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Intermediate Filament Proteins and Actin Isoforms as Markers for Soft Tissue Tumor Differentiation and Origin

I. Smooth Muscle Tumors

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A series of 3 benign and 10 malignant smooth muscle (SM) neoplasms and of 2 malignant fibrous histiocytomas was examined by light microscopy, transmission electron microscopy, two-dimensional gel electrophoresis (2D-GE) and indirect immunofluorescence, using polyclonal monospecific or monoclonal antibodies to desmin, vimentin, cytokeratin, α-SM and α-sarcomeric (α-SR) actins. Benign neoplasms displayed typical light-microscopic features of SM, whereas leiomyosarcomas demonstrated variations in their histologic pattern. In 6 sarcomas, light microscopy suggested a SM differentiation, whereas in the other 4, a predominant nondistinctive spindle-cell pattern was observed. By transmission electron microscopy, all 13 neoplasms showed the minimal essential features of SM differentiation. Immunofluorescence disclosed heterogeneity of cytoskeletal protein expression: 5 neoplasms (3 benign and 2 malignant well-differentiated) expressed desmin, vimentin, and α-SM-actin; 2 malignant neoplasms expressed desmin and vimentin; 1 malignant neoplasm expressed desmin, vimentin and α-SR actin; 1 malignant neoplasm expressed vimentin and α-SR actin; and 4 malignant neoplasms expressed vimentin alone. By 2D-GE, 3 benign and 4 malignant SM neoplasms expressed α, β, and γ actins, and the remaining expressed only β and γ actins. The presence of α-SM actin in all benign neoplasms and in 2 well-differentiated leiomyosarcomas suggests that this actin isoform reflects a high degree of cellular differentiation. In 2 leiomyosarcomas, α-SR actin was detected by immunofluorescence, which suggested a skeletal muscle differentiation of these neoplasms. This study supports the assumption that leiomyosarcomas represent a heterogeneous group of neoplasms and furnishes new criteria for their characterization. (Am J Pathol 1987, 128:91–103)

THE DIAGNOSIS OF soft tissue tumors traditionally is based on histologic patterns. However, it is well established that histogenetically different neoplasms might manifest similar if not identical histologic patterns, and neoplasms of common histogenesis often exhibit a spectrum of various histologic features. This is particularly valid for spindle cell variants, especially smooth muscle (SM) neoplasms.[1] The introduction of cell differentiation markers, and particularly of antibodies for cytoskeletal proteins, has provided a new tool for the study of differentiation and classification of various tumors.[2–6] In general, SM cells show similar morphologic features. However, they are heterogeneous in their content of intermediate filament proteins and actin isoforms.[7–9] With respect to actin isoform expression, vascular SM cells show a predominance of the α-isoform, whereas parenchymal SM cells are characterized by a large proportion of γ-SM actin.[9–13] β- and γ-cytoplasmic isoforms are relatively increased in fetal or in pathologically dedifferentiated SM cells.[9]

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In order to determine whether the heterogeneity of intermediate filament proteins and actin isoforms of normal SM cells is also found in neoplastic cells, we examined a series of 10 leiomyosarcomas, 2 leiomyomas, and 1 intravascular leiomyomatosis in which the diagnoses were established by light and electron microscopy. We employed indirect immunofluorescence with antibodies against desmin, vimentin, cytokeratin, α-SM, and α-sarcomeric (α-SR) actins. The actin isoforms of each neoplasm were also identified biochemically by two-dimensional gel electrophoresis (2D-GE).[14] Our results reveal that SM neoplasms, like normal SM cells, are heterogeneous in their content of intermediate filament proteins and actin isoforms, and allow one to suggest more precisely than possible with morphologic techniques alone the histogenetic origin and the degree of differentiation of these different tumors.

Materials and Methods

Tissue Samples

The neoplasms that formed the basis of this study were obtained from surgical pathology material and consisted of 10 leiomyosarcomas, 2 leiomyomas, and 1 intravascular leiomyomatosis (Table 1). Two typical malignant fibrous histiocytomas served as controls. Freshly excised samples were prepared for light microscopy, indirect immunofluorescence, transmission electron microscopy, and 2D-GE.

Frozen samples from the upper third of normal human esophagus containing both smooth and striated muscle and samples from normal human bowel served as controls for antibody specificity; for internal controls, see below.

Light Microscopy

The samples were fixed in 10% neutral formalin, postfixed in Bouin's solution, and embedded in paraffin. Sections cut at 3–5 μ were stained with hematoxylin-phloxine-saffran (HPS), Alcian blue–periodic acid–Schiff (AB-PAS), Masson's trichrome, and Laidlaw's reticulin. In addition, cryostat sections from specimens prepared for immunofluorescence were also stained with HPS for correlation of histologic and immunohistochemical results.

Transmission Electron Microscopy

Tissue samples from different areas of the neoplasms were cut into 1-mm cubes, fixed for 2 hours at room temperature in 3% glutaraldehyde buffered with 0.1 M phosphate buffer at pH 7.4, and postfixed for 1 hour in 2% osmium tetroxide in the same buffer. The cubes were dehydrated in graded acetones and embedded in Epon 812. Semithin sections were stained with toluidine blue; thin sections were double-stained on copper grids with uranyl acetate and lead citrate and examined in a Philips 201 electron microscope.

Antisera and Indirect Immunofluorescence

As intermediate filament antibodies, we used affinity-purified rabbit anti-desmin,[15] two monoclonal anti-vimentin (Sanbio, Nistelrode, Holland; Behringer Mannheim Biochemica, FRG) antibodies, and two broad-specificity anti-cytokeratin antibodies: one affinity-purified polyclonal antibody raised in guinea pig[16] and one monoclonal antibody (Ortho Diagnostic System Inc., Don Mills, Ontario, Canada). As antiactin isoform antibodies, we used anti-α-SM-1, a monoclonal antibody specific for α-SM actin,[17] and anti-α-SR-1, a monoclonal antibody specific for α-SR and cardiac actins (Skalli et al., in preparation). The specificity of anti-α-SR-1 for α-striated and cardiac muscle was tested by ELISA, immunoblotting, and immunofluorescence (Skalli et al, in preparation).

Immunofluorescence staining was performed on 3–5 μ thick cryostat sections obtained from tissue samples snap-frozen in liquid isopentane. Tissue sections were fixed for 5 minutes in acetone at −20 C and air-dried at room temperature for 30–45 minutes. After rinsing in Tris-buffered saline (TBS), the sections were incubated with the first antibody for 1 hour at room temperature. Affinity-purified rabbit anti-desmin and guinea pig anti-prekeratin were used at an IgG concentration of 0.025 mg/ml and 0.2 mg/ml respectively. Monoclonal antibodies against vimentin, prekeratin, α-SM actin, and α-SR actin were used at a dilution of 1:20, 1:20, 1:10, and 1:10, respectively. After three rinsings in TBS, the sections were incubated for 1 hour at room temperature with the appropriate second antibody. As second antibody, we used FITC-conjugated goat anti-rabbit IgG (Behringwerke, Marburg, FRG) diluted 1:20, fluorescein isothiocyanate (FITC)- or tetramethyl rhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG (Meloy, Springfield, Virginia) diluted 1:20, and FITC-conjugated goat anti-guinea pig IgG (Cappel Laboratories, Cochranville, Pa) diluted 1:30. After three rinsings in TBS, the sections were mounted in buffered polyvinylalcohol.[18]

Photographs were taken with a Zeiss photomicroscope (Carl Zeiss Inc., Oberkochen, FRG) equipped with epiillumination, using a plan apochromate ×40/1.0 objective, on 3 M 640T color slide films (3M Co., Savona, Italy) or HP5 Ilford black and white film (Ilford Company, Basel, Switzerland).

2D-GE

Tissue samples were homogenized in 1% sodium dodecyl sulfate (SDS), 20 mM dithiothreitol (DTT),[19] sonicated (three times at setting 7 of a Branson sonifier, Branson Sonic Power Co, Danbury, Conn) and boiled for 3 minutes. The protein concentration of the sample was determined by the method of Bradford.[20]

Actin isoforms were separated by a first-dimension isoelectric focusing gel (3 mm × 10 cm) according to the method of O'Farrell.[14] The pH gradient was established with 2% preblended ampholytes, pH 4.0–6.5 (Pharmacia Co, Lucerne, Switzerland). According to the manufacturer's instructions, anodic and cathodic solutions were 10 mM glutamic acid and 10 mM glycine, respectively; both solutions were degassed for 30 minutes before electrophoresis. Gels were loaded with 10–20 μg proteins. Focusing was for 14 hours at 1000 volts. Protein separation according to their molecular weight was then done on 10% SDS-polyacrylamide gel electrophoresis (PAGE).[21] Gels were stained for 2 hours with 0.05% Coomassie blue in 50% methanol and 10% acetic acid and destained with 20% methanol, 10% acetic acid.

Results

Smooth and striated muscle of the upper third of human esophagus showed strong cytoplasmic fluorescence with the anti-desmin antibody. Smooth and striated muscle were discriminated with anti-α-SM-1 and anti-α-SR-1, which stained exclusively smooth and striated muscle, respectively. Vimentin antibody stained intensely the submucosal mesenchymal cells and vascular SM cells. Vascular SM cells were also positive with anti-α-SM-1 and to a lesser extent with anti-desmin. The muscularis mucosae and muscularis propria of human bowel revealed an intense cytoplasmic fluorescence with the anti-desmin antibody and a positive but weaker fluorescence with anti-α-SM-1. The epithelial lining of the esophagus and bowel was strongly stained with the anti-cytokeratin antibodies, as were the seromucous glands of the esophageal submucosae.

Small vessels of neoplasms usually displayed an intense staining of SM cells of their wall with anti-α-SM-1, but only some cells were stained with anti-desmin (Figures 1–3). All vascular SM cells, endothelium, and other stromal cells were intensely anti-vimentin reactive (Figures 1–3). The stromal vessels, therefore, served as internal controls for desmin, vimentin, and anti-α-SM-1 fluorescent reactions.

The neoplastic cells of the two malignant fibrous histiocytomas were negative after staining with anti-desmin, anti-α-SM-1, and anti-α-SR-1, but were strongly anti-vimentin reactive. Ultrastructurally, both tumors displayed fibroblastic and histiocytic differentiation.[22]

The principal histologic patterns and ultrastructural features of leiomyosarcomas are summarized in Table 1. Minimal ultrastructural features judged essential to establish the diagnosis of SM differentiation of the neoplasms included remnants of basal lamina, pinocytotic surface vesicles, plasmalemmal attachment plaques, and cytoplasmic bundles of 6-nm-thick microfilaments with associated fusiform dense bodies. Compared with leiomyomas and the intravascular leiomyomatosis, which displayed histologically typical SM differentiation, ie, arrangement of neoplastic cells in intersecting fascicles, abundant acidophilic fibrillar cytoplasm accentuated by Masson's trichrome stain, and/or epithelioid aspects (Cases 11–13 and Figure 6), leiomyosarcomas often revealed variation in pattern. In 6 leiomyosarcomas, histologic and cytologic aspects suggested a widespread SM differentiation (Cases 2, 3, 6, 8, 9, and 10 and Figures 2 and 4); in the other 4, nondistinct spindle cells patterns predominated, interrupted by focal hemangiopericytoma-like, edematous-embryonal, alveolar aspects and areas resembling malignant fibrous histiocytoma (Cases 1, 4, 5, and 7 and Figures 1 and 3). In 2 leiomyosarcomas, extensive stromal hyalinization was observed (Cases 4 and 6). At the ultrastructural level, all neoplasms exhibited variable degrees of SM differentiation, as described above (Figures 1–4 and 6). AB-PAS stain was helpful in recognizing focal myxoid changes (Case 1). PAS revealed cytoplasmic glycogen, usually more abundant in leiomyomas and well-differentiated leiomyosarcomas. Laidlaw's reticulin stain emphasized predominant histologic patterns and showed individual cells or groups of cells surrounded by fine fibrils. The ultrastructural evidence of SM differentiation represented the common denominator of the neoplasms and allowed for inclusion some atypical forms of leiomyosarcomas (Cases 1, 4, 5, and 7). Although SM features were evident in almost every cell in benign neoplasms, these were present in but a minority of cells in the malignant forms. The distinction between leiomyosarcomas and myofibroblastic sarcomas was made mainly on the basis of the nuclear shape, much more indented in the latter tumor.

The results of immunofluorescent staining of SM tumors with anti-vimentin, anti-desmin, anti-α-SM-1 and anti-α-SR-1, as well as the biochemical analysis of the actin isoform composition, are summarized in Table 2. All neoplasms were stained by antivimentin

Table 1—Smooth Muscle Neoplasms: Clinical and Morphologic Features

Case	Age and sex	Localization and maximum diameter (cm)	Histologic diagnosis and predominant patterns	Treatment and follow-up	Ultrastructural Features			
					Basal Lamina (or Remnants)	Pinocytotic vesicles	Attachment plaques	Bundles of Microfilaments with dense bodies
1	27F	Intramuscular, Tongue; 5.0	Leiomyosarcoma with edematous-embryonal and hemangiopericytomalike patterns	Local recurrence and vascular invasion. Dead 18 months after resection	+	+	+	+
2	44M	Stomach; 28.0	Leiomyosarcoma with epithelioid pattern	Local recurrence. Alive, 30 months after resection	++	+++	++	+
3	41M	Small bowel; 9.0	Leiomyosarcoma with epithelioid pattern	Local recurrence, liver metastases. Dead, 54 months after resection	+	+	+	+
4	37M	Thorax wall; 19.0	Leiomyosarcoma with alveolar pattern and hyalinization of the stroma	Retroperitoneal metastases. Dead, 12 months after resection	++	++	++	++
5	66F	Retroperitoneum; 12.0	Leiomyosarcoma with malignant fibrous histiocytomalike patterns	Alive, 14 months after resection	++	++	++	++
6	90F	Retroperitoneum; 5.0	Leiomyosarcoma with hyalinization of stroma	Liver and lung metastases. Dead 2 months after resection	++	+	+	+
7	38F	Intramuscular, thigh; 7.0	Leiomyosarcoma with hemangiopericytomalike patterns	Alive, 14 months after resection	+	+	+	+
8	90F	Intramuscular, thigh; 7.0	Leiomyosarcoma	Alive, 10 months after resection	++	++	++	++
9	26F	Intramuscular, arm; 9.0	Leiomyosarcoma	Alive. Local recurrence 2 months after resection	++	++	++	++
10	23F	Uterus; 8.0	Leiomyosarcoma	Alive, 4 months after hysterectomy	+++	+++	+++	+++
11	46F	Uterus; 8.0	Intravascular leiomyomatosis	Alive, 9 months after hysterectomy	+++	+++	+++	+++
12	64F	Uterus; 4.0	Leiomyoma	Alive, 24 months after hysterectomy	+++	+++	+++	+++
13	41F	Uterus; 3.5	Leiomyoma	Alive, 18 months after hysterectomy	+++	+++	+++	+++

+, poorly developed; ++, moderately developed; +++, well developed.

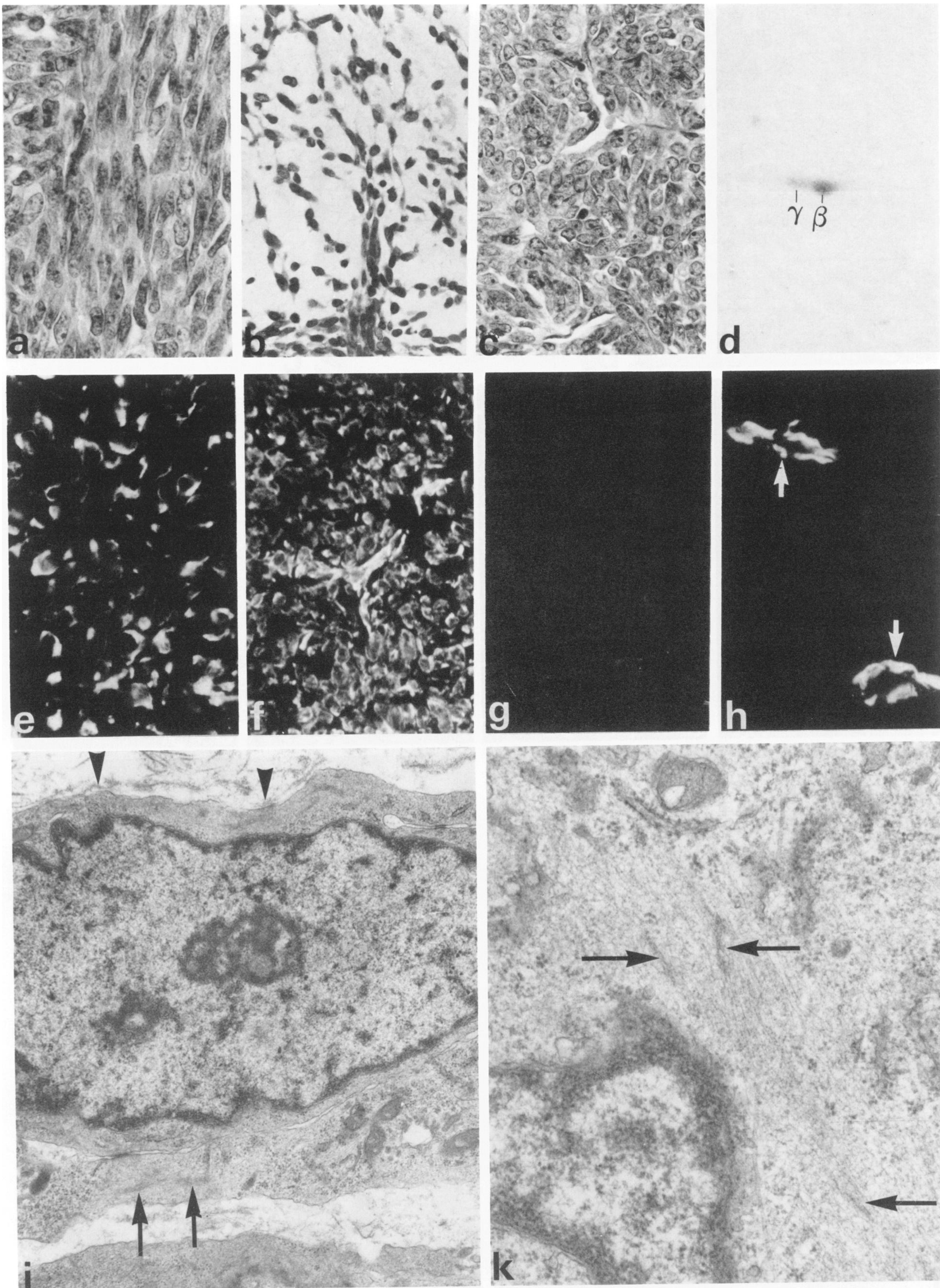

Figure 1—Leiomyosarcoma of the tongue (Case 1). **a–c**—Light-micrographics revealing intersecting fascicles of spindle cell (**a**), edematous-embryonal (**b**), and hemangiopericytomalike patterns (**c**). (HPS, **a,** ×480; **b,** ×300; **c,** ×480) **d**—2D-GE showing β and γ actin spots with a predominance of the β spot. **e–h**—Indirect immunofluorescence micrographs illustrating positive staining for desmin (**e,** ×400) and vimentin (**f,** ×300) and negative staining for α-SR actin (**g,** ×300) and α-SM actin (**f,** ×400). This latter antibody strongly stains SM cells of stromal vessels (*arrows*). **i–k**—Transmission electron micrographs demonstrating SM differentiation, ie, remnants of basal lamina (*arrowheads*) and bundles of cytoplasmic microfilaments with dense bodies (*arrows*).

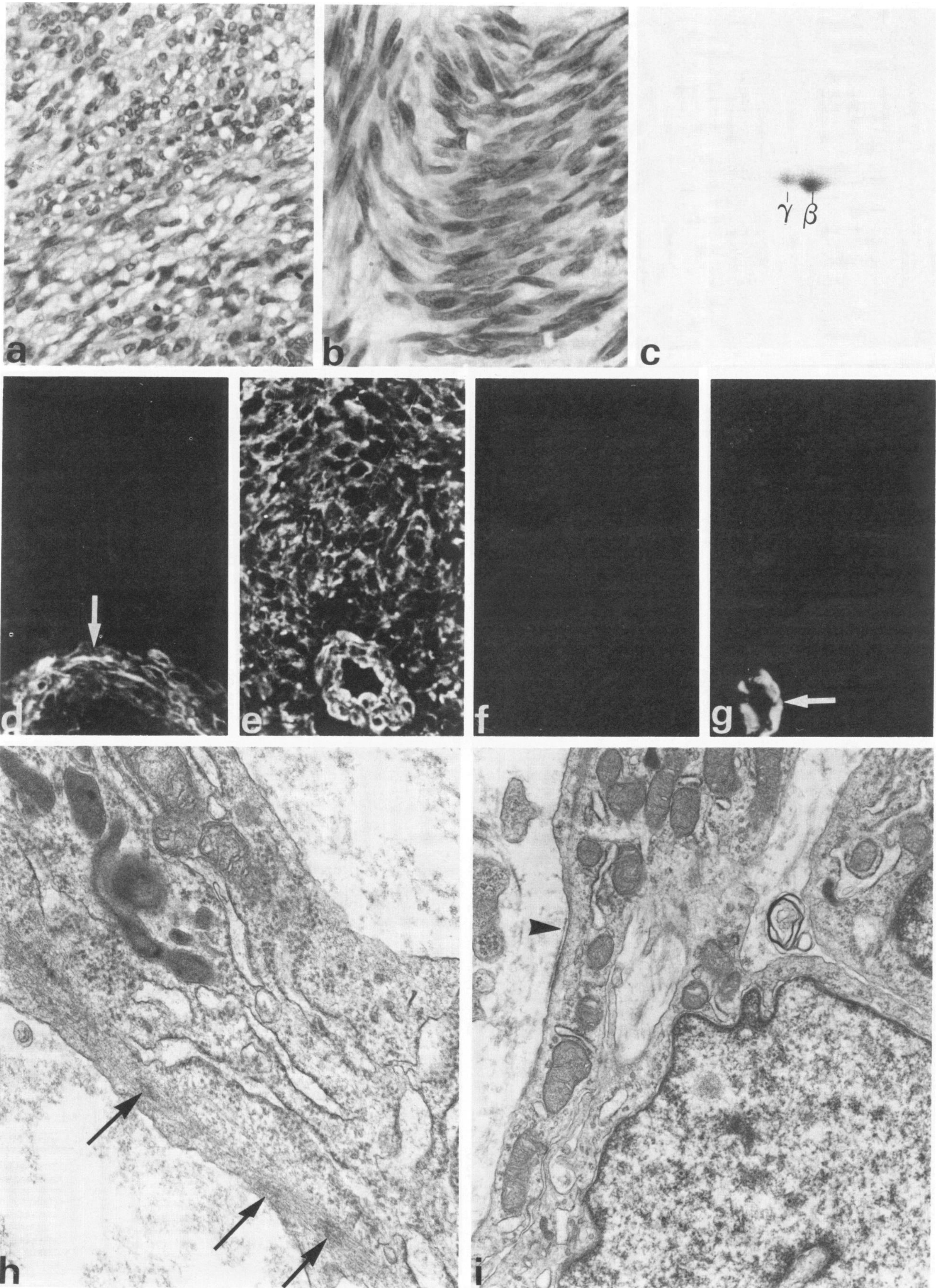

Figure 2—Leiomyosarcoma of the stomach (Case 2). **a–b**—Light micrographs revealing the epithelioid aspect (**a**) and intersecting fascicles of spindle cells (**b**). (HPS, **a**, ×300; **b**, ×480). **c**—2D-GE showing β and γ actin spots with a predominance of the β spot. **d–g**—Indirect immunofluorescence micrographs illustrating negative staining for desmin (**d**, ×300), positive staining for vimentin (**e**, ×300), negative staining for α-SR actin (**f**, ×300) and α-SM actin (**g**, ×300); SM cells of stromal vessels are stained with anti-α-SM-1 and desmin antibodies (*arrows*). **h–i**—Transmission electron micrographs demonstrating SM differentiation, ie, bundles of cytoplasmic microfilaments with dense bodies (*arrows*) and remnants of basal lamina (*arrowhead*). (**h**, ×42,000; **i**, ×17,000)

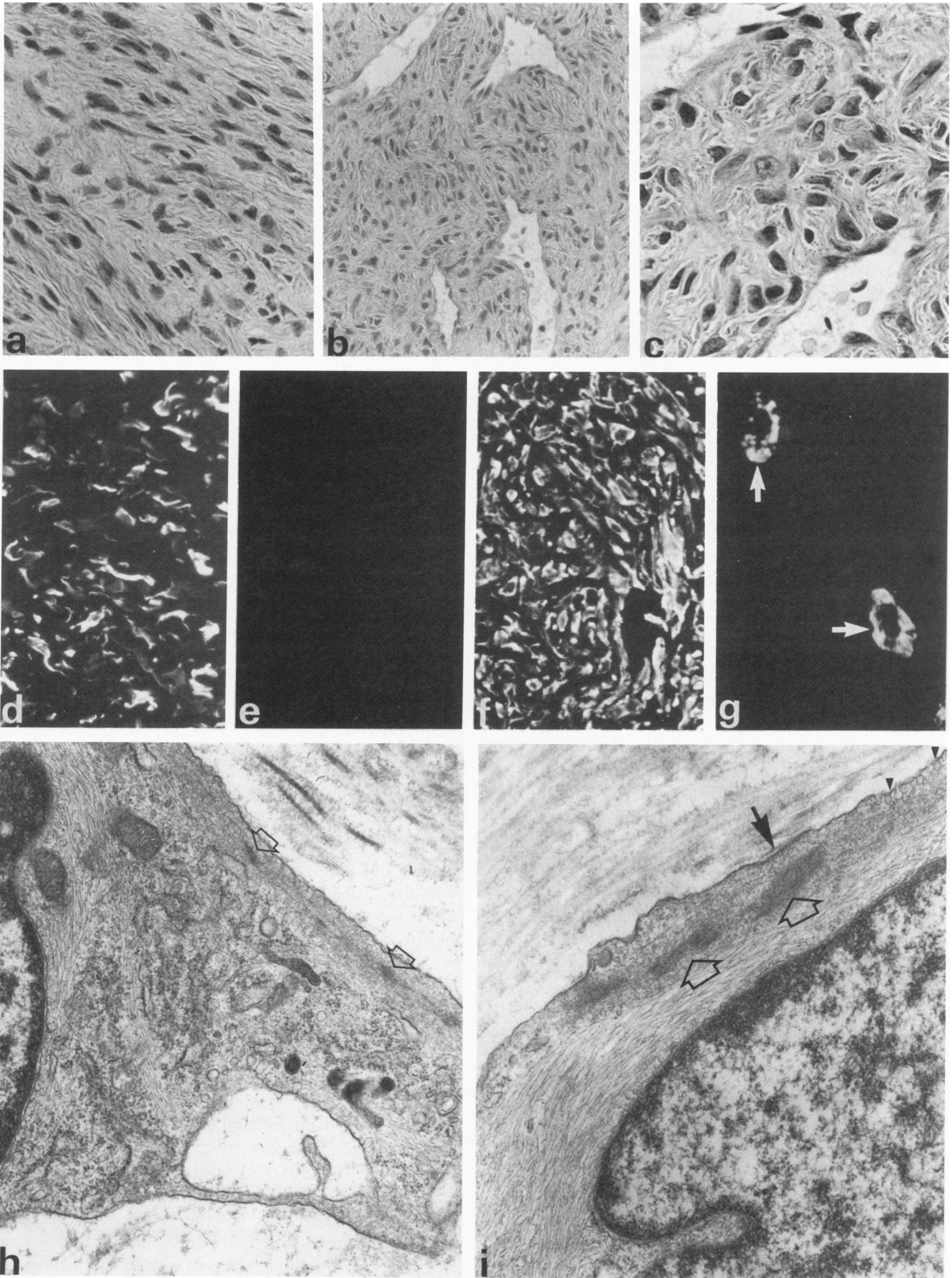

Figure 3—Leiomyosarcoma of the thigh (Case 7). **a–c**—Light micrographs revealing fascicles of spindle cells (**a**) and a hemangiopericytomalike pattern (**b** and **c**). (HPS, **a**, ×300; **b**, ×190; **c**, ×480). **d–g**—Double indirect immunofluorescence micrograph illustrating positive staining for α-SR actin and negative staining for desmin (**d** and **e**, ×300). **f** and **g**—Indirect immunofluorescence micrographs illustrating positive staining for vimentin (**f**, ×300) and negative staining for α-SM actin (**g**, ×300). SM cells of stromal vessels are stained with anti-α-SM-1 (*arrows*). **h–i**—Transmission electron micrographs demonstrating SM differentiation, ie, cytoplasmic bundles of microfilaments with dense bodies (*open arrows*), plasmalemmal attachment plaques, remnants of basal lamina (*arrow*), and pinocytotic vesicles (*arrowheads*). Note the perinuclear bundle of intermediate filaments. (**h** and **i**, ×25,500)

Table 2—Smooth Muscle Neoplasms: Cytoskeletal Features

Case	Indirect immunofluorescence*				Actin isoforms (2D-GE)		
	Vimentin	Desmin	α-SM actin	α-SR actin	α	β	γ
1	++	++	−	−	−	+	+
2	++	−	−	−	−	+	+
3	++	−	−	−	−	+	+
4	+++	+	−	+	±	+	+
5	++	−	−	−	−	+	+
6	+++	−	−	−	−	+	+
7	++	−	−	++	+	+	+
8	++	+	+	−	±	+	+
9	++	++	−	−	−	+	+
10	++	++	++	−	+	+	+
11	++	+++	+++	−	+	+	+
12	+++	+++	+++	−	+	+	+
13	+++	+++	+++	−	+	+	+

*Intensity of fluorescence reaction: −, negative; +, weak; ++, moderate; +++, strong. For Coomassie blue staining: +, present; −, absent. For "±" see Results.

and were negative with anticytokeratin (Figures 1–4 and 6). The 2 uterine leiomyomas and the intravascular leiomyomatosis were positive for anti-desmin and anti-α-SM-1 and negative for anti-α-SR-1 (Figure 6). Five leiomyosarcomas were positive for anti-desmin (Figures 1, 4, and 5), and only 2 were positive for anti-α-SM-1 (Figures 4 and 5); theses 2 cases were also positive for anti-desmin (Figures 4 and 5). Surprisingly, 2 tumors were positive for anti-α-SR-1 (Cases 4 and 7). In particular, the leiomyosarcoma with a predominant hemangiopericytoma-like pattern showed positive staining for anti-α-SR-1 (Case 7, Figure 3). This tumor was negative with both anti-desmin and anti-α-SM-1. The other leiomyosarcoma revealing positive staining for anti-α-SR-1 was anti-desmin reactive, but negative for anti-α-SM-1 (Case 4). In 4 leiomyosarcomas, neoplastic cells were negative for anti-desmin, anti-α-SM-1, and anti-α-SR-1 (Cases 2, 3, 5, and 6 and Figure 2). In no case was positive staining for both anti-α-SM-1 and anti-α-SR-1 observed.

In tumors positive for anti-desmin and anti-α-SM-1, most cells were stained with both antibodies. The same result was observed in the tumor positive for anti-desmin and anti-α-SR-1. However, in the case of intravascular leiomyomatosis, approximately 40% of the cells were positive for anti-desmin and negative for anti-α-SM-1, the remaining 60% of the cells being positive for both antibodies.

The pattern of actin isoforms was also examined by means of 2D-GE. Only two spots were observed in tumors negative for anti-α-SM-1 and anti-α-SR-1 actin. These spots were identified to be β- and γ-actin by co-migration of α-actin with the sample proteins (Figures 1, 2, 4, 5, and 6). In these cases, the ratio of β : γ was approximately 3 : 1. α-Actin was the predominant spot in the two cases of leiomyomas and the case of intravascular leiomatosis (Figure 6). 2D-GE analysis of two leiomyosarcomas positive for anti-α-SM-1 or anti-α-SR-1 (Cases 7 and 10) revealed the clear presence of an α-actin spot; however, in these cases, β-actin was the predominant actin isoform. In 2 cases of leiomyosarcoma positive either for anti-α-SR-1 (Case 4) or anti-α-SM-1 (Case 8), only the β and γ spots of actin could be detected when gels were loaded with 20 μg of protein. However, loading greater amounts (50 μg) of protein on the gels resulted in the appearance of a Coomassie blue-stained α spot. These cases have been graded in Table 2 as ± 2D-GE analysis of the two malignant fibrous histiocytomas revealed the presence of only two actin spots corresponding to β and γ actins. The ratio of β to γ was approximately 2.5 : 1, as previously reported for fibroblastic tissues.[9]

Discussion

Leiomyosarcomas may display a large spectrum of histologic patterns which often coexist within the same neoplasm, yet share common ultrastructural features of SM differentiation.[1] Histologic patterns, however, do not necessarily represent valuable indicators of cellular differentiation. Many examples exist in which epithelial cells are spindle shaped and even arranged in fascicles.[23–25] Alternatively, mesenchymal neoplasms may display epithelioid features.[1,5,26] The purpose of our work was to complement classical morphologic techniques with recently developed cytoskeletal differentiation markers and, if possible, to

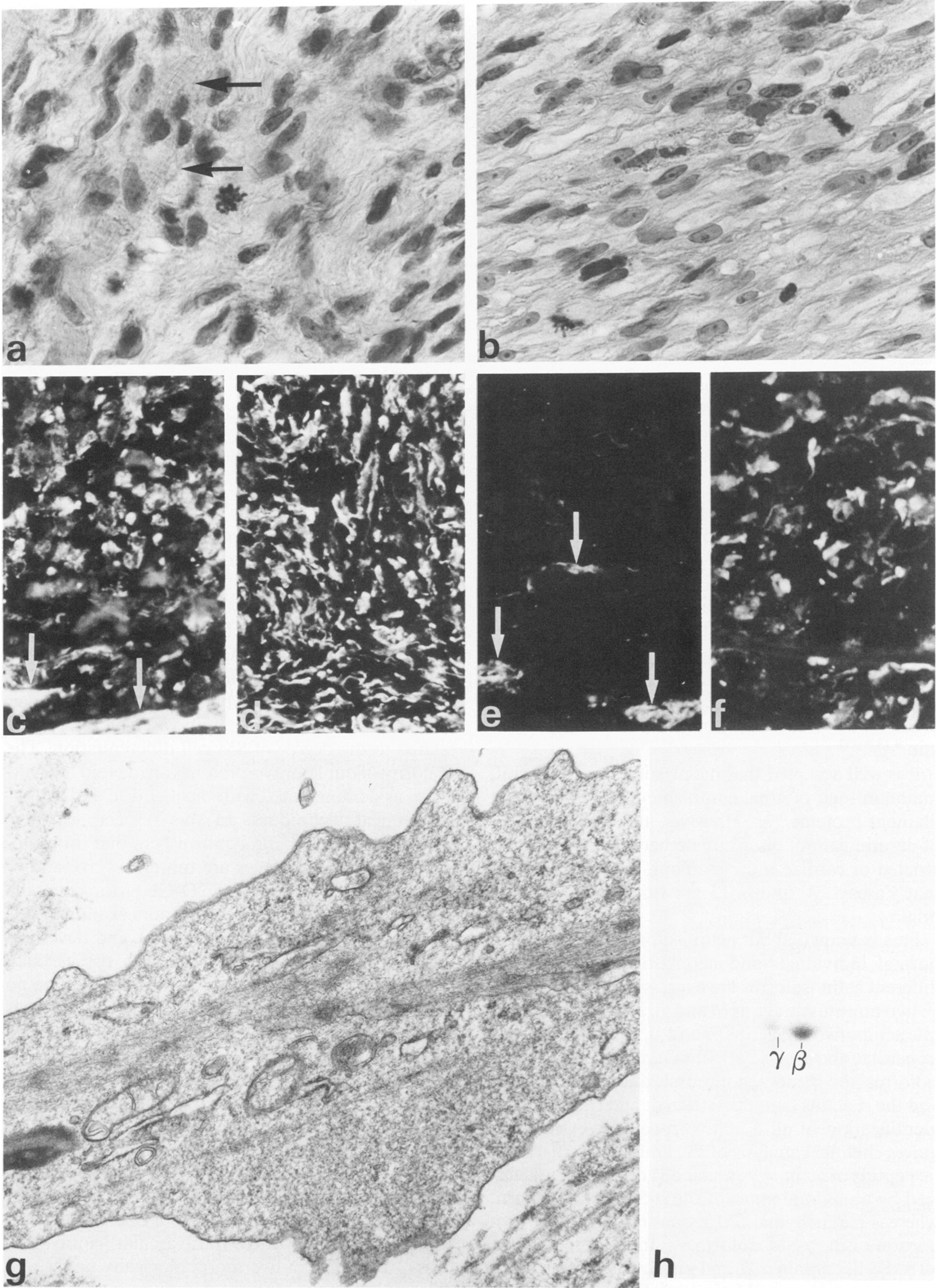

Figure 4—Intramuscular leiomyosarcoma of the thigh (Case 8). **a–b**—Light micrographs revealing a spindle cell sarcoma with a high mitotic index invading skeletal muscle (**a,** *arrows*) and distinct fibrillar cytoplasm (**b**) (HPS, **a,** ×300; **b,** ×480). **c–f**—Indirect immunofluorescence micrographs illustrating positive staining for desmin (**c,** ×300), vimentin (**d,** ×300) and α-SM actin (**f,** ×300) and negative staining for α-SR actin (**e,** ×300). Preexistent skeletal muscle fibers are stained for desmin and α-SR actin (*arrows*). **g**—Transmission electron micrograph demonstrating SM differentiation, ie, cytoplasmic bundles of cytoplasmic microfilaments with dense bodies (×24,750). **h**—2D-GE showing β and γ spots with predominance of the β spot.

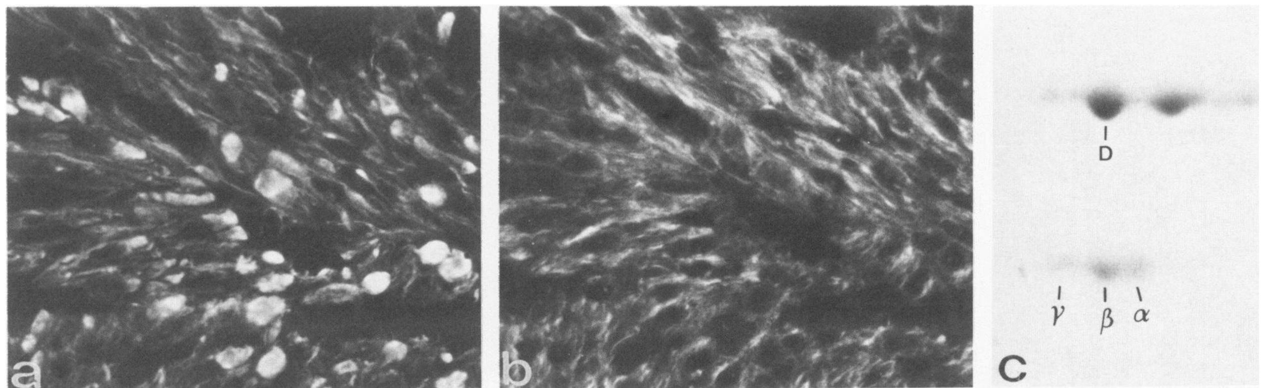

Figure 5—Leiomyosarcoma of the uterus (Case 10). **a–b**—Double indirect immunofluorescence micrographs showing positive staining for both desmin (**a**) and α-SM actin (**b**). Note that desmin and α-SM actin are not necessarily codistributed within tumor cells and that desmin is present in the form of globular accumulation corresponding to whorls of 10-nm filaments at the electron-microscopic level (data not shown). (×400) **c**—2D-GE showing α, β, and γ actins with a predominance of the β spot. Note also the presence of important amounts of desmin (**D**).

correlate the distribution of these markers with the clinical behavior of the tumors.

Parenchymal SM cells of the respiratory, gastrointestinal, and urogenital tracts represent a homogeneous cell population in which desmin is practically the exclusive intermediate filament.[27–29] In contrast, vascular SM cells are heterogeneous with respect to their content of intermediate filaments: most contain vimentin as their sole detectable intermediate filament, and a smaller proportion also expresses desmin.[8,13,30–32]

It is well accepted that neoplastic cells, in general, maintain their original complement of intermediate filament proteins.[2–5,33] However, the demonstration of desmin cannot discriminate between smooth and striated or cardiac muscles. Thus, we feel confident that Cases 1, 4, and 8–13 are tumors of muscular origin.

Less is known about actin isoform distribution in normal individuals and neoplastic situations.[9] Six different actin isoforms are expressed in mammals[10–12]: two nonmuscle actins (β and γ), so-called cytoplasmic actins, two SM actins (α and γ), and two SR actins (α-cardiac and α-skeletal). 2D-GE resolves only three isoforms: the β and γ (nonmuscle and muscle) actins and the α actins (smooth, striated, and cardiac). The identification of all 6 actins in cellular extracts requires chemical analysis of the amino-terminal tryptic peptide of actin.[12] Vascular SM cells are characterized by a predominance of the α-SM actin isoform, whereas parenchymal SM tissues reveal a large proportion of the γ-SM isoform.[9–13] In adult mammals, all SM cells contain α, β, and γ isoforms with a pattern varying in different SM tissues.[9] This pattern varies also during nonneoplastic pathologic conditions such as atheromatosis,[15,34] but changes only slightly in uterine leiomyomas, compared with normal myometrium.[9] It is noteworthy that during the first months of life, the aortic media contains 50% of cells without α-SM actin, whereas α-SM actin negative cells in the adult constitute less than 1%, demonstrating that, at least in vessels, differentiation of SM cells takes place after birth.[17] All these observations suggest that the pattern of actin isoform expression and, in particular, the expression of α-SM actin in vascular SM cells, is related to the degree of SM cell differentiation. The majority of our leiomyosarcomas are devoid of α-SM actin, as demonstrated with biochemical and immunochemical techniques. In this respect, we have shown previously[17] and confirm here that immunohistochemical techniques are more sensitive and of course more selective than 2D-GE. Moreover, the ratio of β to γ actin in all SM tumors examined was approximately 3 : 1 (data not shown), and never less than 2.6, which is typical of fibroblastic tissues,[9] suggesting that the tumoral tissues examined contain no or negligible amounts of γ-SM actin. These findings suggest collectively that either leiomyosarcomas are not of SM origin, or that they lose, to a large extent, this differentiation marker. Thus, the presence of α-SM actin in a leiomyosarcoma could reflect the degree of differentiation and would be easier to detect and evaluate by means of immunochemical techniques than the classic electron-microscopic features, which require extensive study in order to be detected in a minority of cells. The presence of α-SM actin in benign tumors and in the intravascular leiomyomatosis,[35] as well as in two morphologically rather well differentiated leiomyosarcomas, supports this possibility. 2D-GE revealed a predominance of α-actin

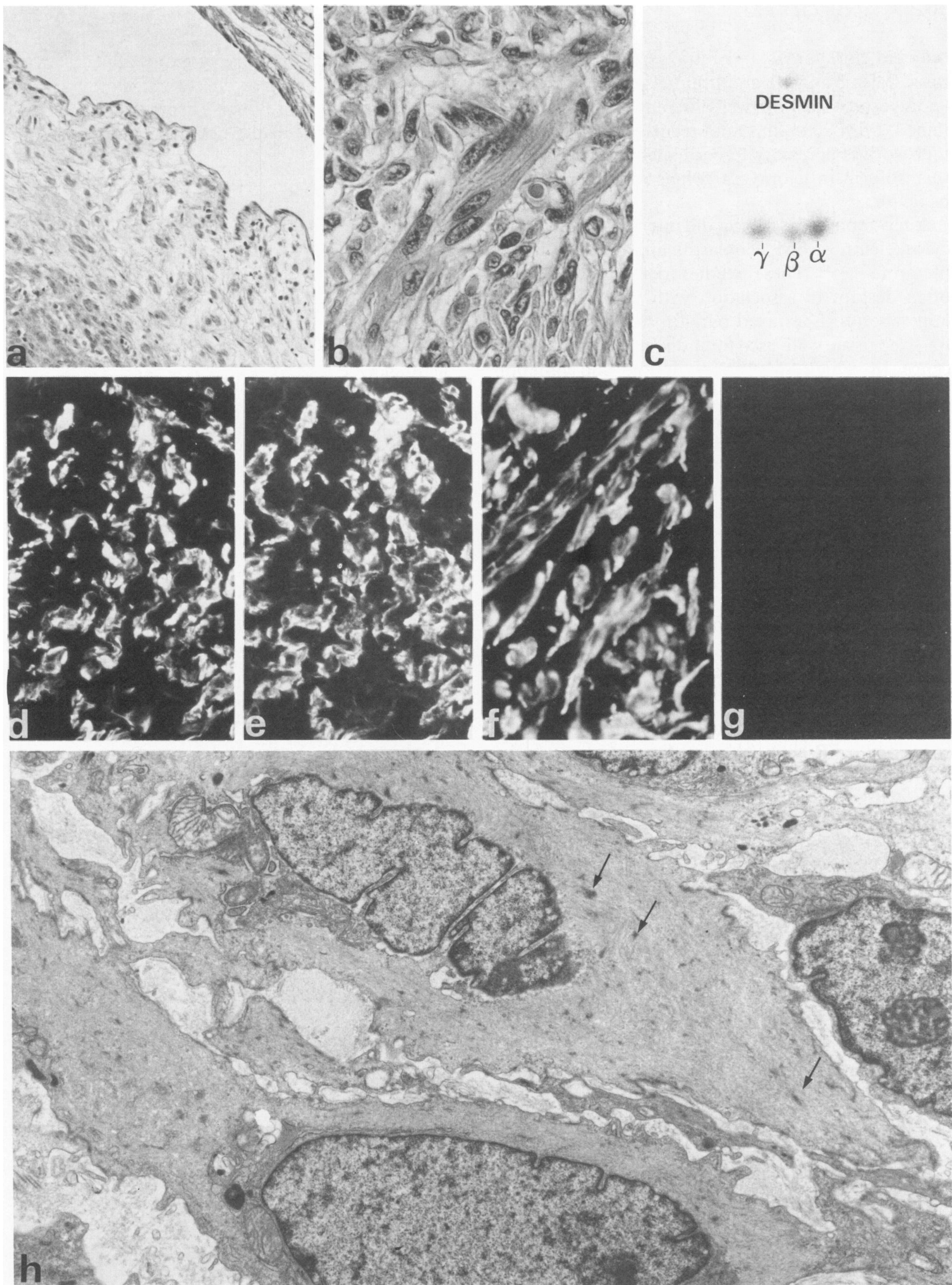

Figure 6—Intravascular leiomyomatosis (Case 11). **a–b**—Light micrographs revealing intravascular growth of the neoplasm (**a**) and SM differentiation with fibrillar cytoplasm (*arrows*). (HPS, **a**, ×120; **b**, ×480) **c**—2D-GE showing α, β, and γ actin spots with a predominance of the α spot. **d–e**—Double indirect immunofluorescence illustrating positive staining for desmin (**d**, ×300) and α-SM actin (**e**, ×300). **f–g**—Indirect immunofluorescence micrographs demonstrating positive staining for vimentin (**f**, ×480) and negative staining for α-SR actin (**g**, ×480). **h**—Transmission electron micrograph showing a high degree of SM differentiation. Neoplastic cells are laden with cytoplasmic microfilaments forming numerous dense bodies (*arrows*). (×6600)

only in leiomyomas and intravascular leiomyomatosis. Cases 8 and 10, an intramuscular and a uterine leiomyosarcoma, both well differentiated, which contained α-SM actin, have not recurred as yet, but further work is necessary to correlate the expression of this isoform in leiomyosarcomas with their clinical behavior.

A few reports describing the intermediate filament cytoskeleton of SM neoplasms are available in the literature.[2,3,5,36] These studies demonstrated most often desmin in association with vimentin. In the largest series,[37] dewaxed paraffin sections of formalin-fixed tissue were used for indirect immunofluorescence. In benign SM tumors, desmin and vimentin were found, but in most malignant SM neoplasms, desmin was present only occasionally and was associated with vimentin. The authors concluded that the presence of desmin might represent an index of differentiation. These results have to be interpreted with some caution because it was shown that for immunohistochemical demonstration of desmin, alcohol-fixed paraffin sections or frozen sections are more suitable than formalin-fixed tissues.[38] In the present study, desmin was present in significant amounts in some aggressive leiomyosarcomas (Cases 1, 4, and 9). In addition, desmin does not allow one to discriminate between leiomyosarcomas and rhabdomyosarcomas.

The observation that 2 cases of leiomyosarcomas contained α-SR actin (or α-cardiac actin) is surprising. Indeed, we first used anti-α-SR-1 as a negative control for anti-α-SM-1. It has been reported that during the development of normal striated muscle, α-cardiac and α-SM actins appear prior to the expression of α-SR skeletal muscle actin,[39] but nothing is presently known about actin isoform expression in the early development of SM of mammals. An alternate explanation could be that these two leiomyosarcomas represent atypical rhabdomyosarcomas lacking ultrastructural evidence of rhabdomyoblastic differentiation, ie, Z-bands or Z-band material. The presence of α-SR actin in tumors with a hemangiopericytoma-like pattern could be further clarified by the study of typical hemangiopericytomas, a work presently in progress. The follow-up of the cases showing expression of α-SR actin suggests that these tumors have a relatively aggressive behavior, compared with those containing α-SM actin; again, further work is needed to confirm this possibility.

The use of cytoskeletal markers has been shown to be useful in the characterization of neoplastic proliferations and in the establishment of their histogenetic origin. Our present study using desmin, α-SM actin, and α-SR actin supports the hypothesis that leiomyosarcomas are a heterogeneous group of neoplasms. It furnishes new crieria for their classification and new information on their biologic characteristics. Finally, it may be helpful in the definition of their clinical behavior.

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