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Epithelial markers in synovial sarcoma

An immunohistochemical study on paraffin embedded tissues

F. Wrba [1], H. Fertl [1], G. Amann [1], E. Tell [2], R. Krepler [1]

[1] Institut für Pathologische Anatomie der Universität Wien, Allgemeines Krankenhaus, Vienna, Austria
[2] Institut für Pathologie, Krankenhaus Amstetten, Amstetten, Austria

Summary. Immunohistochemical studies on synovial sarcomas have proved the potentiality of these neoplasm for epithelial and mesenchymal differentiation and antibodies detecting epithelial cells have been found to be helpful in determining the histological types. In this study different epithelial markers directed against various cytokeratins, HMFG-2 and EMA were investigated on paraffin embedded tissues of 13 cases of synovial sarcomas, with regard to their reliability in unmasking the epithelial components demonstrable in this type of neoplasm. The results lead to three conclusions: firstly, synovial sarcomas possess the capacity for generating different epithelial cell types with uncommon compositions of intermediate filaments as well as of membrane proteins, secondly, these features may be expressed in a heterogenous pattern even within the same tumour and finally, the use of wide range anti-cytokeratin antibodies covering the spectrum of basic as well as acidic type proteins seems to be necessary for the detection of all epithelial components demonstrable in synovial sarcomas.

Key words: Epithelial markers – Synovial sarcoma – Immunohistochemistry

Introduction

Synovial sarcoma is a histologically and clinically well-defined entity which accounts for up to 10% of all malignant soft tissue tumours (Haagensen and Stout 1944; Cadman et al. 1965; Mackenzie 1966 and 1977; Hajdu et al. 1977; Enzinger and Weiss 1988).

Offprint requests to: F. Wrba, Department of Pathological Anatomy, University of Vienna, Allgemeines Krankenhaus, Spitalgasse 4, A-1090 Vienna, Austria

Four main patterns of histological types are recognized (Enzinger and Weiss 1988): the classic biphasic variant which is characterized by sarcomatous and distinct epithelial components, the monophasic fibrous type with predominantly sarcomatous spindel cell elements, the monophasic epithelial type and the poorly differentiated synovial sarcomas composed of solidly packed small oval or spindle – shaped cells with indistinct epithelial differentiation.

Immunohistochemical studies on human synovial sarcomas have proved the potentiality of these neoplasms for epithelial and mesenchymal differentiation and antibodies detecting cytokeratins have been found to be effective tools to determinate the histological types (Miettinen et al. 1982, 1983; Corson et al. 1983; Mukai et al. 1984; Abenoza et al. 1986; Leader et al. 1987; Enzinger and Weiss 1988). However, it is known that, mainly in the monophasic fibrous type, sometimes only a few isolated cells express epithelial properties causing difficulties in histologic diagnosis. For this reason we investigated different epithelial markers concerning their reliability to unmask epithelial components demonstrable in synovial sarcomas. Therefore an immunohistochemical examination of 13 synovial sarcomas was carried out using various antibodies against cytokeratins, human milk fat globule protein – 2 (HMFG-2) and epithelial membrane antigen (EMA).

Materials and methods

The tissue samples were paraffin embedded tissues of 13 synovial sarcomas. Additionally, two normal synovial membranes were investigated.

Sections were stained with haematoxylin & eosin, PAS, diastase-PAS and Gomori's silver impregnation technique. Tumours were classified according to Enzinger and Weiss (1988) as biphasic, predominantly monophasic fibrous if the presence

Table 1. Antibodies used in this study

Antibody	specificity	source
I. lu 5	1, 5, 6, 8, 19, 14 a-keratins of hair follicles	ref. Franke et al. (1987)
II. anti-cytokeratin CAM 5.2	CK # 8, 18, 19	Becton-Dickinson
III. monoclonal antiglandular epithelia	CK # 7	Amersham
IV. monoclonal anticytokeratin	CK # 18	Amersham
V. monoclonal anticytokeratin	CK # 19	Amersham
VI. monoclonal antitype II cytokeratins	CK basic type II, and # 8	Amersham
VII. polyclonal rabbit anti-human keratin	keratins of 56 and 64 kD	Dako Corporation
VIII. rabbit polyclonal anti-keratin (wide spectrum screening)	bovine muzzle epidermal keratin, keratin subunits 58, 56, 52, 60, 51, 48 kD	Dako Corporation
IX. monoclonal KL-1	keratins of 56 kD	Immunotech
X. HMFG-2	human milk fat globule membrane	Seward
XI. anti-human epithelial membrane antigen (EMA)	delipidated extract of human cream	Dako Corporation
Vimentin		Dako Corporation

Table 2. Application of antibodies

Antibody, number	Dilution* incubation time	Method**
I.	1:400, RT, 1.5 h	indirect
II.	1:20, 4 C, over night	indirect
III.	1:5, 4 C, over night	indirect
IV.	1:5, 4 C, over night	indirect
V.	1:50, 4 C, over night	indirect
VI.	1:10, 4 C, over night	indirect
VII.	1:400, 4 C, over night	PAP
VIII.	1:400, 4 C, over night	PAP
IX.	1:100, RT, 30 min	indirect
X.	1:200, RT, 1 h	indirect
XI.	1:400, RT, 1 h	indirect
Vimentin	1:20, RT, 1 h	indirect

* All antibodies were diluted in TBS with 5% human AB serum; RT: room temperature

** All antibodies except # X (HMFG-2) and anti-vimentin were applicated after predigestion with protease 0.5% (Sigma Chemical Co, USA) in TBS for 5 min

of different cell types were in part evident, monophasic fibrous and poorly differentiated synovial sarcomas. There was no case of monophasic epithelial synovial sarcoma in this material. For immunohistochemistry 4 µm sections were deparaffinized in xylene, placed in absolute ethanol and hydrated in graded series of alcohol and Tris-buffered saline (TBS, pH 7.5). In addition, indirect immunoperoxidase technique (Mason et al. 1982) or PAP method (Sternberger 1974) was carried out in a humidity chamber. The antibodies used and their application are outlined in Tables 1 and 2. Peroxidase activity was visualized using 3,3 diaminobenzidine-tetrahydrochloride (DAB, 0,05%, Fluka, USA) and H_2O_2 in TBS as chromogen. After counterstaining in Mayer's haematoxylin sections were dehydrated, cleared and mounted with Entellan (Merck, Austria).

Sections were scored for staining reaction on a scale from − to + + ; − : absence of staining, + : weak but clearly positive staining, + + : strong staining.

Results

A total of 13 synovial sarcomas were investigated. 6 cases were classified as biphasic, 6 cases as predominantly monophasic fibrous, 1 case as monophasic fibrous with poorly differentiated portions. The immunohistochemical results of the various epithelial markers investigated are summarized in Table 3 and represented in Figures 1–2.

Antibody # I revealed strong positivity of all epithelial cells of biphasic synovial sarcomas. In monophasic fibrous type as well as in the case of poorly differentiated type some spindle-shaped cells also showed strong reactivity with antibody # I. Nearly congruous results were obtained with antibody # II directed against cytokeratins # 8, # 18, and # 19 (representing simple epithelium).

According to this pattern antibodies # IV and # V (directed against cytokeratins # 18 and # 19) produced similar results showing expression either of cytokeratin # 18 or of cytokeratin # 19 solely. Case # 3 revealed expression of both cytokeratins and only four cases showed negativity. In general, antibody # VI revealed predominantly weak reactivity, displaying positivity only in 6 cases.

Antibodies # VII and # VIII directed against various cytokeratins of basic and acidic keratin polypeptides (expressed in simple as well as in stratified epithelia; Moll et al. 1982) were positive in nearly all cases, displaying a pattern similar to that of antibody # I.

The antibodies # III, and # IX, directed against cytokeratins # 7, and 56 kD respectively, were consistently negative.

An interesting pattern of immunoreactivity was

Table 3. Summary of immunoreactivities of epithelial markers in 13 synovial sarcomas

Case #/type*	Antibodies used/immunoreactivity**										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
1 b	++	++	−	−	++	++	++	++	−	+	++
2 b	++	+	−	+	−	+	++	++	−	−	+
3 b	++	++	−	++	+	++	++	++	−	−	++
4 pm	++	++	−	+	−	−	++	+	−	−	−
5 pm	+	−	−	−	−	−	+	+	−	−	+
6 pd	++	++	−	++	−	−	+	++	−	−	−
7 b	++	++	−	++	−	+	++	++	−	−	−
8 pm	++	++	−	+	−	−	++	++	−	−	−
9 pm	++	+	−	+	−	−	++	++	−	−	−
10 pm	++	−	−	−	−	−	++	++	−	−	−
11 b	++	++	−	+	−	+	++	++	−	+	++
12 pm	++	−	−	−	−	−	−	−	−	−	+
13 b	++	+	−	−	−	+	++	++	−	−	−

* b: biphasic; pm: predominantly monophasic; pd: monophasic fibrous with poorly differentiated portions
** Absence of staining reaction; + weak but clearly positive staining; ++ strong positive staining reaction

seen in case # 13. Though antibodies # II and # VI (directed against cytokeratins # 8, # 18, # 19) showed weak positivity, no similar reaction of an appropriate monospecific antibody (# IV, # V) was obvious.

HMFG-2 was found in two cases (# 1 and # 11), staining a few tubule lining epithelial cells, whereas positivity for EMA was observed in 6 cases staining tubule lining cells as well as some spindle-shaped cells (Fig. 2).

In normal synovial lining cells neither cytokeratin expression nor HMFG-2 nor EMA reactivity could be observed (data not shown; data according to Enzinger and Weiss 1988). In all cases the sarcomatous portions revealed strong immunoreactivity for vimentin. Cytokeratin coexpression with vimentin was obvious in three cases (# 1, # 8 and # 12) by showing positivity in gland – like structures as well as in spindle-shaped cells (not shown).

All antibodies showed strong positive reaction with the appropriate type of epithelium (formalin fixed and paraffin embedded tissues of skin, gallbladder, stomach, and breast) used as positive controls.

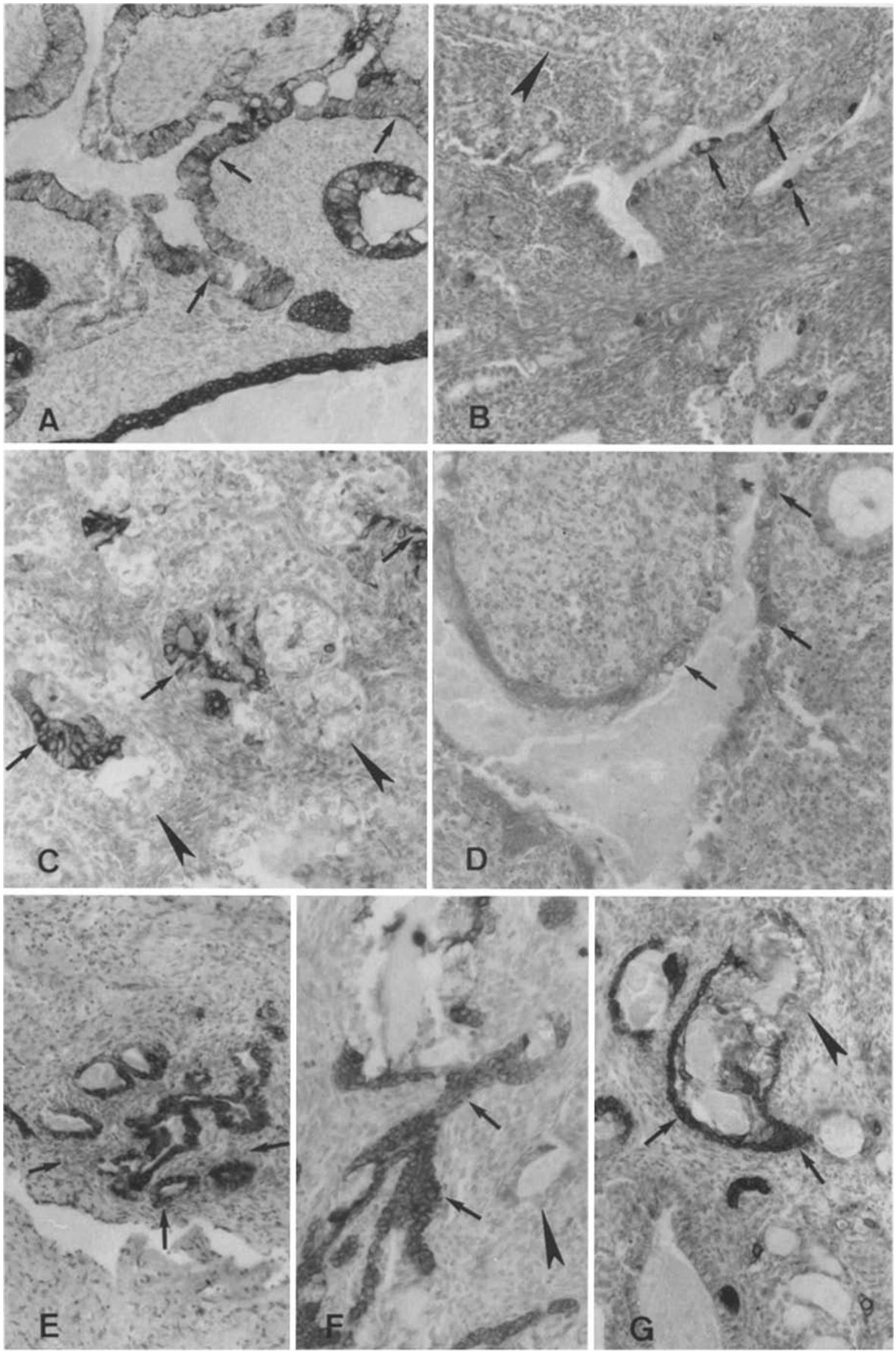
Negative control studies performed by omission of the respective first antibody were consistently negative.

Discussion

Synovial sarcomas are well defined neoplasms of mesenchymal origin with the potentiality for epithelial cell differentiation. Therefore, antibodies directed against cytokeratins have proved to be valuable for histological classification of this neoplasm (Miettinen et al. 1982 and 1983; Corson et al. 1983; Mukai et al. 1984; Abenoza et al. 1986; Leader et al. 1987; Enzinger and Weiss, 1988). Based on the fact, that sometimes only a few cells may carry epithelial features, a comparison of immunoreactivities of different epithelial markers was carried out.

Cytokeratin filaments are characterized by a distinct biochemical diversity, represented in human tissues by at least 19 different polypeptides (Moll et al. 1982). The keratins can be subdivided into two distinct groups: type I keratins (acidic keratins; MW 40–56,5 kD) and type II keratins (basic keratins; MW 53–67 kD) (reviewed by Fuchs 1988). Cytokeratins tend to be expressed as specific pairs of type I and type II subunits. Keratins # 8 and # 18, for instance, are characteristic of simple epithelium, whereas keratins # 5 and # 14 are found in the stratified squamous epithelia. Some simple epithelia also express keratins # 7, # 17 or # 19 (Moll et al. 1982, 1988).

The main finding was that synovial sarcomas displayed cytokeratin patterns revealing attributes of simple as well as of stratified epithelium in a clear heterogenious manner even within the same neoplasm. Therefore only antibodies covering a wide range of different cytokeratins of acidic as well as basic type seem to be appropriate for detection of epithelial components of synovial sarcomas. In particular, in almost all of the cases examined in this study comparisons of the different immunoreactivities have shown a close correlation between the antibodies # I, # II, # VII and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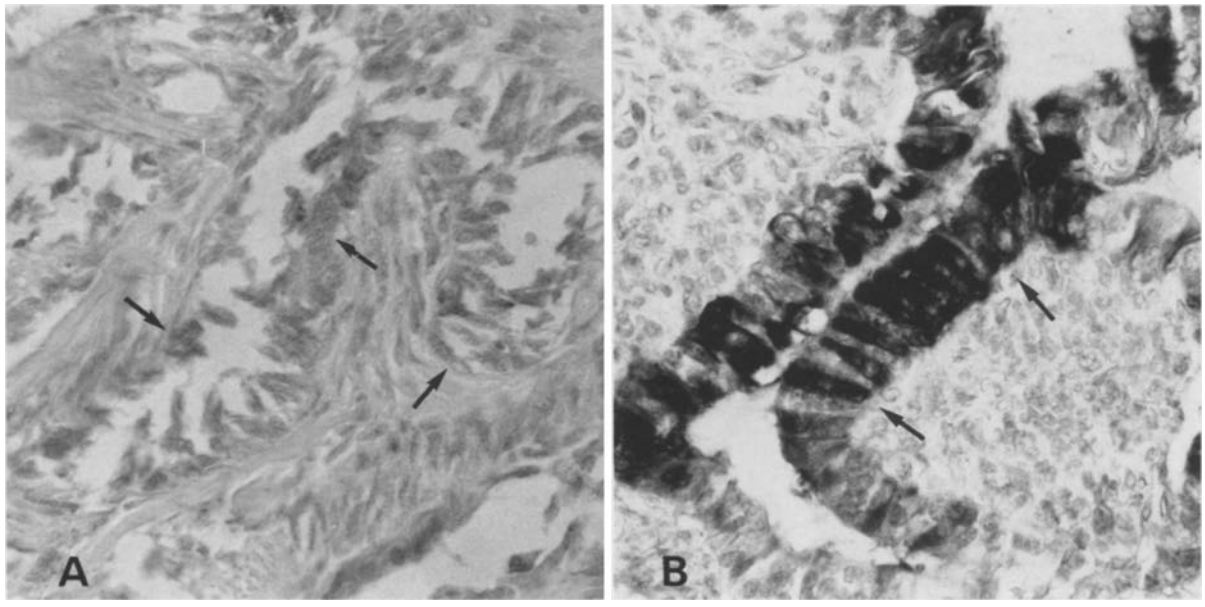

Fig. 2A, B. Immunoperoxidase reaction of (**A**) HMFG-2 (*arrows*, case # 1, ×100) and (**B**) EMA (*arrows*, case # 11, ×120) in pseudoglandular structures of a biphasic synovial sarcoma

VIII regardless of whether tubule lining cuboidal cells or spindle shaped cells were compared. However, cytokeratin # 7 (antibody # III) and cytokeratin of 56 kD molecular weight (antibody # IX) considered to be characteristic of glandular and stratified epithelium could not be detected. How far fixation may have influenced the immunoreactivities by alteration of certain epitopes is subject of further investigation.

With regard to HMFG-2 and EMA, not only epithelial cells but also spindle – shaped cells reacted positively with this antibodies. However, it is known that these markers bind to antigens expressed on epithelial tumours predominantly of glandular origin and it has been reported that HMFG-2 seems to be a reliable marker for adenocarcinomas of the breast (Wrba et al. 1987).

Our results of cytokeratin expression, HMFG-2 and EMA reactivity in a spectrum of 13 synovial sarcomas have led to three main conclusions, firstly that synovial sarcomas possess intrinsic capacities for generating different epithelial cell types of uncommon compositions of intermediate filaments as well as of membrane proteins, secondly that this feature may be expressed in a heterogenious pattern even within the same tumor, and finally that the use of wide range anti-cytokeratin antibodies covering the spectrum of basic as well as acidic type proteins is necessary for the detection of all epithelial components demonstrable in this type of neoplasm.

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Fig. 1A–G. Immunoperoxidase reaction of anti-cytokeratin antibodies in different portions of biphasic synovial sarcoma (case # 3; ×50). **A** Antibody # I; strong staining of all epithelial cells lining pseudoglandular structures. **B** Antibody # II; heterogeneity of immunoreaction: positively stained cells (*arrows*) opposite to negative pseudoglandular structures (*arrowhead*). **C** Antibody # IV; heterogeneity of immunoreaction: positively stained (*arrows*) and negative cells (*arrowheads*). **D** Antibody # V; scattered weak positively stained cells (*arrows*) in between predominantly negative epithelia. **E** Antibody # VI; group of positively stained pseudoglandular structures (*arrows*). **F, G** Antibodies # VII and # VIII; heterogeneity of immunoreaction: strong positive (*arrows*) and negative (*arrowheads*) cells

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