α -Inhibin Immunoreactivity in Soft-Tissue Neoplasia

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Antibodies directed against α -inhibin have been previously reported as staining both sex cord-stromal neoplasms as well as adrenal cortical tumors. This relatively restricted immunoreactivity pattern is useful in the assessment of retroperitoneal masses, especially in a setting of limited tissue (*e.g.*, needle biopsy). However, no study to date has evaluated α -inhibin immunoreactivity in soft-tissue neoplasms, which frequently enter the differential diagnosis of retroperitoneal masses. We investigate the incidence of α -inhibin staining in a variety of soft-tissue neoplasms by using formalin-fixed, paraffin-embedded tissue sections from 282 previously classified soft-tissue neoplasms with anti- α -inhibin (Serotec, 1:75). A modified avidin-biotin complex method was used after heat-induced epitope retrieval. Cytoplasmic granular staining was considered positive. Of the 282 tumors studied, a total of 8 (2.8%) demonstrated positive staining with anti- α -inhibin antibody. These included 4 of 25 liposarcomas (16%), 2 of 18 angiosarcomas (11%), 1 of 48 lipomas (2.1%), and 1 of 1 rhabdomyoma (100%). Negative staining was noted among hemangiomas (0/28), schwannomas (0/32), leiomyomas (0/16), fibrosarcomas (0/2), fibromas (0/11), dermatofibromas (0/9), neurofibromas (0/6), synovial sarcomas (0/15), rhabdomyosarcomas (0/10), Triton tumors (0/2), and malignant fibrous histiocytomas (0/59). We conclude that rare soft-tissue tumors, especially those exhibiting either lipomatous or vascular differentiation, demonstrate α -inhibin immunoreactivity. These findings re-emphasize the need for a well-construed antibody panel when immunohistochemical methods are employed in the evaluation of retroperitoneal neo-

plasms. However, the rarity of α -inhibin expression by soft-tissue neoplasms provides further support for its overall specificity as a marker of adrenal cortical differentiation in the biopsy evaluation of a retroperitoneal mass.

KEY WORDS: Immunohistochemistry, Inhibin, Soft tissue neoplasms.

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Inhibins are heterodimeric hormones within the transforming growth factor- β superfamily of primarily gonadal origin that regulate pituitary follicle-stimulating hormone secretion by feedback inhibition (1-3). The distribution of these hormones, particularly the α subunit (α -inhibin), has been shown to include gonadal tissue (4, 5), adrenal cortex (6, 7), and pituitary (8). Neoplasms shown to express α -inhibin include gonadal neoplasms (predominantly sex cord-stromal tumors, Refs. 9-14; neoplasms of the adrenal cortex, Refs. 15-17; and pituitary adenomas, Ref. 8). The diagnostic role of anti- α -inhibin antibodies in evaluation of tumors of the female genital tract has been extensively studied (14, 18-22). More recently, immunohistochemical detection of α -inhibin in adrenal cortical neoplasms has been shown to assist in their distinction from other tumors, particularly renal carcinoma (15, 16).

In the biopsy evaluation of retroperitoneal masses, soft-tissue neoplasms frequently enter the differential diagnosis. Distinguishing between a soft-tissue neoplasm, especially one exhibiting epithelioid differentiation, and a poorly differentiated adrenal cortical neoplasm may pose a diagnostic dilemma, particularly with a limited specimen (*e.g.*, needle biopsy). Although the use of anti- α -inhibin antibodies has been studied and subsequently has become accepted practice when evaluating lesions in which adrenal cortical tumors are a component of the differential diagnosis, no studies to date have evaluated α -inhibin expression in soft-tissue neoplasms. In this study, we assess the incidence of α -inhibin expression in a large cohort of previously classified benign and malignant soft-tissue neoplasms.

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METHODS

Two hundred eighty-two neoplasms were retrieved from the archival files of the Ohio State University Medical Center. Each case was reviewed for diagnostic accuracy. A representative block of formalin-fixed, paraffin-embedded tissue from each case was selected for immunohistochemical study. Five-micrometer sections were cut, heated to 55° C for 1 hour, deparaffinized in Americlear (American Scientific, McGaw Park, IL), and dehydrated in graded ethanols. Endogenous peroxidase activity was quenched by immersion in absolute methanol containing 0.6% (v/v) hydrogen peroxide for 30 minutes. Slides were rehydrated in graded ethanols and distilled water. Antigen retrieval was performed by steamer method (30 minutes), with target retrieval solution (DAKO). Slides were then immunostained on a DAKO autostainer with anti- α -inhibin (Clone R1, Seratec; 1:75) by using a modified streptavidin-binding technique (LSAB+, DAKO). A chromogen precipitin was added using the 3,5' diaminobenzidine kit (hybrid 3,3'-diaminobenzidine, K3466; DAKO). After a brief rinse in tap water, sections were counterstained with Harris' hematoxylin and coverslipped using a synthetic mounting medium. Granular cytoplasmic staining was considered positive. A formalin-fixed section of one adrenal cortex served as a positive control. Negative controls were prepared by substituting nonimmune mouse ascites fluid for the primary antibody. All positive and negative controls stained appropriately. Positive results were confirmed by using the above-stated protocol with the addition of a biotin-blocking agent (DAKO) after incubation with the primary antibody to eliminate any false-positive results that were secondary to endogenous biotin activity.

RESULTS

Of the 282 tumors included in this study, only 8 (2.8%) demonstrated positive staining with anti- α -inhibin antibody (Table 1). These included 4 of 25 liposarcomas (16%; Fig. 1A–B), 2 of 18 angiosarcomas (11%; Fig. 2A–B), 1 of 48 lipomas (2.1%), 1 of 1 rhabdomyoma (100%), and 2 of 18 angiosarcomas (11%). In the positive cases, staining was granular and cytoplasmic and was either diffuse or zonal in distribution. Negative staining was noted in hemangiomas (0/28), schwannomas (0/32), leiomyomas (0/16), fibrosarcomas (0/2), fibromas (0/11), dermatofibromas (0/9), neurofibromas (0/6), synovial sarcomas (0/15), rhabdomyosarcomas (0/10), Triton tumors (0/2), and malignant fibrous histiocytomas (0/59).

TABLE 1. Anti-Inhibin A Immunoreactivity

Tumor	Total Number	Number Positive
Liposarcoma	25	4
Lipoma	48	1
Rhabdomyoma	1	1
Angiosarcoma	18	2
Hemangioma	28	0
Schwannoma	32	0
Leiomyoma	16	0
Fibrosarcoma	2	0
Fibroma	11	0
Dermatofibroma	9	0
Neurofibroma	6	0
Synovial sarcoma	15	0
Rhabdomyosarcoma	10	0
Triton tumor	2	0
Malignant fibrous histiocytoma	59	0

DISCUSSION

Using routine immunohistochemical methods, α -inhibin expression is limited to a relatively narrow spectrum of human tissues and neoplasms. This specificity has been particularly useful in identifying adrenal cortical differentiation in tumors (15). In biopsy evaluation of a retroperitoneal mass, the differential diagnosis may include both adrenal cortical neoplasms and soft-tissue neoplasms, particularly those tumors exhibiting clear cell morphology. Clinical and radiographic findings may prove useful, but this is not universally true. In addition, such information may not be available to the surgical pathologist at the time of evaluation. Immunohistochemical studies, including evaluation for α -inhibin expression, can augment routine histological evaluation, particularly in settings of limited material (e.g., core needle biopsy), of relatively paucicellular lesions, or of obscured architectural features.

This study demonstrates that a small percentage of benign and malignant soft-tissue neoplasms do express α -inhibin. The tumors with the greatest proclivity to express this marker were liposarcomas (16%) and angiosarcomas (11%). No α -inhibin staining was observed among tumors of smooth muscle, fibrous, or nerve sheath–neural differentiation. The absence of staining among smooth muscle neoplasms (leiomyomas) in our study parallels the findings of a recent study evaluating the immunophenotype of a variety of uterine smooth muscle and endometrial stromal neoplasms (23). That study identified no α -inhibin immunoreactivity among 10 leiomyomas, 9 highly cellular leiomyomas, 9 epithelioid smooth muscle tumors, or 9 leiomyosarcomas.

The etiology of α -inhibin positivity among soft-tissue neoplasms cannot be conclusively identified. Possibilities include true but aberrant α -inhibin expression due to divergent differentiation in a particular neoplastic lineage, cross-reactivity of the an-

FIGURE 1. **A**, liposarcoma with round cell features (hematoxylin and eosin, original magnification 200 $\times$). **B**, same neoplasm depicted in Figure 1A, immunostained with anti- α -inhibin showing characteristic granular cytoplasmic staining. (Anti- α -inhibin with hematoxylin counterstain, original magnification 400 $\times$.)

tibody with a similar but undescribed epitope, or false positivity due to endogenous biotin activity or other assay-related technical problem (24). The latter is not likely because endogenous biotin was quenched in all cases before antibody application and because all positive and negative controls stained appropriately.

When immunohistochemical methods are necessary for the evaluation of a retroperitoneal mass, we strongly recommend use of a limited and directed panel of antibodies based on histomorphologic features. Similar to the case of α -inhibin, melan A (A103) expression is extremely sensitive and specific for adrenal cortical or other steroid cell tumors (25). Vimentin coexpression is also expected in neoplasms of adrenal cortical differentiation (26). Cy-

tokertatin immunoreactivity would suggest either renal carcinoma or metastatic carcinoma from a distant site. However, $\leq 10\%$ of adrenal cortical carcinomas have detectable cytokeratin expression (27), with evidence suggesting variability of detection based on epitope retrieval technique (28). Further support for renal carcinoma may be provided by expression of CD10, which is positive in the majority of clear cell and papillary subtypes (27). Exclusion of vascular neoplasms would be supported by absence of expression of CD31, a sensitive and specific marker of vascular endothelial differentiation (29).

This study shows that rare soft-tissue neoplasms demonstrate α -inhibin immunoreactivity. However, the low incidence of positivity in soft-tissue neo-

FIGURE 2. **A,** Photomicrograph depicting an angiosarcoma exhibiting areas of epithelioid differentiation (hematoxylin and eosin stain, original magnification 200 $\times$). **B,** Positive immunostaining for α -inhibin in the neoplasm shown in Figure 2A (anti- α -inhibin with hematoxylin counterstain, original magnification 200 $\times$).

plasms supports the overall specificity of α -inhibin for adrenal cortical differentiation in the evaluation of retroperitoneal neoplasms. The inclusion of α -inhibin in a well-constructed panel of antibodies can further assist in the correct identification of such neoplasms and avoid misclassification.

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