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ORIGINAL ARTICLE

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Actin isoform pattern expression: a tool for the diagnosis and biological characterization of human rhabdomyosarcoma

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Abstract The diagnosis and characterization of rhabdomyosarcoma requires the use of combined histological and immunohistochemical criteria due to the variety of its histological patterns. The identification of actin isoform expression is accepted as a useful adjunct to the diagnosis and classification of soft tissue tumors. Using a new antibody specific for α-cardiac actin, obtained according to a recently described strategy for the production of polyclonal antibodies against actin isoforms [9], we have analyzed a series of 17 rhabdomyosarcomas, including all histological subtypes. In addition, we have evaluated the presence in these tumors of α-skeletal and α-smooth muscle actins. All specimens examined revealed a positive immunostaining for α-cardiac actin. Tumoral cells of eight cases also expressed α-smooth muscle actin and only three cases (all embryonal subtypes) were positive for α-skeletal actin. Our results indicate that immunohistochemical screening for α-cardiac actin expression is a useful tool for the diagnosis of rhabdomyosarcoma. They also suggest that the expression of α-skeletal actin is valuable in determining the subtype and possibly the state of differentiation of these tumors.

Keywords Cytoskeleton · Immunohistochemistry · Muscle development · Actin antibodies

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Introduction

In mammalians, actin isoforms are expressed in a tissue-specific pattern that is strictly conserved, although no specific functions have up to now been clearly defined for each of them [23]. Actin isoform sequence differences concern few amino acids, essentially located at the N-terminal portion of the molecule; this renders problematic the production of specific monoclonal and/or polyclonal antibodies. Despite these difficulties, few actin isoform-specific antibodies have been produced by different laboratories including ours [9, 17, 19, 35] and have proven useful for the study of biological features of different tissues [10, 21]; they have also been successfully utilized in the diagnosis of soft tissue tumors [32, 36]. We have recently developed a new strategy to purify polyclonal specific antibodies against actin isoforms [9]. Here, we describe the production of an α-cardiac actin-specific antibody (anti α-CA1) and its application to the diagnosis and characterization of rhabdomyosarcoma (RMS).

RMS, the most common soft-tissue malignancy of childhood and adolescence, is a highly heterogeneous group of tumors with respect to their clinical, morphological and differentiation features. The most accepted histological classification comprises botryoid, embryonal, alveolar and pleomorphic subtypes [14]; in addition a spindle cell variant has been described [6, 31]. Differences among subtypes are not only histological but also include clinical behavior and follow-up (for review see [14, 29]). The common feature of RMS is the propensity for the formation of neoplastic skeletal fibers; thus, the diagnosis is usually based, in addition to conventional histology, on immunohistochemical and ultrastructural investigations that identify this phenotype. Myoglobin, β-enolase and, more recently, myogenin and MyoD1 – two transcriptional regulatory proteins expressed early in skeletal muscle differentiation [38] – have been reported as myogenic markers [7, 13]. A strong immunostaining for myogenin has been associated with the alveolar subtype of RMS [13]; however, a negative staining in a pro-

portion of cases has been noted [7]. Although all RMSs revealed immunoreactive for MyoD1, the nuclear specificity often associated with a nonspecific background staining and reactivity of nonmyoid tissues as well as in other tumors, i.e., Wilms' tumor with myogenous differentiation, ectomesenchymomas [24] and small cell carcinoma with skeletal muscle differentiation [16], hinder its utility in paraffin-embedded routine diagnostic screening [7]. Antibodies against cytoskeletal proteins, actin and desmin in particular, have proven useful for the establishment of RMS diagnosis [2, 8, 11, 12, 15, 22, 28, 36]. We have previously reported that RMSs express transcripts for α-cardiac and α-smooth muscle actin but not α-skeletal actin mRNA [33]; however, results obtained from molecular biology studies are neither easily quantifiable nor routinely applicable during diagnostic procedures. Using anti α-CA1, in combination with two other antibodies specific respectively for α-smooth muscle actin [35] and α-skeletal actin [9] previously developed in our laboratory, we have investigated the differential expression of actin isoforms in 17 primary RMSs. Our results demonstrate that this approach facilitates the diagnosis of RMS and helps in the characterization of the principal subtypes of this tumor.

Materials and methods

Patient population

A total of 17 RMSs were obtained from the files of either the Division of Clinical Pathology, Geneva, Switzerland; the Institute of Anatomic Pathology, Tor Vergata University of Rome, Italy, or the Department of Oncology, University of Bologna, Italy. The main characteristics of patients are reported in Table 1. All tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin. For all tumors, the diagnosis of RMSs had been made on the basis of clinical and histological data using accepted classification criteria [14] and confirmed in some cases by immunohistochemical staining for established myogenic markers. Control tissues included: uterine leiomyoma (10); neuroblastoma (1); leiomyosarcoma (8), of which seven were uterine and one was from the skin; benign fibrous histiocytoma including dermato-fibroma (8) and lymphoma (1).

Anti α-CA1 production and immunopurification

Anti α-CA1 was prepared following an experimental strategy adapted from the one previously used in order to prepare an antibody against α-skeletal actin (anti α-SKA1) [9]. Briefly, the NH_2-terminal decapeptide of α-cardiac actin (Ac-DEEDTTALVC-COOH) was coupled to maleimide activated keyhole limpet hemocyanin (KLH, Pierce, Rockford, Ill.) through its cysteine residue, according to the instructions of the manufacturer. The coupled peptide was used for antiserum production in a rabbit (performed by Biodesign International, Kennebunk, Me.). The antiserum was first affinity purified using Sulfolink beads (Pierce) coupled with the α-cardiac actin decapeptide. Elution fraction was dialyzed overnight against phosphate-buffered saline solution and then successively loaded on (1) a Sulfolink column coupled with the NH_2-terminal decapeptide of α-skeletal actin, (2) a column coupled with the NH_2-terminal decapeptide of β-cytoplasmic actin and (3) finally a column coupled with the NH_2-terminal decapeptide of γ-smooth muscle actin to remove the population of antibodies cross-reacting with these three isoforms. The antibodies present in the final flow-through were finally concentrated on the column coupled with the α-cardiac decapeptide, and the resulting elution fraction represented anti-αCA1.

Electrophoretic and immunoblot analysis

Antibody specificity was determined by immunoblotting of the following whole-tissue homogenates: rat striated muscle, rat myocardium, human blood platelets, rat aorta and chicken gizzard

Table 1 Clinicopathologic, morphological and immunohistochemical information of patients with rhabdomyosarcoma (RMS). *LTF* lost to follow-up, *DOD* died of disease, *NED* no evidence of disease, *AWD* alive with disease

Case	Age (y) /sex	Localization	Morphological diagnosis	Status	α-Actin isoform expression in tumor cells			Desmin
					α-CARD	α-SK	α-SM	
1	2.5/Male	Prostate	Embryonal	DOD (26 months)	+++	+++	–	+++
2	5/Female	Pelvic cavity	Embryonal	DOD (7 months)	+++	+	–	+++
3	24/Male	Left jaw	Embryonal	DOD (3 months)	+++	–	–	++
4	15/Female	Anterior clinoid	Embryonal	LTF	++	–	++	+
5	17/Female	Orbit	Embryonal	LTF	+++	–	++	+++
6	2/Male	Right lung	Embryonal	DOD (10 months)	+++	+++	–	+++
7	22/Male	Orbit	Embryonal	NED (3 years)	+++	–	–	+++
8	9/Male	Urethra/bladder	Embryonal, botryoid	LTF	+++	–	–	+
9	15/Male	Nasal cavity	Predominantly alveolar	AWD (2 years)	+++	–	++	+++
10	6/Male	Para-testicular	Alveolar	AWD (3 years)	+++	–	+	+++
11	23/Female	Pterygo-palatine pit	Alveolar	AWD (1 year)	+++	–	–	+
12	16/Male	Head-neck region	Alveolar	DOD (7 days)	+++	–	–	+++
13	5/Male	Tongue	Mixed embryonal, alveolar	NED (6 years)	+++	–	++	++
14	49/Male	Striated muscle/ upper part of the back	Pleomorphic	LTF	+++	–	+	+++
15	43/Male	Striated muscle/ lumbar region	Spindle cells	AWD (6 years)	+	–	–	+
16	21/Female	Hand	Alveolar	DOD (2 years)	++	–	++	–
17	25/Female	Shoulder	Alveolar	AWD (3 years)	++	–	++	++

(40 µg per lane was loaded for Coomassie blue staining and 2 µg per lane for immunoblotting). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in 10% polyacrylamide gels [25] and the proteins were electroblotted to nitrocellulose according to Towbin et al. [39]. Nitrocellulose membranes were incubated with anti α-CA1 diluted in Tris-buffered saline solution containing 3% bovine serum albumin (BSA) and 0.1% Triton X-100 for 2 h at room temperature. After three washes with Tris-buffered saline, a second incubation was performed with peroxidase-conjugated affinity-purified goat anti-rabbit IgG (Jackson Immunoresearch Laboratories, West Grove, Pa.) at a dilution of 1:10,000 in Tris-buffered saline containing 0.1% BSA and 0.1% Triton X-100. Peroxidase activity was developed using the ECL Western-blotting system (Amersham, Rahn AG, Zürich, Switzerland), according to the instructions of the manufacturer.

Light microscopy and immunohistochemistry

Four-micron sections of formalin-fixed and paraffin-embedded fragments of RMSs were either stained with hematoxylin-eosin or used for immunohistochemistry with anti α-SKA1 (1:40 dilution in Tris-buffered saline [9]), anti α-smooth muscle actin (α-sm1 [35]; 1:200 dilution in Tris-buffered saline), anti α-CA1 (1:20 dilution in Tris-buffered saline) and monoclonal antibody against human desmin (Clone 33, Dako, Glostrup, Denmark; 1:20 dilution in Tris-buffered saline). Immunoperoxidase staining was performed essentially as previously described [9]. After staining, sections were observed using a Zeiss Axiophot photomicroscope. Images were acquired with a high sensibility Axiocam color camera (Carl Zeiss, Oberkochen, Germany), stored on optical disks (Sony Corp, Tokyo, Japan) and printed with a digital Fujifilm Pictography 4000 printer (Fujifilm, Tokyo, Japan).

Indirect immunofluorescence and confocal laser scanning microscopy

Rat hearts were embedded in OCT 4583 (Miles Scientific, Naperville, Ill.) and frozen in pre-cooled liquid isopentane. Three-micron cryostat sections were fixed in acetone at –20°C for 5 min and air-dried for 2 h at room temperature. Sections were double-stained with purified anti α-CA1 (1:20 dilution in phosphate-buffered saline solution) and monoclonal antibody against human desmin (Dako, 1:10 dilution). Subsequently, tissue sections were incubated with TRITC-conjugated anti-rabbit and fluorescein-labeled anti-mouse antibodies (both from Jackson ImmunoResearch Labs, Inc.), mounted in buffered polyvinyl alcohol [27] and observed with a confocal laser scan fluorescence inverted microscope (model LSM 510, Carl Zeiss). Images were stored on optical disks (Sony Corp) and printed with a digital Fujifilm Pictography 4000 printer (Fujifilm).

Scoring of the staining

For each tumor specimen, the antibody-stained sections were carefully examined by two independent observers and staining was scored as follows: – all tumor cells negative, + 1–10% tumor cells positive, ++ 10–50% tumor cells positive, +++ 50–100% tumor cells positive.

Results

Purification and determination of the specificity of anti α-CA1

As shown previously for anti-SKA1 [9], immunization with hemocyanin-coupled α-cardiac actin NH_2-terminal decapeptide resulted in high titer antibodies showing an important cross-reactivity with all actin isoforms (data not shown). The first step of purification consisted of loading the immune serum on Sulfolink beads coupled with the decapeptide used for immunization. To test the cross-reactivity of the eluted antibodies with actin isoforms, we used extracts of rat striated muscle for α-skeletal actin; rat myocardium for α-cardiac actin; human blood platelets for β and γ cytoplasmic actins present at the ratio of 5 to 1 [26]; rat aorta for α-smooth muscle actin; and chicken gizzard for γ-smooth muscle actin. Sequences of actin isoforms are perfectly conserved in higher vertebrates [40], hence species differences did not interfere with our results. Western-blot analysis (Fig. 1A, panel b) showed that the antibody population eluted from the beads coupled with the α-cardiac decapeptide strongly reacted with α-cardiac actin but also with the other isoforms.

Following a strategy similar to one recently reported [9], antibody populations recognizing epitopes common to the other actin isoforms were then eliminated by sequential passages on columns coupled with NH_2-terminal decapeptide of actin isoforms sharing similar amino acid sequences with α-cardiac actin (see Materials and methods). Reactivity of the antibody following α-skeletal, β-cytoplasmic and γ-smooth muscle actin columns are presented in Fig. 1A, panels c, d and e, respectively. The resulting purified antibody population recognized the actin isoform present in the cardiac muscle extract but did not cross react with actin from platelets, aorta and gizzard (Fig. 1A, panel e). The antibody also recognized the skeletal muscle homogenate, albeit to a clearly lesser extent (Fig. 1A, panel c, lane 1).

α-Cardiac actin has been shown to be expressed in both skeletal and cardiac tissue during mouse development, to be detectable in adult skeletal muscle and to represent about 5% of the sarcomeric actin mRNA in adult mouse [18]. Therefore, because all extracts were blotted after loading with similar amounts of actin (Fig. 1A, panel a), it appeared likely that the reactivity of purified anti α-CA1 with the skeletal muscle extract is due to the fraction of muscle α-cardiac actin present in this tissue. To unequivocally test specificity, purified anti α-CA1 was incubated with synthetic peptides corresponding to the NH_2-terminal sequences of the six isoforms before immunoblotting analysis (Fig. 1B). The decapeptide of α-cardiac actin used for immunization completely blocked the reactivity of purified anti α-CA1; however, peptides corresponding to the NH_2-terminal sequence of the other actins including α-skeletal actin had no effect (Fig. 1B). Anti α-CA1 was then tested by means of immunofluorescence on rat cardiac muscle. Confocal microscopy demonstrated that, as expected, desmin and anti α-CA1 stainings are located in the Z-bands and I-bands of myocytes, respectively (Fig. 1C); no colocalization between these two antigens was observed. The staining distribution in the myocardium was uniform with the exception of areas in which, according to our previous results, α-skeletal actin was present instead of α-cardiac actin [9] (data not shown).

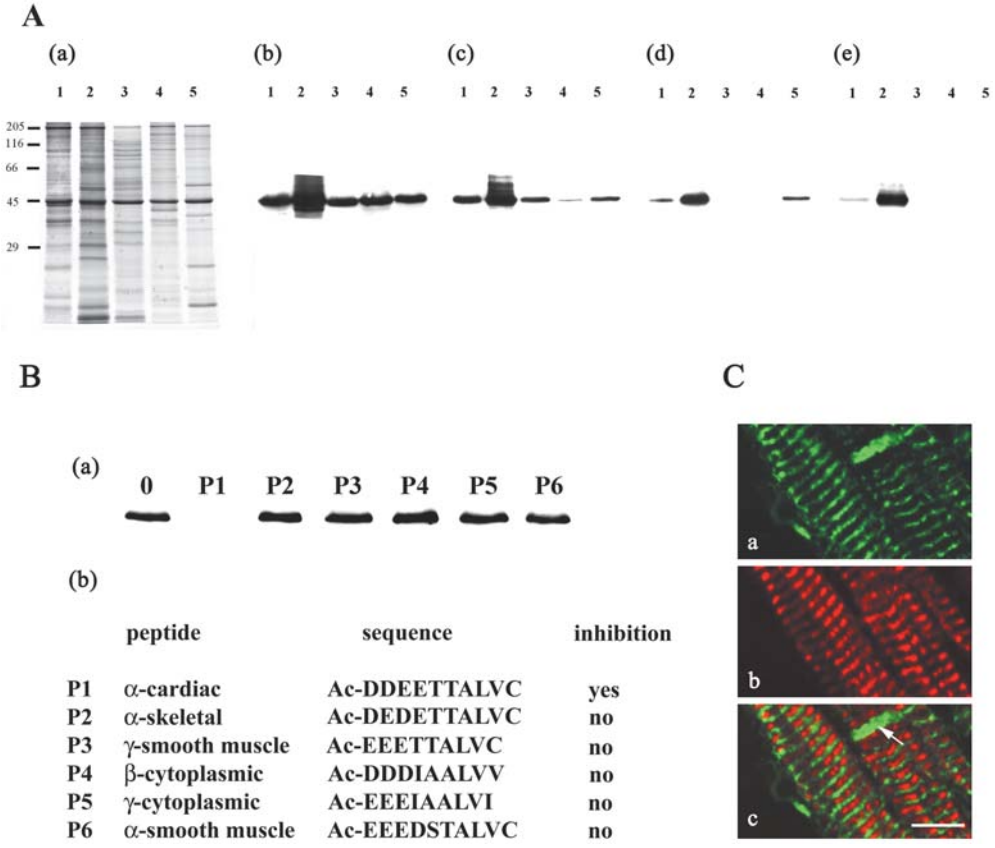

Fig. 1 Characterization of anti α-CA1 specificity by Western-blot analysis and immunofluorescence. **A** Specificity of the antibody was assessed at different steps of purification by immunoblots on appropriate tissue or cell extracts. *a* Coomassie blue staining of a 10% gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of rat skeletal muscle (*lane 1*), rat myocardium (*lane 2*), human blood platelets (*lane 3*), rat aorta (*lane 4*) and chicken gizzard (*lane 5*) (40 μg per lane). *b–d* Western blots immunostained with anti α-CA1 at different purification steps. *b* The serum was run on beads coupled with the NH_2-terminal decapeptide of α-cardiac actin; then the fractions recovered from beads were further run on beads coupled with α-skeletal (*c*), β-cytoplasmic (*d*) and γ-smooth (*e*) NH_2-terminal decapeptides. The final antibody reacts strongly with cardiac and to a lesser extent with skeletal muscle homogenates (*e*). **B** Inhibitory effect of decapeptides corresponding to the NH_2-terminal sequence of actin isoforms on purified anti α-CA1 reactivity was tested by Western blot. Rat cardiac muscle homogenate was subjected to SDS-PAGE followed by Western blotting with purified anti α-CA1 alone (*lane 0*) or mixed with each of the peptides P1 to P6. Only P1 blocked the reactivity of anti α-CA1. **C** Adult rat cardiac muscle sections were double-labeled by indirect immunofluorescence with a monoclonal antibody against desmin (*a*) and the purified anti α-CA1 (*b*). Tissues were observed with a confocal laser scan fluorescence microscope. The overlay of images *a* and *b* presented in *c* shows no colocalization of these antigens. Anti α-CA1 is restricted to I-bands, while anti-desmin stains Z-lines and intercalated disks (*arrow*). *Scale bar* 5 μm

Immunochemical staining of tumor specimens

Results of immunohistochemical investigation are summarized in Table 1. All RMSs tested except one revealed a positive staining for desmin but significant differences in the level of expression of this protein were observed: an important number of positive cells was present in nine cases, while in three others the number of positive cells was between 10% and 50%, and in four cases it was less than 10%.

We have previously shown by means of an antibody recognizing both α-skeletal and α-cardiac isoforms that a series of embryonal, alveolar and pleomorphic RMSs expressed α-sarcomeric actin [35]. The availability of specific anti α-SKA1 [9] and anti α-CA1 allowed us to investigate separately their expression in RMSs. As shown in Table 1, all tumors were positive for α-cardiac actin that was expressed in different proportions: 13 of 17 cases were strongly α-cardiac actin positive (between 50% and 100% of tumoral cells, see Fig. 2b, e). In only one case (spindle-cell RMS), the positivity was less than 10% (case 15, Fig. 3c); some vessels in this subtype were positive for this actin isoform (Fig. 3d see below). The majority of RMSs did not express α-skeletal actin. Figure 2 shows a typical example of an embryonal (*left column*) and an alveolar (*right column*) RMS stained with anti α-CA1 and anti α-SKA1. Both cases are strongly positive for α-cardiac actin (Fig. 2b, e) and negative for α-skeletal actin (Fig. 2c, f). In three RMSs (all embryonal), we found a coexpression of the two sarcomeric actin isoforms within the same area of the tumor (Fig. 3a and b). Tumoral cells positive for α-skeletal actin corresponded to differentiated rhabdomyoblasts with relatively abundant cytoplasm (Fig. 3b). The expression of α-smooth muscle actin was likewise investigated, as this actin isoform has been previously found in tumoral

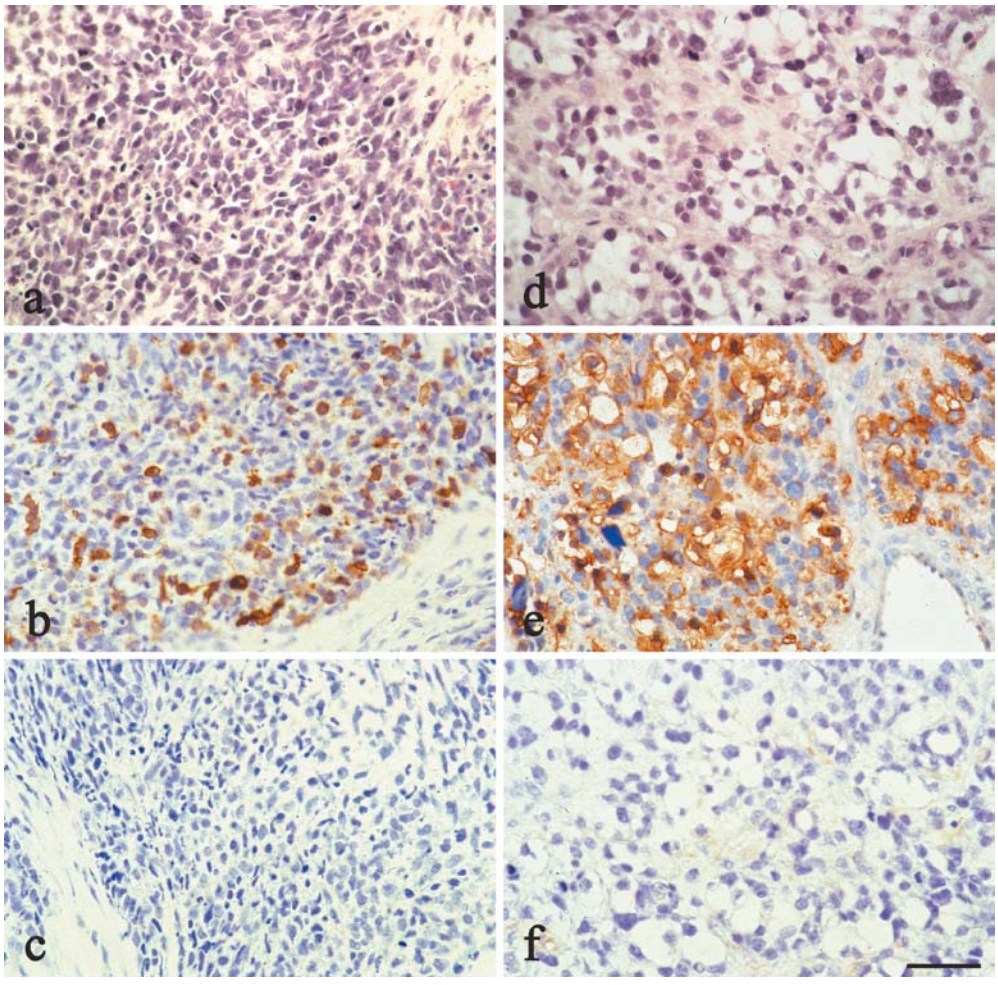

Fig. 2. Representative staining for α-cardiac and skeletal actins of alveolar and embryonal rhabdomyosarcoma (RMS). Serial sections of embryonal (case 5) and alveolar RMS (case 10) were stained with hematoxylin and eosin (**a** and **d**), anti α-CA1 (**b** and **e**) and anti α-SKA1 (**c** and **f**) antibodies. Both tumors are clearly positive for α-cardiac actin and negative for α-skeletal actin. *Scale bar* 200 μm

cells of some RMSs [35]. α-Smooth muscle actin was detectable in tumor cells of eight cases with levels of expression generally lower than those found for the other actin isoforms (Fig. 3e). It is interesting to note that the three cases of embryonal RMSs positive for α-skeletal actin were negative for α-smooth muscle actin (Table 1). No differences were present in the frequency of α-smooth muscle actin expression comparing alveolar and embryonal subtypes of RMSs. As expected, fibroblastic cells of the stroma reaction, when present in the tumor specimen, were strongly positive for α-smooth muscle actin (Fig. 3f). Staining for α-cardiac actin in control tumors was negative, with the exception of leiomyoma in which a slight positivity was seen in about 30% of cells in five cases and leiomyosarcoma in which a positivity was seen in about 20% of cells in two cases. In addition, in rare occasions, small arterial vessels were clearly positive both within the tumor and in adjacent apparently normal connective tissue.

Discussion

During the last few years, antibodies specific for a single actin isoform have become progressively available [9], although some of them (e.g., against γ-smooth muscle or γ-cytoplasmic actins) are still lacking. The use of such antibodies has allowed exploration of the function of these slightly different but well-conserved and tissue-specific proteins; for example, evidence has been produced that α-smooth muscle actin plays a role in regulating the motility [30] and the contractility [3, 20] of myofibroblastic cells. The production of an antibody against α-cardiac actin has been previously described, but its specificity is dependent on the experimental conditions, with obvious difficulties in interpretation, particularly in routine diagnostic procedures [17]. We have recently developed a strategy, which has allowed us to produce antibodies strictly specific for α-skeletal [9] and α-cardiac (present paper) actins, despite the extremely small sequence difference distinguishing these two isoforms [40]. The success in producing such specific anti-actin antibodies suggests that this strategy may be attempted to obtain a complete panel of antibodies specific for each isoform.

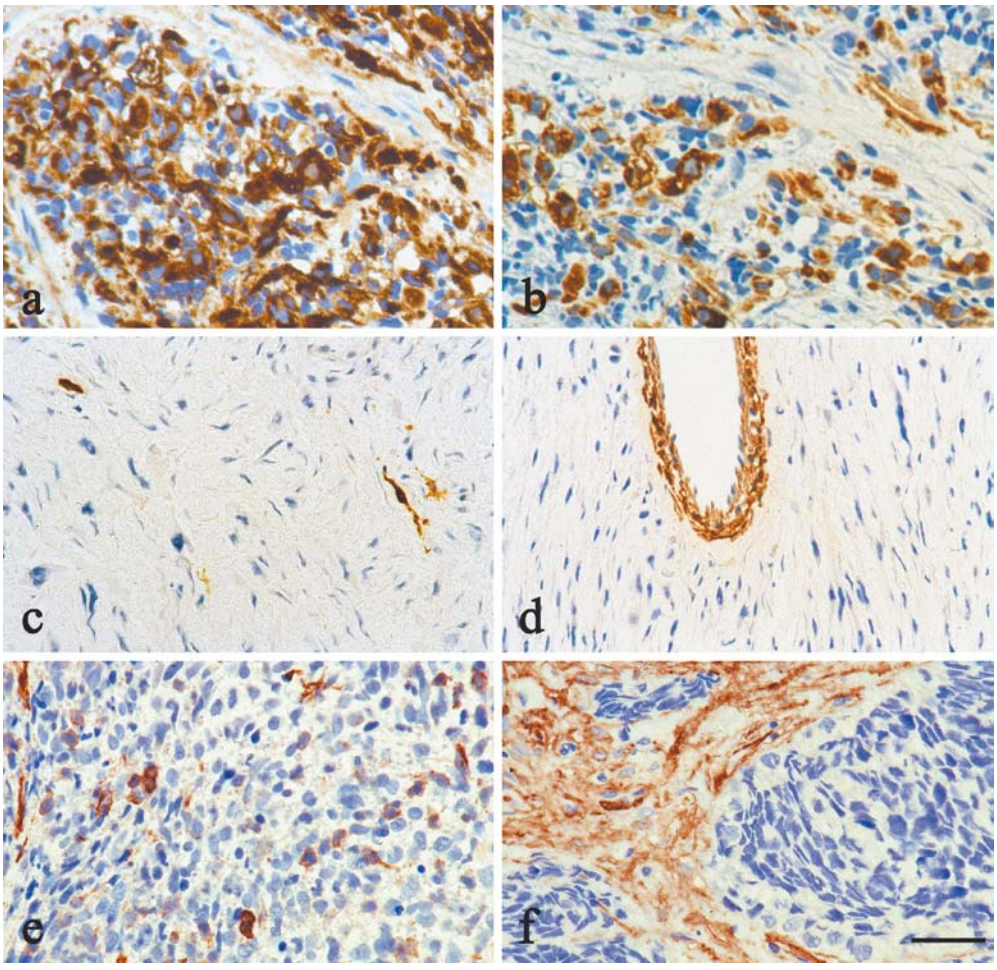

Fig. 3 Actin isoforms distribution in different rhabdomyosarcomas (RMSs). **a**, **b** Representative pictures of a RMS positive for α-skeletal actin. Serial sections of case 1 were immunostained with anti α-CA1 (*a*) and anti α-SKA1 (*b*). Both antibodies strongly react with tumor cells. **c**, **d** α-Cardiac staining of spindle-cell RMS. A few cells of case 15 are positive throughout the tumor (*c*); interestingly, the wall of a small vessel reacts with anti α-CA1 (*d*). **e**, **f** α-Smooth muscle actin staining of RMS. Tumoral cells of case 5 reveal positive staining for α-smooth muscle actin (*e*). Stromal myofibroblasts of case 7 are positive for α-smooth muscle actin (*f*). *Scale bar* 200 μm

The antibodies against α-skeletal and α-smooth muscle actins have already proven their usefulness for the study of myocardial hypertrophy mechanisms in both experimental animals [9, 10] and humans [37]. The use of these antibodies in combination with anti α-CA1 should be of further help in order to precisely investigate skeletal and cardiac muscle development and disease.

In this work we have focused on a practical aspect of actin antibody utilization, i.e., the diagnosis of RMS, which remains difficult to establish without ambiguity. Immunohistochemical markers, in particular those recognizing cytoskeletal proteins, have improved the accuracy of the diagnosis of soft tissue tumors including RMS [2, 8, 11, 12, 15, 22, 28, 33, 36]. Traditionally, markers for myosin heavy chain or desmin have been used to distinguish RMS from other soft tissue sarcomas with similar histological patterns. However, these markers, in particular desmin, do not have optimal specificity for RMS and also present heterogeneous expression among RMSs [29]. Here we show that anti α-CA1 is a general marker of all subtypes of this tumor in accordance with previous results at the mRNA level [33]. All control soft tissue and small cell tumors examined were negative with the exception of most leiomyomas, few leiomyosarcomas and, in normal tissues, some arterial vessels. Hence, with these limitations, we confirm the specificity of α-cardiac actin positivity in the characterization of RMS. The presence of α-cardiac actin in arterial vessels has been previously reported [5]; further studies are needed in order to establish the role of this isoform in smooth muscle cells.

By means of an antibody recognizing both α-cardiac and α-skeletal actins, we have previously shown that sarcomeric actin expression is increased in well-differentiated relative to poorly differentiated RMSs [36]. In rat experimental RMS, it has also been shown, using biochemical techniques, that the pattern of actin isoform expression reflects the differentiation state of tumor cells [4]. Thus, the availability of specific antibodies will be important for the characterization of the degree of differentiation of RMS, including during the monitoring of therapeutic strategies [8]. The constant presence of α-cardiac actin and the fact that α-smooth muscle and α-skeletal actin isoforms are expressed in only some cases of our series of tumors raise the question of the relationship between actin isoform gene expression and biological behavior of tumoral cells. It is well established that α-cardiac and α-smooth muscle actin are transiently expressed during the development of skeletal muscle [34, 41], while α-skeletal actin represents the most differentiated isoform. Hence, the actin isoform ex-

pression pattern in RMSs mimics different developmental stages of skeletal muscle: RMSs positive for only α-cardiac and α-smooth muscle actins mimic an early developmental stage, whereas the positivity for α-skeletal actin in three embryonal cases reflects a higher differentiation of these tumors. This possibility is compatible with the histological features of RMS showing that rhabdomyoblasts with cross-striations are seen in 50–60% of embryonal RMS and more rarely in other subtypes [1].

In conclusion, we have produced a specific α-cardiac actin antibody that represents a useful adjunct for the diagnosis of RMS. Moreover it appears possible to use our panel of actin isoform specific antibodies, in addition to the already known markers of this tumor (for review see [29]), in order to characterize the different subtypes on the basis of their differentiation features; this may improve the understanding of the biology and evolution of RMS.

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References

1. Agamanolis DP, Dasu S, Krill CE (1986) Tumors of skeletal muscle. Hum Pathol 17:778–795
2. Altmannsberger M, Osborn M, Treuner J, Holscher A, Weber K, Shauer A (1982) Diagnosis of human childhood rhabdomyosarcoma of antibodies to desmin, the structural protein of muscle specific intermediate filaments. Virchows Arch 39: 203–215
3. Arora PD, Narani N, McCulloch CA (1999) The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. Am J Pathol 154:871–882
4. Babai F, Skalli O, Schurch W, Seemayer TA, Gabbiani G (1988) Chemically induced rhabdomyosarcomas in rats. Ultrastructural, immunohistochemical, biochemical features and expression of alpha-actin isoforms. Virchows Arch 55:263–277
5. Bea F, Bar H, Watson L, Blessing E, Kubler W, Kreuzer J, Jahn L (2000) Cardiac alpha-actin in smooth muscle cells: detection in umbilical cord vessels and in atherosclerotic lesions. Basic Res Cardiol 95:106–113
6. Cavazzana AO, Schmidt D, Ninfo V, Harms D, Tollot M, Carli M, Treuner J, Betto R, Salviati G (1992) Spindle cell rhabdomyosarcoma. A prognostically favorable variant of rhabdomyosarcoma. Am J Surg Pathol 16:229–235
7. Cessna MH, Zhou H, Perkins SL, Tripp SR, Layfield L, Daines C, Coffin CM (2001) Are myogenin and myoD1 expression specific for rhabdomyosarcoma? A study of 150 cases, with emphasis on spindle cell mimics. Am J Surg Pathol 25:1150–1157
8. Cintorino M, Vindigni C, Del Vecchio MT, Tosi P, Frezzotti R, Hadjistilianou T, Leoncini P, Silvestri S, Skalli O, Gabbiani G (1989) Expression of actin isoforms and intermediate filament proteins in childhood orbital rhabdomyosarcomas. J Submicrosc Cytol Pathol 21:409–419
9. Clement S, Chaponnier C, Gabbiani G (1999) A subpopulation of cardiomyocytes expressing alpha-skeletal actin is identified by a specific polyclonal antibody. Circ Res 85:e51–e58
10. Clement S, Pellieux C, Chaponnier C, Pedrazzini T, Gabbiani G (2001) Angiotensin II stimulates alpha-skeletal actin expression in cadiomyocytes in vitro and in vivo in the absence of hypertension. Differentiation 69:66–74
11. de Jong A, van Kessel van Vark M, Albus Lutter C, van Raamsdonk W, Voûte P (1985) Skeletal muscle actin as tumor marker in the diagnosis of rhabdomyosarcoma in childhood. Am J Surg Pathol 9:467–474
12. Denk H, Krepler R, Artlieb U, Gabbiani G, Rungger-Brandle E, Leoncini P, Franke WW (1983) Proteins of intermediate filaments. An immunohistochemical and biochemical approach to the classification of soft tissue tumors. Am J Pathol 110:193–208
13. Dias P, Chen B, Dilday B, Palmer H, Hosoi H, Singh S, Wu C, Li X, Thompson J, Parham D, Qualman S, Houghton P (2000) Strong immunostaining for myogenin in rhabdomyosarcoma is significantly associated with tumors of the alveolar subclass. Am J Pathol 156:399–408
14. Enzinger FM, Weiss SW (2001) Rhabdomyosarcoma. In: Weiss SW, Goldblum JR (eds) Soft tissue tumors, 4th edn, Mosby, St Louis, pp 785–835
15. Eusebi V, Ceccarelli C, Gorza L, Schiaffino S, Bussolati G (1986) Immunocytochemistry of rhabdomyosarcoma. The use of four different markers. Am J Surg Pathol 10:293–299
16. Eusebi V, Damiani S, Pasquinelli G, Lorenzini P, Reuter VE, Rosai J (2000) Small cell neuroendocrine carcinoma with skeletal muscle differentiation: report of three cases. Am J Surg Pathol 24:223–230
17. Franke WW, Stehr S, Stumpp S, Kuhn C, Heid H, Rackwitz HR, Schnolzer M, Baumann R, Holzhausen HJ, Moll R (1996) Specific immunohistochemical detection of cardiac/fetal alpha-actin in human cardiomyocytes and regenerating skeletal muscle cells. Differentiation 60:245–250
18. Garner I, Sassoon D, Vandekerckhove J, Alonso S, Buckingham ME (1989) A developmental study of the abnormal expression of alpha-cardiac and alpha-skeletal actins in the striated muscle of a mutant mouse. Dev Biol 134:236–245
19. Gimona M, Vandekerckhove J, Goethals M, Herzog M, Lando Z, Small JV (1994) Beta-actin specific monoclonal antibody. Cell Motil Cytoskeleton 27:108–116
20. Hinz B, Celetta G, Tomasek JJ, Gabbiani G, Chaponnier C (2001) α-smooth muscle actin expression upregulates fibroblast contractile activity. Mol Biol Cell 12:2730–2741
21. Hinz B, Mastrangelo D, Iselin CE, Chaponnier C, Gabbiani G (2001) Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. Am J Pathol 159:1009–1020
22. Jong AS, van Vark M, Albus-Lutter CE, van Raamsdonk W, Voute PA (1984) Myosin and myoglobin as tumor markers in the diagnosis of rhabdomyosarcoma. A comparative study. Am J Surg Pathol 8:521–528
23. Khaitlina SY (2001) Functional specificity of actin isoforms. Int Rev Cytol 202:35–98
24. Kumar S, Perlman E, Harris CA, Raffeld M, Tsokos M (2000) Myogenin is a specific marker for rhabdomyosarcoma: an immunohistochemical study in paraffin-embedded tissues. Mod Pathol 13:988–993
25. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
26. Landon F, Huc C, Thome F, Oriol C, Olomucki A (1977) Human platelet actin. Evidence of beta and gamma forms and similarity of properties with sarcomeric actin. Eur J Biochem 81:571–577
27. Lennette DA (1978) An improved mounting medium for immunofluorescence microscopy (letter). Am J Clin Pathol 69: 647–648
28. Miettinen M, Lehto VP, Badley RA, Virtanen I (1982) Expression of intermediate filaments in soft-tissue sarcomas. Int J Cancer 30:541–546
29. Parham DM (2001) Pathologic classification of rhabdomyosarcomas and correlations with molecular studies. Mod Pathol 14:506–514
30. Ronnov Jessen L, Petersen OW (1996) A function for filamentous alpha-smooth muscle actin: retardation of motility in fibroblasts. J Cell Biol 134:67–80

31. Rubin BP, Hasserjian RP, Singer S, Janecka I, Fletcher JA, Fletcher CD (1998) Spindle cell rhabdomyosarcoma (so-called) in adults: report of two cases with emphasis on differential diagnosis. Am J Surg Pathol 22:459–464
32. Schurch W, Skalli O, Seemayer TA, Gabbiani G (1987) Intermediate filament proteins and actin isoforms as markers for soft tissue tumor differentiation and origin. I. Smooth muscle tumors. Am J Pathol 128:91–103
33. Schurch W, Bochaton-Piallat ML, Geinoz A, d'Amore E, Laurini RN, Cintorino M, Begin LR, Boivin Y, Gabbiani G (1994) All histological types of primary human rhabdomyosarcoma express alpha-cardiac and not alpha-skeletal actin messenger RNA. Am J Pathol 144:836–846
34. Schwartz RJ, Rothblum KN (1981) Gene switching in myogenesis: differential expression of the chicken actin multigene family. Biochemistry 20:4122–4129
35. Skalli O, Ropraz P, Trzeciak A, Benzonana G, Gillessen D, Gabbiani G (1986) A monoclonal antibody against α-smooth muscle actin: a new probe for smooth muscle differentiation. J Cell Biol 103:2787–2796
36. Skalli O, Gabbiani G, Babai F, Seemayer TA, Pizzolato G, Schurch W (1988) Intermediate filament proteins and actin isoforms as markers for soft tissue tumor differentiation and origin. II. Rhabdomyosarcomas. Am J Pathol 130:515–531
37. Suurmeijer AJH, Clément S, Francesconi A, Bocchi L, Angelini A, Van Veldhuisen DJ, Spagnoli LG, Gabbiani G, Orlandi A (2002) Alpha actin isoform distribution in normal and failing human heart: a morphological, morphometrical and biochemical study. J Pathol (in press)
38. Tonin PN, Scrable H, Shimada H, Cavenee WK (1991) Muscle-specific gene expression in rhabdomyosarcomas and stages of human fetal skeletal muscle development. Cancer Res 51: 5100–5106
39. Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354
40. Vandekerckhove J, Weber K (1979) The complete amino acid sequence of actins from bovine aorta, bovine heart, bovine fats skeletal muscle and rabbit slow skeletal muscle. Differentiation 14:123–133
41. Woodcock-Mitchell J, Mitchell JJ, Low RB, Kieny M, Sengel P, Rubbia L, Skalli O, Jackson B, Gabbiani G (1988) α-Smooth muscle actin is transiently expressed in embryonic rat cardiac and skeletal muscles. Differentiation 39:161–166