Intermediate filaments in smooth muscle tumours

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SUMMARY Antisera to the intermediate filaments vimentin and desmin react with fixed paraffin embedded tissue. Benign uterine myomas contain both classes of filaments. Gastrointestinal "smooth muscle tumours" however often lack desmin even when they appear histologically benign. In the sarcomas examined vimentin was the only class of intermediate filament present. The diagnostic and histogenetic implications of these findings are discussed.

Intermediate filaments are present in a wide variety of cells and although they are morphologically and structurally similar in different cell types, it has been possible to divide them into subclasses according to antigenic determinants and biochemical properties. The main classes so far defined are vimentin (mainly in mesenchymal cells), desmin in muscle, prekeratin in epithelial cells, neurofilaments in neurones and glial fibrillary acid protein in glial cells.^{1,2}

It should, however, be noted that in certain circumstances epithelial cells may contain both prekeratin and vimentin,³ and that with muscle cells there is a change in the class of filament during development from vimentin to desmin, both being demonstrable at one stage.¹

It seemed of interest to examine some of the tumours classified histologically as smooth muscle tumours to determine the class(es) of intermediate filament present and to see whether these correlated with histological features or with malignancy.

Material and methods

Rabbit antiserum against desmin was kindly provided by Dr Badley. This had been raised against chicken gizzard desmin⁴ and was purified by affinity chromatography. It showed strong cross reaction with human desmin.

Rabbit antiserum against human fibroblast vimentin⁵ was kindly provided by Dr Virtanen.

Preliminary studies established that both antisera were active against dewaxed paraffin sections and that the reaction was reduced by pretrypsinisation. The method used was a conventional indirect immunofluorescence using FITC-conjugated goat

antirabbit IgG (Miles) as the second layer. The sections were viewed on a Leitz Orthoplan with epillumination. Optimum titres of antisera were determined using sections of intestine as substrate.

Positive fluorescence was obtained both in tissue fixed with formalin and with formol mercury and tissue blocks up to 40 years old gave satisfactory results though in many cases there was yellow autofluorescence from the tissue which caused some difficulty with black and white photography.

A comparison between formol mercury and formol-fixed paraffin sections and frozen sections of the same tissue showed best results in the fixed tissues with formol mercury marginally the best.

Blocks were obtained from departmental files and in each instance the diagnosis was reviewed on restrained sections. Cases studied are tabulated below:

Uterine myomas	5	Oesophageal leiomyoma	2
"Bizarre" leiomyomas	2	Gastric leiomyoblastoma	1
Uterine leiomyosarcomas	2	Gastric "smooth muscle tumour"	4
Subcutaneous angioleiomyoma	1	Small intestinal "smooth muscle tumour"	4

Details of the alimentary smooth muscle tumours are given in Table 1. Blocks of skin, gall bladder, oesophagus, stomach, intestine, heart, skeletal muscle were also studied.

Results

On normal tissues the results obtained were as shown in Tables 2 and 3.

Table 1 *Gastrointestinal smooth muscle tumours*

Case	Site	Age (yr)	Sex	Tumour size (mm)	Mitoses per 10 HPC	Outcome	Histology
1	Gastric	64	M	35 × 35 × 35	14	Metastasis to liver and mesentery	Malignant leiomyoblastoma
2	Gastric	57	M	40 × 40 × 15	5	Follow-up awaited	Leiomyosarcoma
3	Gastric	66	M	60 × 60 × 20	5	Well at 2 yr	Leiomyosarcoma
4	Gastric	61	M	30 × 30 × 30	<1	Well at 1 yr	Leiomyoma
5	Gastric	63	F	90 × 60 × 30	8	Metastasis to liver and mesentery. Died	Leiomyosarcoma
6	Small intestinal	81	M	30 × 25 × 25	0	No follow-up available	Leiomyoma
7	Small intestinal	54	M	110 × 75 × 65	3	Died at 1 yr of myocardial infarct	Leiomyosarcoma
8	Small intestinal	26	F	20 × 20 × 20	0	Well at 5 yr	Leiomyoma
9	Small intestinal	35	F	35 × 20 × 20	0	Well at 1 yr	Leiomyoma
10	Oesophagus	52	M	35 × 25 × 15	0	Well at 3 yr	Leiomyoma
11	Oesophagus	32	M	100 × 40 × 40	0	Well at 3 yr	Leiomyoma

Table 2 *Distribution of desmin*

Desmin-positive	Desmin-negative
Gastrointestinal smooth muscle	Fibroblasts
Uterine smooth muscle	Endothelium
Arrector pili muscle of skin	
Vascular smooth muscle	
Cardiac muscle	
Skeletal muscle	

Table 3 *Distribution of vimentin*

Vimentin-positive	Vimentin-negative
Fibroblasts	Cardiac muscle
Endothelium	Skeletal muscle
Vascular smooth muscle	Non-vascular smooth muscle

UTERINE TUMOURS

Benign myomas showed strong cellular staining for desmin (Fig. 1). Vimentin staining was present though weaker.

The bizarre leiomyomas stained positively for both desmin and vimentin. The staining was stronger for the former and strikingly in excess of the normal uterine smooth muscle (Figs. 2 and 3). Study of the adjacent sections suggested that both vimentin and desmin were present in the same cells, but since both sera were from the same species, this could not be formally established by double fluorescence.

Both leiomyosarcomas of the uterus were negative for desmin and stained very weakly for vimentin.

GASTROINTESTINAL SMOOTH MUSCLE TUMOURS

Both benign oesophageal myomas were desmin positive and weakly positive for vimentin.

With the smooth muscle tumours of the stomach and intestine desmin was usually absent, the

Fig. 1 Uterine myoma showing strong staining in the cytoplasm for desmin with negative nuclear images. Immunofluorescence × 400.

antiserum staining only vessel walls (Fig. 4) or residual muscle of the lamina propria. In one case there was weak patchy staining of tumour cells (Fig. 5). This was a small intestinal vimentin-positive tumour (case 7) which on the basis of pleomorphism and moderate number of mitoses had been considered malignant. Vimentin positivity was observed with seven out of nine lesions. It was usually rather weak.

ANGIOLEIOMYOMA

In the example studied both vimentin and desmin were present.

Fig. 2 *Bizarre leiomyoma of uterus. Haematoxylin and eosin $\times 400$.*

Fig. 3 *Bizarre leiomyoma of uterus showing strong staining for desmin. Immunofluorescence $\times 300$.*

Fig. 4 *Absence of desmin in gastric smooth muscle tumour. The vessel wall by contrast is positively stained. Immunofluorescence $\times 400$.*

Fig. 5 Weak patchy staining for desmin in an intestinal smooth muscle tumour which appeared histologically malignant. Immunofluorescence $\times 400$.

Discussion

The observed distribution of desmin and vimentin in normal tissue was essentially similar to that previously described. The main point deserving comment is the presence of both desmin and vimentin in blood vessel walls. In mammalian aorta the position is somewhat complex with the smooth muscle consisting of a heterogeneous population of cells containing either vimentin or desmin or both proteins.⁶ Other examples of coexistence of vimentin and desmin in the same cell are found only in cultured cells or developing muscle.

Our findings on the intermediate filaments of uterine myomas extend the observations of Gabbiani *et al.*⁶ Study of adjacent sections suggested that both vimentin and desmin were present in the same cell, but since the antisera were from the same species this could not be formally established by double immunofluorescence. By contrast sarcomas of uterus and most gastrointestinal "muscle" tumours contained only vimentin.

The presence of cells in vascular smooth muscle which are heterogeneous in their intermediate filament composition raises the possibility that benign smooth muscle tumours of subcutis, oesophagus, and uterus originate from blood vessel walls. This possibility is strengthened by the fact that so far in other neoplastic transformations no changes of intermediate filament type have been observed from the original tissue. An alternative interpretation would be that the

malignant smooth muscle tumours show a differentiation comparable to that of the primitive myoblast which contains only vimentin whereas the benign myomas are analogous to a later stage of myoblast development when both desmin and vimentin are present.

Spindle cell tumours of the gastrointestinal tract have always caused difficulty to the pathologist. They are conventionally designated as "smooth muscle tumours" because of the similarity of the cells to the smooth muscle cells, the demonstration of myofibrils in some cases and an apparent origin within the muscle coats. From a negative point of view there is an absence of features that would satisfactorily place them in any other category.

It could be argued that these tumours are a heterogeneous group and that only those showing myofibrils should be classified as "smooth muscle." Similar arguments have been employed against classifying tumours as rhabdomyosarcomas when they lack cross striations, but they have not won widespread acceptance.⁷

As Morson and Dawson have pointed out, it is difficult to forecast metastasis on the basis of histological criteria, some bland tumours behaving aggressively. They suggest⁸ that one should not attempt to subcategorise the group as leiomyomas and leiomyosarcomas but it seems more logical to attempt to find additional criteria.

Unfortunately the intermediate filament class is not of value for this purpose. Desmin was present in

both the oesophageal tumours which appeared histologically benign but of the remaining tumours only one contained small patches of desmin-positive cells and this appeared clearly malignant. Vimentin was present in most tumours both benign and malignant, but staining was often weak (probably reflecting the paucity of intermediate filaments present).

It seems that desmin is an index of differentiation in smooth muscle tumours, found in a majority of cells only in benign tumours. Desmin and vimentin occur together in benign smooth muscle tumours. In malignant smooth muscle tumours desmin appears only occasionally and in patches: where an intermediate filament is demonstrable it is vimentin.

Although the presence of desmin does not guarantee that a smooth muscle tumour is benign, in its absence such a tumour should be regarded with caution.

References

- ¹ Lazarides E. Intermediate filaments as mechanical integrators of cellular space. *Nature* 1980;**283**:249–56.
- ² Schmid E, Tapscott S, Bennett GS *et al.* Differentiation 1979;**15**:27–40.
- ³ Werner W, Franke WW, Schmid E, Winter S, Osborn M, Weber K. Widespread occurrence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates. *Exp Cell Res* 1979;**123**:25–46.
- ⁴ Badley RA, Lloyd CW, Woods SA, Carruthers L, Allcock C, Rees D. Mechanisms of cellular adhesion. *Exp Cell Res* 1978;**117**:231–44.
- ⁵ Virtanen I, Lehto VP, Lehtonen E, Vartio T, Stenman S. Expression of Intermediate Filaments in Cultured Cells. *J Cell Sci* 1981;**50**:45–63.
- ⁶ Gabbiani G, Schmid E, Winter S, *et al.* Vascular smooth muscle cells differ from other smooth muscle cells: predominance of vimentin filaments and a specific-type actin. *Proc Natl Acad Sci USA* 1981;**78**:298–302.
- ⁷ Ashton N, Morgan G. Embryonal sarcoma and embryonal rhabdomyosarcoma of the orbit. *J Clin Pathol* 1965;**18**:699–714.
- ⁸ Morson BC. "Non-epithelial tumours" and "Tumours." In: Morson BC, Dawson IMP, eds. *Gastrointestinal pathology* 2nd ed. Oxford: Blackwells, 1979:189 and 423.

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