Comparison of β enolase and myoglobin as histological markers of rhabdomyosarcoma

JANICE A ROYDS,* S VARIEND,† WR TIMPERLEY,‡ CB TAYLOR*

From the *Department of Biochemistry, University of Sheffield, the †Department of Histopathology, Children's Hospital, Sheffield, and the ‡Department of Neuropathology, Royal Hallamshire Hospital, Sheffield

SUMMARY A comparative study of β enolase and myoglobin as markers of muscle differentiation in rhabdomyosarcoma was carried out, using an immunoperoxidase peroxidase antiperoxidase technique. Material from 26 cases of childhood rhabdomyosarcoma was studied and subdivided into embryonal and alveolar types. Positive cytoplasmic staining for β enolase was seen in 85% of tumours studied (91% alveolar, 79% embryonal), whereas positive staining for myoglobin was detected in only 69% of tumours (82% alveolar, 64% embryonal). β Enolase and myoglobin are useful in the histological diagnosis of rhabdomyosarcoma, and of the two, β enolase seems to be the more sensitive.

The differential diagnosis of rhabdomyosarcoma from other paediatric small cell neoplasms such as neuroblastoma, Ewing's sarcoma, and lymphoma is often difficult. Accurate diagnosis is essential to select optimum treatment. Unfortunately, in poorly differentiated tumours the diagnostic accuracy, based on overall cellular pattern and the presence of rhabdomyoblasts and cross striations, is low.¹ As the classification of these tumours is based on their histogenesis the development of tissue specific markers should improve diagnostic accuracy. Several protein constituents of striated muscle have been suggested as possible markers for rhabdomyosarcoma—namely, desmin, creatine phosphokinase (muscle type), and myoglobin.^{2,3} Kahn *et al* showed that of these markers, myoglobin had the highest specificity.⁴

Myoglobin is a protein of low molecular weight that transports and stores oxygen and is found exclusively in striated muscle. Its presence has been shown in rhabdomyosarcomas with varying degrees of sensitivity, ranging from 37% of tumours⁴ to 89%.³ Intermediate levels of sensitivity of 47.8% and 76% were shown by de Jong *et al*⁵ and Corson and Pinkus,² respectively.

In a previous study we investigated the use of skeletal muscle specific isoenzyme of the glycolytic enzyme enolase β enolase as a diagnostic marker in rhabdomyosarcoma and found that it had an overall sensitivity of 86%.⁶ We therefore carried out a com-

parative study of these two most promising markers of muscle differentiation in rhabdomyosarcoma—namely, β enolase and myoglobin.

Material and methods

ANTISERA

Anti- $\beta\beta$ enolase serum was produced in rabbits using protein purified from human skeletal muscle as previously described.⁶ This antiserum was used at a dilution of 1/100. A commercially available rabbit antihuman myoglobin antiserum was used (Boehr- ing Institut, West Germany) at a dilution of 1/100.

HISTOLOGY

Material from 26 cases of childhood rhabdomyosarcoma was obtained from the files of the Sheffield Children's Hospital. Diagnosis was established using conventional clinical and histological criteria as previously described.⁶

Sections (6 μ m) were cut, dewaxed, and stained for β enolase and myoglobin using the immunoperoxidase peroxidase antiperoxidase technique with 3,3'-diaminobenzidine as a final indicator.⁷ Normal rabbit serum or antiserum adsorbed with the appropriate antigen were used as negative controls. Sections of skeletal muscle were included in each batch to serve as positive controls.

Results

The 26 tumours diagnosed as primary or metastatic

Anti- β enolase and anti-myoglobin positive staining of childhood rhabdomyosarcoma

Case no	β enolase	Myoglobin
<i>Alveolar</i>		
1	3+	3+
2	3+	3+
3	2+	1+
4	3+	3+
5	2+	1+
6	2+	1+
7	3+	0
8	2+	0
9	occasional cell 1+	occasional cell 1-
10	0	1+
11	3+	3+
<i>Embryonal</i>		
12	0	0
13	0	2+
14	3+	2+
15	1+	1+
16	1+	1+
17	1+	2+
18	1+	0
19	2+	3+
20	1+	2+
21	trace	0
22	1+	0
23	1+	1+
24	3+	4+
25	0	0
<i>Unclassifiable</i>		
26	2+	0

rhabdomyosarcoma were subdivided into embryonal and alveolar types. One tumour was not classifiable. There were no tumours with a pleomorphic pattern. The Table summarises the results of the staining for β enolase and myoglobin. No staining was found in the control sections when normal rabbit serum or adsorbed antiserum was used.

Positive cytoplasmic staining for β enolase was seen in 85% of tumours (91% alveolar, 79% embryonal) (Fig. 1), and some of the strongest staining was found in the large multinucleate cells. Intracellular myoglobin was detected in 69% of tumours (82% alveolar, 64% embryonal) (Fig. 2).

Twenty four of the 26 tumours were positive for one or both of these markers of skeletal muscle differentiation. Of the two tumours reacting negatively for both markers, one was a poorly differentiated myxoid tumour from the middle ear and the other a myxoid tumour from the bladder, which, rather surprisingly, showed myoblastic differentiation on conventional histological examination. Only two of the neoplasms stained for myoglobin but not for β enolase, and one of these (Case 13; Table) was a metastatic deposit from a primary tumour (Case 1) that was negative for both markers. Six of the tumours were positive for β enolase but negative for myoglobin.

Fig. 1 Section of rhabdomyosarcoma stained for β enolase showing considerable variation in intensity of staining of rhabdomyoblasts. Original magnification $\times 750$.

Fig. 2 Section of rhabdomyosarcoma stained for myoglobin. Original magnification $\times 750$.

Discussion

Positive cytoplasmic staining for β enolase was seen in 85% of tumours studied in this series, whereas positive staining for myoglobin was detected in only 69% of tumours. There may be several reasons why β enolase is a more sensitive marker than myoglobin. Firstly, the presence of each marker in tumour cells may relate to the degree of differentiation, and,

secondly, myoglobin may need a slightly higher degree of cellular differentiation for its expression. β Enolase is present in human fetal muscle cells from about the 10th to 12th weeks of gestation with concentrations approaching those of adult muscle by the 28th weeks,⁷ whereas myoglobin is present only in low concentrations in fetal muscle cells, increasing mainly during postnatal maturation.⁸

A possible correlation between myoglobin staining and degree of differentiation has been reported in rhabdomyosarcoma.^{3,9} Furthermore, myoglobin occurs at a greater concentration in slow twitch than in fast twitch muscle.³ Slow twitch muscle develops later than the fast twitch muscle type and only after innervation.¹⁰ The presence of specific components of slow twitch muscle therefore represents a high degree of differentiation. In a study by de Jong *et al* all the cases of rhabdomyosarcoma were negative for another protein characteristic of slow twitch muscle—namely, antislowl myosin.⁵ Twenty of 23 cases of rhabdomyosarcoma in the same study, however, were positive for antifat myosin. Other reasons for differences in sensitivity may be differences in the stability of myoglobin and β enolase and variations in the sensitivity of the staining technique for the different markers.

β Enolase and myoglobin, either singly or together, are useful in the histological diagnosis of rhabdomyosarcoma. They are both sensitive markers and seem to be specific for striated muscle as no other soft tissue tumour has yet been shown to stain positively for either β enolase or myoglobin.^{2,6} Of the two, β enolase seems to be the more sensitive.

We thank Mrs Anne Davis, Mr A Buxton, and Miss P Little for technical help and Mrs P Kirk for typing the manuscript.

We gratefully acknowledge financial support from the Wellcome Trust (Grant 11353/1.5).

References

- ¹ Bale PM, Parsons RE, Stevens MM. Diagnosis and behaviour of juvenile rhabdomyosarcoma. *Hum Pathol* 1983;14:596–611.
- ² Corson JM, Pinkus GS. Intracellular myoglobin—a specific marker for skeletal muscle differentiation in soft tissue sarcomas. *Am J Pathol* 1981;103:384–9.
- ³ Brooks JJ. Immunohistochemistry of soft tissue tumours. Myoglobin as a tumour marker for rhabdomyosarcoma. *Cancer* 1982;50:1757–63.
- ⁴ Kahn HJ, Yeger H, Kassim O, *et al*. Immunohistochemical and electron microscopic assessment of childhood rhabdomyosarcoma. *Cancer* 1983;51:1897–903.
- ⁵ De Jong ASH, Van Raamsdonk W, Van Vark M, Vonte PA, Albus-Lutter CLE. Myosin and myoglobin as tumour markers in the diagnosis of rhabdomyosarcoma. *Am J Surg Pathol* 1984;8:521–8.
- ⁶ Royds JA, Variend S, Timperley WR, Taylor CB. An investigation of beta enolase as a histological marker of rhabdomyosarcoma. *J Clin Pathol* 1984;37:905–10.
- ⁷ Royds JA, Parsons MA, Taylor CB, Timperley WR. Enolase isoenzyme distribution in the human brain and its tumours. *J Pathol* 1982;137:37–49.
- ⁸ Kagen LJ, Christian CL. Immunologic measurements of myoglobin in human adult and fetal skeletal muscle. *Am J Physiol* 1966;211:656–60.
- ⁹ Kagawa N, Sano T, Inaba H, Mori K, Hizawa K. Immunohistochemistry of myoglobin in rhabdomyosarcomas. *Acta Pathol Jpn* 1983;33:515–22.
- ¹⁰ Rubinstein NA, Kelly AM. Development of muscle fibre specialisation in the rat hind limb. *J Cell Biol* 1981;90:128–44.

Requests for reprints to: Dr WR Timperley, Department of Neuropathology, Royal Hallamshire Hospital, Sheffield S10 2JF, England.