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The use of vimentin antibodies in the diagnosis of malignant mesothelioma

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Summary. An immunohistochemical investigation of vimentin, an intermediate filament, was carried out on formalin fixed paraffin embedded sections of 44 malignant mesotheliomas of the pleura and 24 pulmonary adenocarcinomas, in order to assess its value in differential diagnosis. Seventy-five percent of the malignant mesotheliomas showed positive staining for vimentin. An unexpected finding was the presence of vimentin in 46% of the pulmonary adenocarcinomas. In either case there was no correlation between the presence of vimentin and the histological types or grades of differentiation. The overall level of vimentin staining was significantly greater in the malignant mesotheliomas than in the pulmonary adenocarcinomas but no single antibody dilution was found to provide clear cut separation of the two groups. Vimentin does not appear to be a simple discriminatory marker of malignant mesothelioma.

Key words: Malignant mesothelioma – Pulmonary adenocarcinoma – Vimentin – Immunohistochemistry

Introduction

Diagnostic differentiation between malignant mesothelioma and adenocarcinoma still remains a significant problem in pathology. The extreme variation in histological appearance of mesothelioma on the one hand and the differing grades of differentiation exhibited by adenocarcinoma on the other have combined to make histological diagnosis impossible on occasion. This problem occurs both on surgical and autopsy material and its solution is important because of the increased therapeutic use of pleuropneumonectomy and the assessment of financial compensation. This has led to the formation of mesothelioma panels composed of "experts".

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Ancillary procedures such as mucin histochemistry and electron microscopy have been advocated (Wagner et al. 1962; Kannerstein et al. 1973; Wang 1973) but both suffer from significant limitations (Whitaker and Shilkin 1984). The advent of tumour specific and histogenetic immunohistological markers has provided a more useful adjunct in this situation. We have found either carcinoembryonic antigen (CEA) or pregnancy specific $beta_1$-glycoprotein immunoreactivity to be present in more than 95% of pulmonary adenocarcinomas whereas no mesothelioma tested so far has given a positive result (Gibbs et al. 1985). On the basis of such exclusion criteria it is possible to distinguish more accurately mesothelioma from adenocarcinoma. Nevertheless, the situation is not altogether satisfactory and a search for suitable positive markers of mesothelioma is necessary.

Several groups, including ours, have employed antibodies to cytokeratins (a class of intermediate filament present in epithelial cells) as positive immunohistological markers for mesothelioma and reactive mesothelium. However, the results have been variable, some groups of workers finding keratin positivity in both adenocarcinoma and mesothelioma (Gibbs et al. 1985; Nagle et al. 1983; Ramaekers et al. 1983a) whilst others have claimed keratin positivity in mesothelioma only (Corson and Pinkus 1982; Said et al. 1983). These results could be explained by the use of different fixatives (Ramaekers et al. 1983a) or by keratin heterogeneity (Muijen et al. 1984; Wu et al. 1982).

Vimentin is another chemically distinct class of intermediate filament found predominantly in mesenchymal cells (Franke et al. 1979) and as a major component of mesothelial cells (Wu et al. 1982). The recent availability of monoclonal antibodies to vimentin has enabled us to investigate its potential usefulness in the differential diagnosis of mesothelioma from pulmonary adenocarcinoma.

Materials and methods

Conventional 5 μ thick sections were taken from stored formalin-fixed paraffin embedded post-mortem tissue of 44 malignant mesotheliomas and 24 pulmonary adenocarcinomas as the substrate for analysis. The mesotheliomas comprised 20 purely epithelial tumours, 18 mixed epithelial and sarcomatous tumours and 6 purely sarcomatous tumors. The adenocarcinomas comprised 8 well differentiated bronchogenic carcinomas, 5 moderately differentiated bronchogenic carcinomas, 7 poorly differentiated bronchogenic carcinomas, 1 bronchiolo-alveolar tumour and 3 adenosquamous carcinomas. All were autopsy cases. A modified version (Jasani and Williams 1982) of the DNP-labelled antibody technique (Jasani et al. 1981) employing a coupled enzyme system was used to give high sensitivity immunoperoxidase labelling of the tissue sections. Briefly, sections were first exposed overnight at 4° C, to DNP-labelled primary antibody, diluted with 20% normal rabbit serum. The DNP groups were then detected using a polyvalent IgM monoclonal bridge antibody (1:100 dilution of an ascites preparation – 45 min at 4° C) followed by DNP-peroxidase conjugate (1:800 dilution of a standard preparation: Newman and Jasani 1984) and DNP-glucose oxidase (1:12 dilution: Jasani and Williams 1982) both for 45 min at 20° C. The staining reaction developed by leaving the sections overnight at 20° C in diaminobenzidine solution (1.6 mg/ml in phosphate-buffered saline, PBS) containing beta-D glucose (15 mg/ml). The latter in the presence of the specifically tissue bound glucose oxidase generates hydrogen peroxide causing highly efficient and sharply selective deposition of diaminobenzidine polymeric product (Fig. 1).

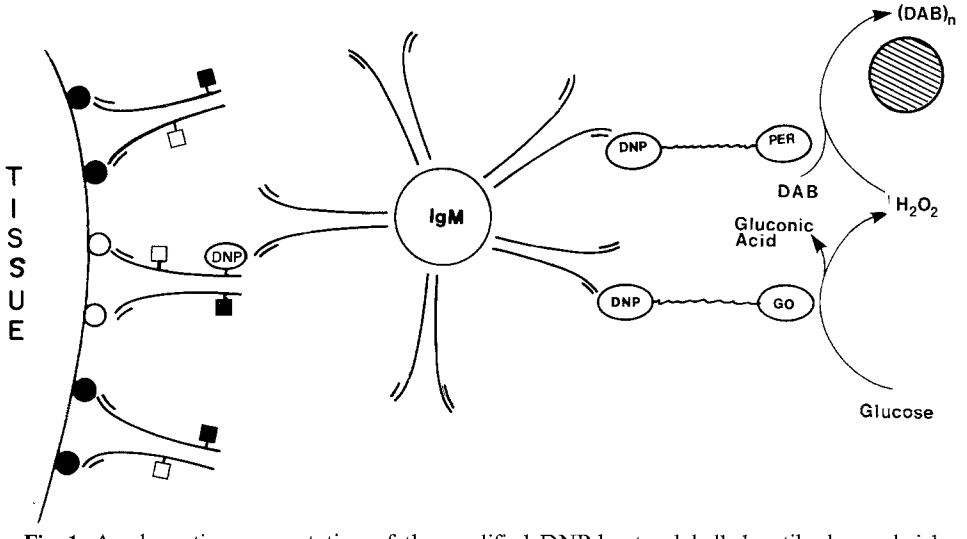


Fig. 1. A schematic representation of the modified DNP-hapten labelled antibody sandwich procedure. The specific and non-specific antigen tissue sites are indicated by open (○) and closed (●) circles, respectively. The non-specific antigenic sites (●) are blocked by unlabelled immunoglobulins (□, ■) from the blocking serum, whilst the DNP labelled specific antibody (□, ■, DNP) binds to the specific tissue antigen sites. The DNP is then localised with IgM monoclonal anti-DNP bridge antibody in conjunction with DNP-Peroxidase (DNP-PER) and DNP-glucose oxidase (DNP-GO) added in the last step. Diaminobenzidine (DAB) glucose solution, generates the nascent hydrogen peroxide (H_2O_2) with resultant deposition of polymerised DAB (○)

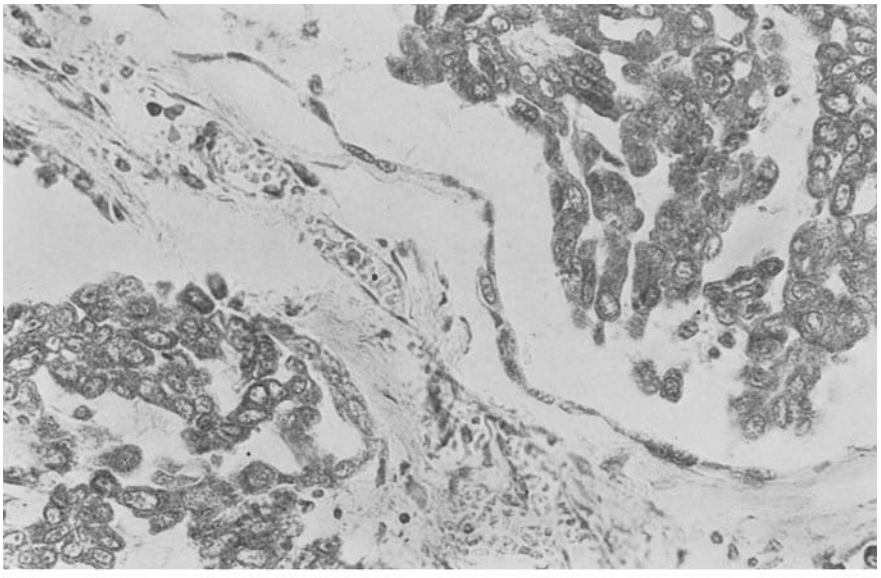
Primary antibodies used included two monoclonals to vimentin purchased from Eurodiagnostic BV, Amsterdam, Holland, and Labsystems OY, Helsinki, Finland, respectively and a polyclonal antibody to carcinoembryonic antigen (Dakopatts, Glostrup, Denmark).

Optimal dilutions for the Eurodiagnostic anti-vimentin (aEDV) and the Labsystem anti-vimentin (aLSV) antibodies were found to be 1:3000 and 1:800 respectively of the commercially recommended stock solutions. Protease treatment did not produce any significant enhancement of staining of either antibody and was not utilised in this study. The anti-carcinoembryonic antigen (aCEA) antibody was used at an optimal dilution of 1:2000 of the commercially recommended stock solution. No attempt was made to remove any reactivity to normal cross-reacting antigen (NCA).

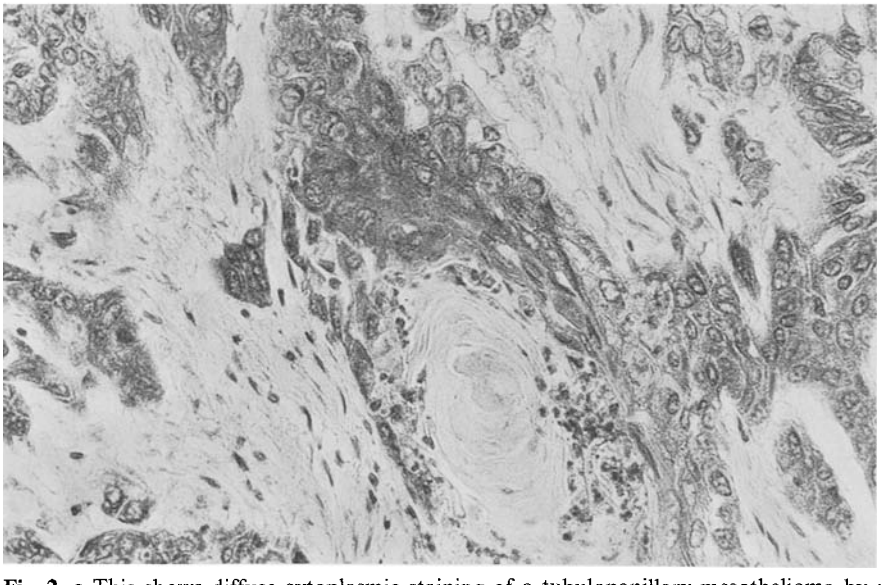
Immunoperoxidase staining (Fig. 2) was assessed on a single blind basis by one observer (A.G) and recorded as positive or negative with respect to a given antibody according to the presence or absence of a brown reaction product over the tumour cells significantly more intense than the background (e.g. collagen matrix). The positive sections were graded subjectively as follows: + (weak) = up to 5% of cells stained; + + (moderate) = 5 to 33% of cells stained; + + + (strong) = greater than 33% of cells stained.

Results

The results of staining with the various antibodies for mesotheliomas and pulmonary adenocarcinomas are shown in Table 1. The results obtained with aEDV and aLSV were very similar; therefore only the results for aLSV are shown. Seventy-five percent of malignant mesotheliomas were positive

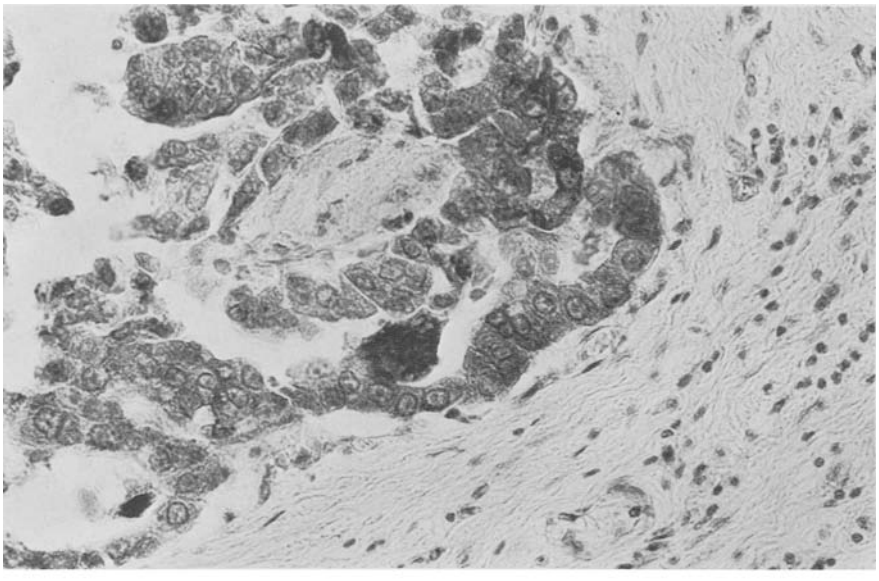


a



b

Fig. 2. a This shows diffuse cytoplasmic staining of a tubulopapillary mesothelioma by anti-vimentin antibody (aLSV) ×400. **b** A well differentiated adenosquamous carcinoma exhibiting diffuse cytoplasmic staining of the tumour cells by antivimentin antibody (aLSV). Note the negative staining of the centrally located keratin pearl. ×440. **c** A moderately differentiated adenocarcinoma showing strong positive staining by anti-CEA antibody (aCEA). ×440. **d** Epithelial type of mesothelioma showing absence of staining by anti-CEA antibody (aCEA). ×440



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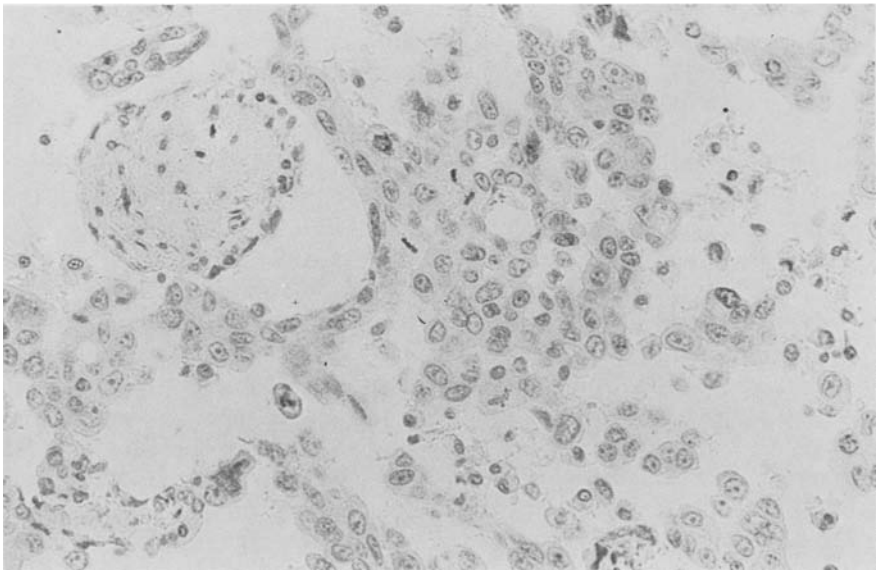

d

Table 1. Results of immunostaining for aLSV and aCEA

		Grade of staining				
		0	+	++	+++	Total
aLSV	– Mesothelioma	11	8	11	14	44
	Adenocarcinoma	13	2	6	3	24
aCEA	– Mesothelioma	44	0	0	0	44
	Adenocarcinoma	3	5	6	10	24

for vimentin whereas only 46% of pulmonary adenocarcinomas were. There was also a statistically significant tendency for the degree of staining to be greater in the mesotheliomas than in the adenocarcinomas (Mann-Whitney test: $P<0.032$). Vimentin positivity was found in all histological types of mesothelioma and adenocarcinoma regardless of grade except for the bronchioloalveolar tumour which was negative. The results for CEA staining show a highly significant difference between mesotheliomas and pulmonary adenocarcinomas. The three CEA negative adenocarcinomas included 1 well differentiated and 1 poorly differentiated bronchogenic carcinoma and 1 adenosquamous carcinoma.

Discussion

The main finding of this study is that the majority of malignant mesotheliomas are positive for vimentin (75%) but a considerable proportion of pulmonary adenocarcinomas also exhibit vimentin immunoreactivity (46%). This contradicts commercial claims that the intracellular presence of vimentin is exclusive to sarcomas. The discrepancy might be explained in a number of ways.

Misdiagnosis is unlikely since all mesotheliomas tested fulfilled established macroscopical and microscopical criteria (McCaughey 1965) and all were CEA negative (Gibbs et al. 1985; Wang et al. 1979; Whitaker et al. 1982). All histological grades of adenocarcinoma evinced vimentin immunoreactivity.

It is possible that the technique employed in this investigation is detecting otherwise sub-threshold amounts of vimentin immunoreactivity within the adenocarcinoma. The immunoperoxidase technique used has been shown to possess a very high sensitivity of detection, partly a result of the hapten anti-hapten bridge method (Jasani et al. 1981) and partly due to the inclusion of DNP-labelled glucose oxidase as an intrinsic source of hydrogen peroxide. The efficiency of diaminobenzidine polymerisation product formation mediated by peroxidase in the presence of the small amounts of nascent hydrogen peroxide is much greater than in the presence of exogenously added hydrogen peroxidase (Venkatachalam and Fahimi (1969); Tice and Wollman (1972); Jasani et al., in preparation.) Serial dilution of the monoclonal anti-vimentin antibodies failed to produce a primary antibody concentration capable of separating the two types of tumour. Erlandson (1984) has reported contamination of commercial antibodies to intermediate filaments. Although not specified, this is more likely to pertain to polyclonal antibodies produced to impure intermediate antigens rather than the monoclonal antibodies utilised in this study. The fact that both anti-vimentin antibodies obtained from separate sources gave identical results also makes this unlikely. The same argument militates against the possibility of inadvertant detection of an antigenic determinant allegedly shared by all classes of intermediate filaments (Pruss et al. 1981).

There is the possibility of co-expression of intermediate filaments e.g. vimentin expression by cytokeratin positive epithelial cells. Franke et al.

(1979) and Ramaekers et al. (1983c) have demonstrated the presence of vimentin and keratin in normal and neoplastic epithelial cells respectively after they have been subjected to in-vitro culture. It is noteworthy that whenever co-expression of intermediate filaments occurs one of them is always vimentin (Erlandson 1984). This may be because vimentin is fundamental to the structural integrity of the cell nucleus (Lazarides 1980); the function of which comes into particular prominence during the proliferative phase of the cell cycle (Franke et al. 1979). Furthermore, when cytokeratin and vimentin are present concomitantly within a given cell usually vimentin is detected in small quantities (Erlandson 1984). Our data support this observation since the staining scores for the adenocarcinomas were significantly lower than those for the mesotheliomas.

One can speculate that pulmonary adenocarcinomas have a greater propensity to co-express vimentin than other types of carcinoma studied to date, perhaps, because they are highly proliferative or have become so by the time diagnosis is made. Limited studies we have performed so far have shown that a considerable proportion of pulmonary adenocarcinomas and mesotheliomas used in this study stain for both vimentin and keratin.

The results of this study indicate that vimentin is of no use as a discriminatory marker between mesothelioma and pulmonary adenocarcinoma and the dictum that staining of a given tumour by specific monoclonal antibodies to intermediate filament proteins provides incontrovertible evidence of histogenetic type (Baumal et al. 1984) is no longer tenable. Nevertheless, in cases where a tumour is both CEA and BGP negative, positive vimentin immunoreactivity would provide valuable confirmatory evidence in favour of a mesothelioma. Furthermore, vimentin whenever coexpressed, might provide a useful prognostic marker.

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