected, namely the lens-forming epitheloid cells and the germinal epithelium of the testis [14]. Cells in tissue culture usually develop vimentin-containing IF-structures [6, 13] in addition to the originally present IF.

The characteristic IF of a given cell type is retained when the cells have been kept in culture over prolonged periods of time. An example of this phenomenon is observed in HeLa cells. These epithelial cells, originating from a cervical carcinoma, were brought into culture almost 30 years ago. Despite this, they still exhibit IF of the keratin-type as well as the culture-associated vimentin IF [19–21]. Most important for our studies is the fact that cells retain their tissue-characteristic IF. When the proteins of the IF are isolated (on the basis of their insolubility) and further purified by SDS-gel electrophoresis, monospecific antibodies against them can be raised. These antibodies have two important properties that make them valuable tools in the identification of tissues and the study of

Accepted 6 July 1982.
*This study was supported by the Dutch Cancer Foundation, Koningin Wilhelmina Fonds, grant No. NUKC 81-12.
†Correspondence should be addressed to Dr. Frans C.S. Ramaekers, Dept of Pathology, Geert Grooteplein Zuid 24 6524 GA Nijmegen, The Netherlands.

tumorigenesis. First, they do not cross-react with filament proteins other than those against which they were raised. Thus antibodies raised against vimentin do not cross-react with prekeratin, desmin, neurofilaments or glial IF. The second important property is that they are not species-specific (compare, for example, ref. [13]). Use of such antisera in tumor characterization has recently been described for experimental rat liver tumors by Bannasch *et al.* [22]. Keratin sera have been described to be useful in the identification and characterization of human neoplastic epithelial outgrowths [23–25]. This paper reports our results in the use of both keratin and vimentin antibodies for the differential diagnosis of human malignant tumors. Special emphasis is placed on metastatic tumors.

METHODS

Fixed frozen sections (4–7 μm thick) from fresh human tissues obtained from surgery were used in this study.

In the indirect immunofluorescence technique the primary antibodies included: (i) antibodies directed against keratin from human epidermal calluses raised in rabbits and antibodies raised in a guinea pig against keratin from bovine hoof epidermis [9]; (ii) vimentin antibodies directed against bovine calf lens IF-protein raised in rabbits and antibodies raised in a guinea pig directed against the fibroblast 57,000-molecular weight IF-protein [13]. All sera exhibit broad cross-reactivity between species (hamster, rat and man) and are highly specific for either epithelial tissues (keratin) or tissues of mesenchymal origin (vimentin). As a second antibody FITC-conjugated swine anti-guinea pig IgG or goat anti-rabbit IgG was used. The indirect immunofluorescence microscopy was performed essentially as described elsewhere [26]. Some typical examples of human tumor tissues used in our experiments are presented in Table 1. The diagnosis was made on paraffin sections, on parallel frozen sections and in some cases by electron microscopy.

RESULTS AND DISCUSSION

From the results summarized in Table 1 it is obvious that malignant tumors derived from epithelial tissue are positive with the keratin antibodies by immunofluorescence, while cells of mesenchymal origin show bright staining with vimentin antibodies. The tumors obtained by surgery can therefore be distinguished by these sera, as demonstrated in Figs 1 and 2. Furthermore, they also demonstrate that carcinomas do not react with the vimentin antisera and sarcomas do not react with the keratin antisera. Using the technique described above we were, for example, able to distinguish between carcinomatous and sarcomatous tissue in a mixed tumor of the uterus and the subsequent carcinomatous metastases in para-aortic lymph nodes [Fig. 2(a, b) and Table 1]. Since the results of our immunofluorescence studies are in good agreement with those obtained by conventional histological and electron microscopical methods, we are convinced that antibodies directed against IF proteins will be a useful supplemental tool in tumor determination.

The technique described above may confirm diagnoses and eliminate diagnostic uncertainty or doubts. Advantages of this method for tumor characterization can be summarized as follows: 1. specificity as a result of reliable immunochemical markers; 2. the method is relatively fast. Within one hour after obtaining and sectioning the tissue the nature of tumors can be identified. This is especially important if metastases are found during surgery and the metastatic tumor has to be traced back to its primary source; 3. tumor tissue can be obtained by percutaneous thin-needle aspiration, since only a few cells are needed for identification. In this way discomfort and risk to the patient can be minimized as surgical intervention may become unnecessary.

However, before applying this method routinely some questions have to be answered. One of these questions concerns the possible expression of new IF-systems in primary tumors or their metastases. As already mentioned, it is well known that nearly all normal and neoplastic cells growing *in vitro* express vimentin in addition to their original IF-system [6, 13]. This phenomenon could interfere with diagnostic specificity. For example, metastatic tumor cells circulating in blood or growing in ascitic fluid may express vimentin when present in this 'suspension medium' and then retain this second IF-system in subsequent metastatic outgrowths. Our results obtained with human tumor metastases, however, indicate that metastatic outgrowths (blood-borne or via the lymphatic route) express only the tissue-specific IF.

Finally, an interesting feature shown in Table 1 concerns the presence of vimentin and the absence of keratin in a seminoma, a germ-cell tumor thought to be derived from the seminiferous 'epithelium'. This result is in agreement with the observations by Franke *et al.* [27], who showed that the seminiferous epithelium in the testes does not contain keratin IF or desmosomes.

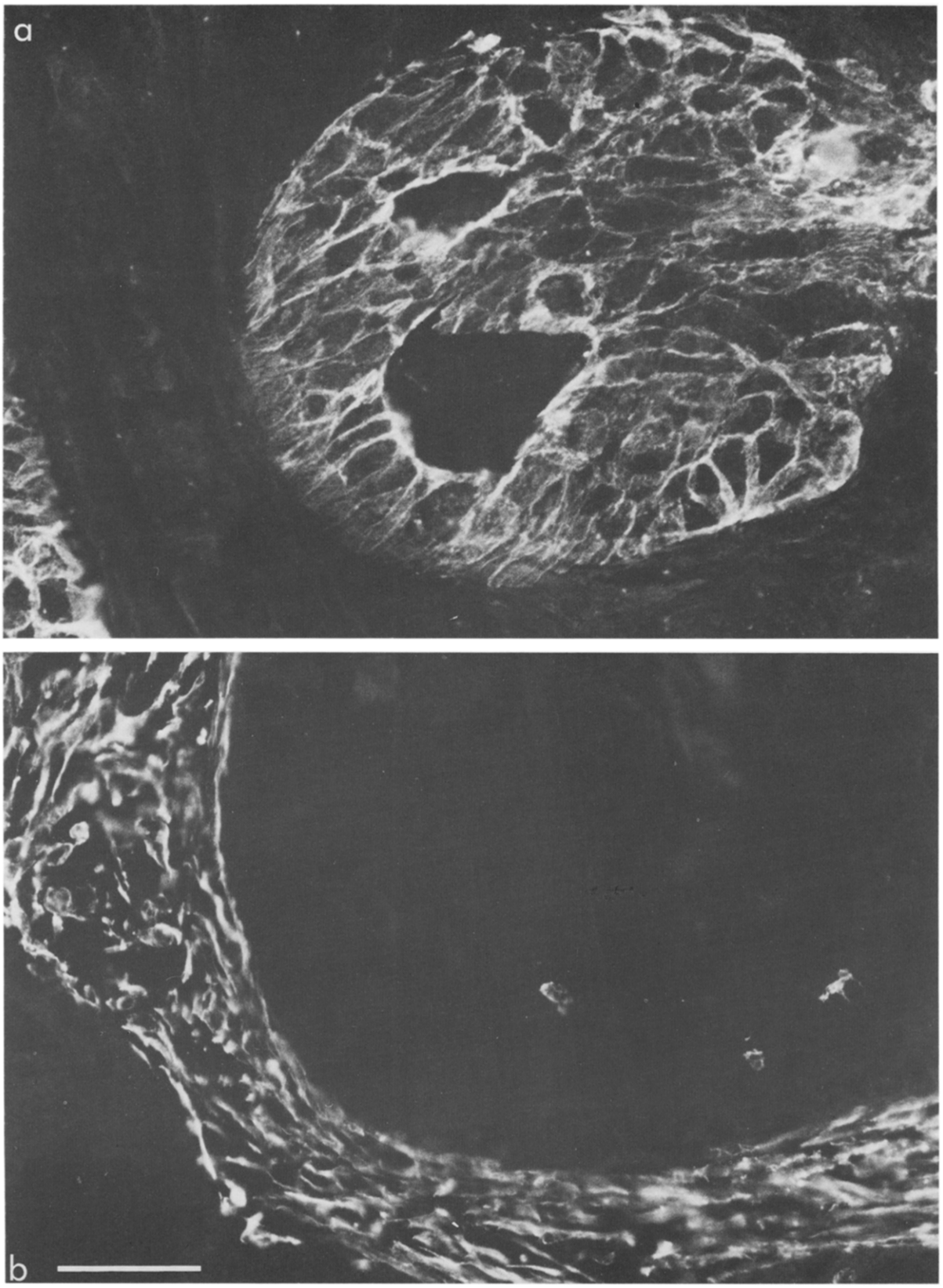

Fig. 1. *Immunofluorescence microscopy of frozen sections from human intestine. (a) Adenocarcinoma reacts positively with keratin antibodies while (b) stromal connective tissue reacts positively with vimentin antibodies. Note the absence of vimentin staining in the epithelial tumor cells in panel (b) and negative stroma with keratin antibodies in panel (a). Bar indicates* 50 μm.

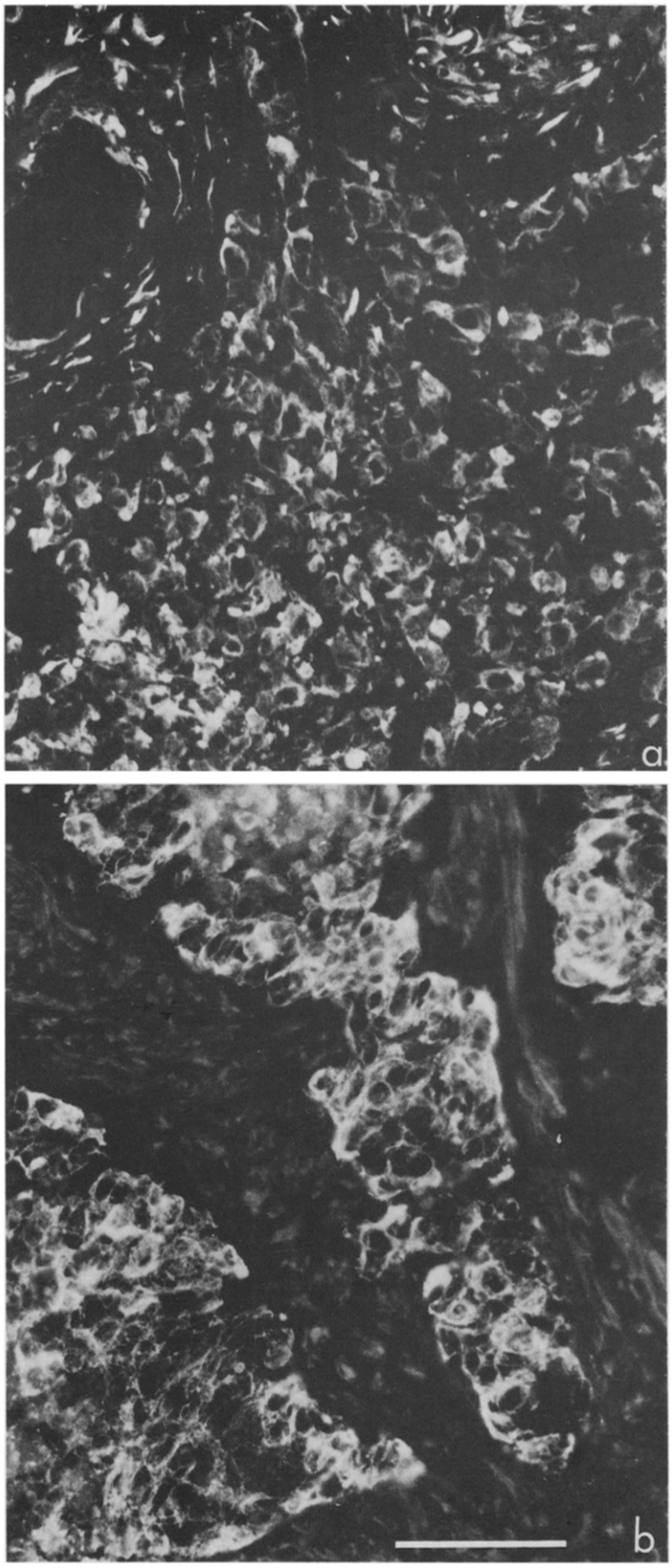

Fig. 2. Immunofluorescence microscopy on frozen sections of (a) human mixed mesodermal sarcoma of the uterus reacting positively with antibodies directed against vimentin. Smooth muscle cells of the myometrium do not react with this antibody. (b) Metastasis of an adenosquamous carcinoma from the uterus within a para-aortic lymph node reacting strongly positive with antibodies directed against keratin. The lymphatic tissue present between the neoplastic outgrowths stains weakly with vimentin antibodies while the tumor tissue is completely negative for vimentin antibodies (not shown). Bar indicates 50 μm.

Table 1. Differential staining of human neoplastic tissues with antibodies directed against vimentin and keratin

Material	Diagnosis	Immunofluorescence			
		Vimentin		Keratin	
		Tumor cells	Stroma	Tumor cells	Stroma
Oral cavity	Squamous cell carcinoma	-	+	+	-
Stomach	Schirrhous carcinoma	-	+	+	-
	Adenocarcinoma of cardia	-	+	+	-
Rectum	Adenocarcinoma	-	+	+	-
Para-aortic lymph node	Metastasis of uterine adeno-squamous carcinoma	-	+	+	-
Axillary lymph node	Metastasis of invasive lobular mamma carcinoma	-	+	+	-
Abdominal lymph node	True histiocytic lymphoma	+	+	-	-
Urinary bladder	Carcinoma *in situ*	-	+	+	-
Humerus	Osteosarcoma	+	+	-	-
Female brast	Lobular mamma carcinoma	-	+	+	-
Corpus uteri	Mixed mesodermal sarcoma	+	+	-	-
	Adenoacanthoma	-	+	+	-
Ovary	Brenner tumor	-	+	+	-
	Cystadenocarcinoma papilliferum serosum	-	+	+	-
Vulva	Malignant melanoma	+	+	-	-
Vagina	Poorly differentiated adenocarcinoma (endometrioid carcinoma)	-	+	+	-
Testis	Anaplastic seminoma	+	+	-	-
	Metastatic adenocarcinoma from gastrointestinal tract	-	+	+	-
Penis	Squamous cell carcinoma	-	+	+	-
Pleura	Mesothelioma	-	+	+	-
Omentum	Metastatic anaplastic adenocarcinoma from stomach	-	+	+	-
	Metastatic poorly differentiated papillary adenocarcinoma from gall bladder	-	+	+	-
	Metastasis from cystadenocarcinoma papilliferum serosum	-	+	+	-
Mesenterium	Metastatic poorly differentiated papillary adenocarcinoma from gall bladder	-	+	+	-
Pelvis	Metastasis from cystadenocarcinoma papilliferum serosum	-	+	+	-

In conclusion, our data confirm and extend those of other investigators (see, for example, refs [28, 29]) and support the use of intermediate filament antibodies in the differential diagnosis of human metastatic tumours as well as tumors in experimental systems.

Acknowledgements—The authors are grateful to Prof. Dr. W. W. Franke (Heidelberg) for generous gifts of the guinea pig antisera used in this study and Dr. T. Feltkamp-Vroom for providing us with some of the tumor tissues. Dr. C. J. Herman provided helpful insights and many helpful discussions during this work. We thank Theo Hafmans for excellent help with photography and Yvonne Lawson for typing the manuscript.

REFERENCES

1. GOLDMAN RD, POLLARD TD, ROSENBAUM JL. *Cell Motility*. Books A–C, Cold Spring Harbor Conferences on Cell Proliferation, Cold Spring Harbor Laboratory, 1976, Vol. 3.
2. LAZARIDES E. Intermediate filaments as mechanical integrators of cellular space. *Nature (Lond)* 1980, **283**, 249–256.
3. DAVISON PF, HONG BS, COOKE F. Classes of distinguishable 10 nm cytoplasmic filaments. *Nature (Lond)* 1977, **109**, 471–474.
4. GILBERT D. 10 nm Filaments. *Nature (Lond)* 1978, **272**, 577–578.
5. FRANKE WW, SCHMID E, OSBORN M, WEBER K. Different intermediate-sized filaments distinguished by immunofluorescence microscopy. *Proc Natl Acad Sci USA* 1978, **75**, 5034–5038.
6. FRANKE WW, SCHMID E, BREITKREUZ D *et al.* Simultaneous expression of two different types of intermediate-sized filaments in mouse keratinocytes proliferating *in vitro*. *Differentiation* 1979, **14**, 35–50.
7. BRECHER S. The occurrence and possible role of 80–100 Å filaments in PtK_1 cells. *Exp Cell Res* 1975, **96**, 303–310.
8. FRANKE WW, GRUND C, OSBORN M, WEBER K. The intermediate-sized filaments in rat kangaroo PtK_2 cells. *Cytobiology* 1978, **17**, 365–391.
9. FRANKE WW, WEBER K, OSBORN M, SCHMID E, FREUDENSTEIN C. Antibody to prekeratin. *Exp Cell Res* 1978, **116**, 429–445.
10. SUN TT, SHIH C, GREEN H. Keratin cytoskeleton in epithelial cells of internal organs. *Proc Natl Acad Sci USA* 1979, **76**, 2813–2817.
11. FRANKE WW, APPELHANS B, SCHMID E, FREUDENSTEIN C, OSBORN M, WEBER K. Identification and characterization of epithelial cells in mammals by immunofluorescence microscopy using antibodies to prekeratin. *Differentiation* 1979, **15**, 7–25.
12. FRANKE WW, SCHMID E, OSBORN M, WEBER K. Intermediate-sized filaments of human endothelial cells. *J Cell Biol* 1979, **81**, 570–580.
13. FRANKE WW, SCHMID E, WINTER S, OSBORN M, WEBER K. Widespread occurrence of intermediate-sized filament of the vimentin-type in cultured cells form diverse vertebrates. *Exp Cell Res* 1979, **123**, 25–46.
14. RAMAEKERS FCS, OSBORN M, SCHMID E, WEBER K, BLOEMENDAL H, FRANKE WW. Identification of the cytoskeletal proteins in lens forming cells, a special epithelioid cell type. *Exp Cell Res* 1980, **127**, 309–327.
15. LAZARIDES E, HUBBARD BD. Immunological characterization of the subunit of the 100 Å filaments from muscle cells. *Proc Natl Acad Sci USA* 1976, **73**, 4344–4348.
16. YEN SH, DAHL D, SCHACHNER M, SHELANSKI ML. Biochemistry of the filaments of brain. *Proc Natl Acad Sci USA* 1976, **73**, 529–533.
17. JORGENSEN AO, SUBRAMANIAN L, TURNBULL C, KALNINS VI. Localization of the neurofilament protein in neuroblastoma cells by immunofluorescent staining. *Proc Natl Acad Sci USA* 1976, **73**, 3192–3196.
18. RUEGER DC, HUSTON JS, DAHL D, BIGNAMI A. Formation of 100 Å filaments from purified glial fibrillary acidic protein *in vitro*. *J Mol Biol* 1979, **135**, 53–68.
19. HYNES RO, DESTREE AT. 10 nm Filaments in normal and transformed cells. *Cell* 1978 **13**, 151–163.
20. FRANKE WW, SCHMID E, WEBER K, OSBORN M. HeLa cells contain intermediate-sized filaments of prekeratin type. *Exp Cell Res* 1979, **118**, 95–109.
21. FELIX H, STRAULI P. Different distribution pattern of 100 Å filaments in resting and locomotive leukaemia cells. *Nature (Lond)* 1976, **261**, 604–605.
22. BANNASCH P, ZERBAN H, SCHMID E, FRANKE WW. Liver tumors distinguished by immunofluorescence microscopy with antibodies to proteins of intermediate filaments. *Proc Natl Acad Sci USA* 1980, **77**, 4948–4952.
23. BATTIFORA H, SUN TT, BAHU RM, RAO S. The use of anti-keratin antiserum as a diagnostic tool: thymoma versus lymphoma. *Human Pathol* 1980, **11**, 635–641.

24. SCHLEGEL R, BANKS-SCHLEGEL S, MCLEOD JA, PINKUS GS. Immunoperoxidase localization of keratin in human neoplasms, a preliminary survey. *Am J Pathol* 1980 **101,** 635–641.
25. KREPLER R, DENK H, FRANKE WW. Presence and distribution of cytokeratin in various human neoplastic and non-neoplastic disorders. *Eur J Cell Biol* 1980, **22,** 378 (abstract).
26. RAMAEKERS FCS, PUTS JJG, KANT A, MOESKER O, JAP PHK, VOOIJS GP. The use of antibodies directed against intermediate filaments in the characterization of human tumors. *Cold Spring Harbor Symp Quant Biol* 1981, **46,** 331–339.
27. FRANKE WW, GRUND C, SCHMID E. Intermediate-sized filaments present in Sertoli cells are of the vimentin type. *Eur J Cell Biol* 1979, **19,** 269–275.
28. GABBIANI G, KAPANCI Y, BARAZZONE P, FRANKE WW. Immunochemical identification of intermediate-sized filaments in human neoplastic cells. *Am J Pathol* 1981, **104,** 206–216.
29. ALTMANNSBERGER M, OSBORN M, SCHAUER A, WEBER K. Antibodies to different intermediate filament proteins, cell type-specific markers on paraffin-embedded human tissues. *Lab Invest* 1981, **45,** 427–434.