

Estrogen receptor β expression in vascular neoplasia: an analysis of 53 benign and malignant cases

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The importance of estrogen in vascular neoplasia is suggested by a predilection for women and a tendency for rapid growth during pregnancy. Although early experiments using radioligand assays demonstrated estrogen receptor (ER) expression, these findings were not confirmed by subsequent immunohistochemical studies which were performed with antibodies raised against ER α . A newly discovered estrogen receptor subtype, ER β , has not been previously characterized in vascular lesions. In order to verify the expression of estrogen receptors in vascular neoplasms as well as to clarify the inconsistency between radioligand and early immunohistochemical studies, we examined a series of 53 benign and malignant vascular neoplasms for ER β expression. All of the subtypes of vascular neoplasia examined had nuclear expression of ER β . The majority of cases (94%) displayed 2+ to 3+ staining. The discrepancy between radioligand studies and previous immunohistochemical studies is attributable to the use of antibodies raised against ER α , which is not expressed in vascular lesions, and not ER β , which is broadly expressed in both benign and malignant vascular neoplasms. Although ER β may be of limited diagnostic use in vascular neoplasia due to its broad expression, the potential exists for a therapeutic approach using ER agonists.

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The role of estrogen in vascular neoplasia has long been suspected due to the observation that vascular neoplasms tend to grow rapidly during pregnancy and in adolescence.¹ These clinical observations were initially substantiated by early experiments in the 1980s using radioligand assays, which confirmed the presence of the estrogen receptor (ER) in vascular tumors;^{2,3} however, with the advent of immunohistochemical techniques, subsequent studies performed in the 1990s with antibodies raised against the ER refuted these earlier data and it appeared that the newer technology had exposed the limitations of the older biochemical assays.^{4–7} Recently, an additional ER subtype, estrogen receptor beta (ER β),⁸ was identified and the previous ER against which the original antibodies were raised was renamed estrogen receptor alpha (ER α). Although ER α has been assessed immunohisto-

chemically in vascular neoplasms and found to be absent, ER β has not been previously characterized in these entities. In order to verify the expression of ER in vascular tumors as well as to clarify the inconsistency between radioligand and early immunohistochemical studies, we examined a series of 53 benign and malignant vascular neoplasms for ER β expression.

Materials and methods

In all, 22 cases of angiosarcoma, three cases of Kaposi sarcoma, one case of epithelioid hemangioendothelioma, two cases of spindle-cell hemangioma, four cases of infantile hemangioendothelioma, 13 cases of hemangioma, seven cases of lymphangioma and one case of papillary endothelial hyperplasia were identified and retrieved from the paraffin archives of the University of Chicago. Formalin-fixed paraffin-embedded specimens were cut into 4- μ m sections and mounted on positively charged slides. Sections were deparaffinized, rehydrated, then washed in Tris-buffered saline (TBS) and subjected to heat epitope retrieval

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in a microwave. Slides were then incubated in 1% hydrogen peroxide in methanol for 5 min to block endogenous peroxidase activity, followed by incubation for 20 min in a protein-blocking solution to reduce nonspecific antibody binding. The primary anti-human polyclonal antibody (Biogenex, San Ramon, CA, USA, 1:50) was applied for 1 h at room temperature. Slides were then incubated for 30 min at room temperature with anti-mouse or anti-rabbit IgG conjugated to a horseradish peroxidase (HRP)-labeled polymer (DAKO Envision™ + System, DAKO Corp., Carpinteria, CA, USA), treated for 5 minutes with 3-3'-diaminobenzidine (DAB) chromogen, counterstained with hematoxylin, and coverslipped. Negative controls received a nonimmune polyclonal rabbit antiserum or monoclonal mouse antibody as appropriate.

Slides were evaluated for strong nuclear staining and scored as 0, 1+ (<25%), 2+ (25–50%), or 3+ (>50%) by two pathologists (ATD and AGM). Ovarian follicles or granulosa cell tumors served as positive controls for ER β .

Results

All of the subtypes of vascular neoplasia examined displayed nuclear expression of ER β (Table 1, Figure 1). The majority of cases (93%) displayed 2+ to 3+ nuclear staining intensity. There was no significant difference between benign and malignant lesions. Specifically, staining intensity, percentage of tumor stained and pattern of expression were similar in both sets of tumors. Only one case of angiosarcoma was negative, with all others having 2+ to 3+ staining intensity. Normal endothelium (Figure 2) and the proliferating endothelium of papillary endothelial hyperplasia were also strongly positive for ER β .

The cases included approximately equal numbers of male subjects and (24 M:29 F) and there was no significant difference in the presence or degree of ER β expression across genders (Table 2).

Discussion

The ER and other steroid hormone receptors are members of the nuclear receptor superfamily of

ligand-activated transcription factors.⁹ Following ligand binding, the ER undergoes dimerization, complexes with coregulatory molecules and binds to the promoter area of targeted genes.¹⁰ Regulation of ER function depends on the relative agonistic and antagonistic properties of the ligands and the combination of repressing and stimulating coregulatory molecules present in the cell. In the 1970s, antibodies to the ER were produced with subsequent development of paraffin-stable monoclonal antibodies in the 1990s. In 1996, a second ER protein was cloned from rat prostate and ovary⁸ and was consequently identified in humans.¹¹ The originally defined ER has since been designated ER α , while the more recently discovered receptor has been designated ER β . ER β displays significant homology with ER α in the transcription activating and ligand binding domains, although the domains involved in the binding of coactivators and corepressors show substantially less homology. ER β has a number of isoforms as a result of differential splicing, which appear to have some tissue specificity.^{12,13}

ER α and ER β have different tissue distributions, although with considerable overlap in breast and other organs. ER β is present in most CNS cells, prostate, ovary, connective tissues of many organs and lymphocytes,^{14,15} decreasing its diagnostic utility. Modulation of estrogen binding depends on the variable distribution of receptor types and subtypes, their varying avidity for ligands, and a constellation of coactivators and corepressors. For example, U2OS osteosarcoma cell lines transfected with inducible ER α or ER β and studied with an Affymetrix GeneChip™ array were found to have fewer than a quarter of their estradiol-, raloxifene- and tamoxifen-regulated genes in common.¹⁶ The differential function of the two ER genes is illustrated in the placenta, where ER β is expressed in syncytiotrophoblast, ER α in cytotrophoblast and where they appear to promote proliferation and differentiation, respectively.¹⁷

Clinical measurement of ER protein has been a routine practice since the 1970s; however, as biomedical science has advanced, the methodology has changed over the years, with varying results. Early assays were based on ligand-binding techniques, which involved either radioligand binding or

Table 1 ER β expression in vascular neoplasms

Diagnosis (53)	0	1+	2+	3+
Angiosarcoma (22)	1/22 (5%)	0	4/22 (18%)	17/22 (77%)
Kaposi sarcoma (3)	0	0	1/3 (33%)	2/3 (67%)
Epithelioid hemangioendothelioma (1)	0	0	0	1/1 (100%)
Spindle-cell hemangioma (2)	0	0	0	2/2 (100%)
Infantile hemangioendothelioma (4)	0	2/4 (50%)	0	2/4 (50%)
Hemangioma (13)	0	1/13 (8%)	2/13 (15%)	10/13 (77%)
Lymphangioma (7)	0	0	0	7/7 (100%)
Papillary endothelial hyperplasia (1)	0	0	0	1/1 (100%)
	1/53 (2%)	3/53 (5%)	7/53 (13%)	42/53 (80%)

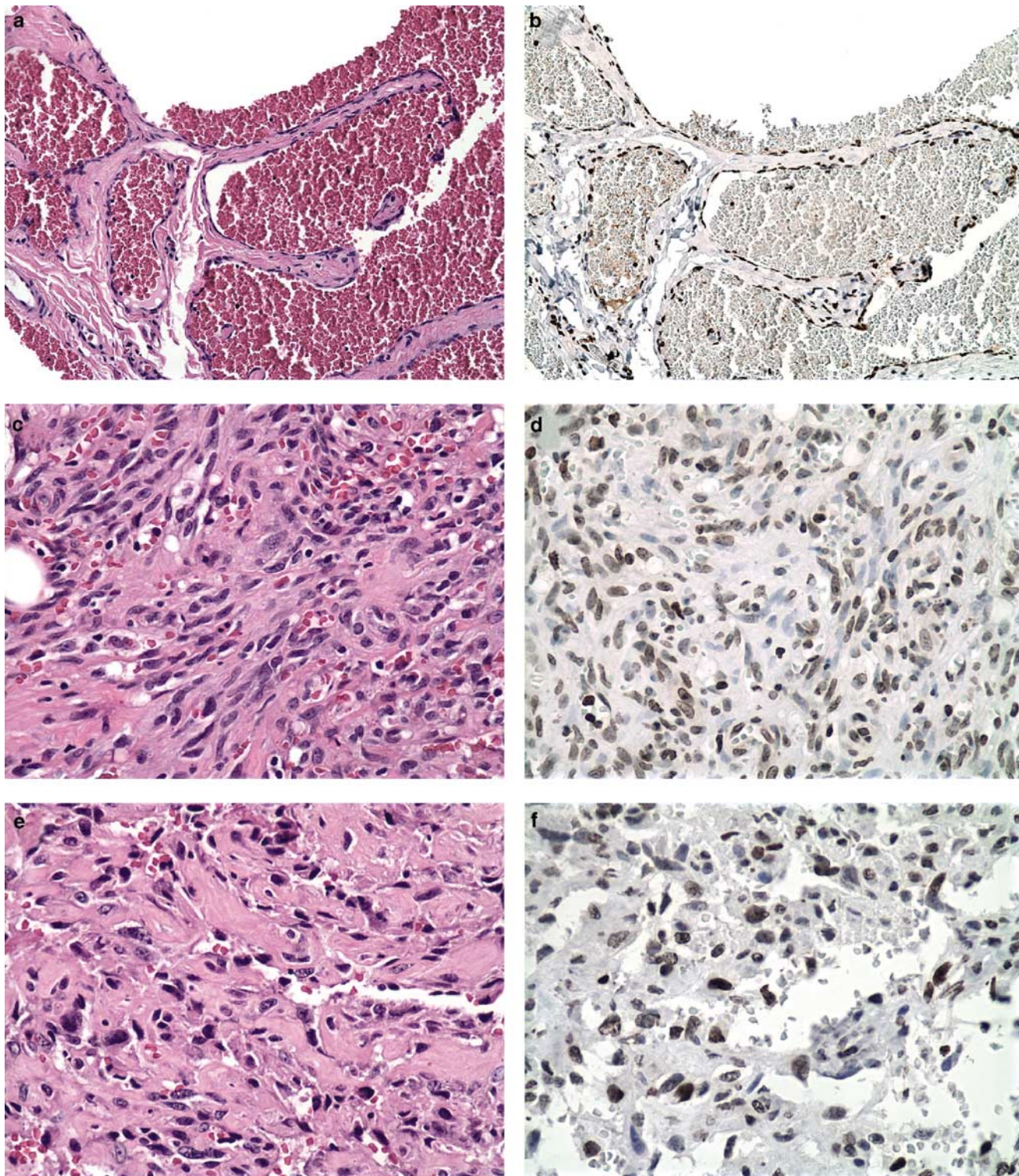


Figure 1 ER β expression in (a, b) capillary hemangioma; (c, d) Kaposi sarcoma; (e, f) angiosarcoma.

fluorescent hormone binding. In the 1980s, these methods were supplanted by monoclonal antibody-based techniques, such as immunohistochemistry and immunoenzyme assays.^{18,19} Although initial comparisons of the methods in breast carcinoma

found a good correlation between the two techniques, no systematic comparison was made in mesenchymal lesions.²⁰ Since ligand-binding assays assess binding of a radiolabeled or fluorescein-labeled ligand, this technique measures both ER α

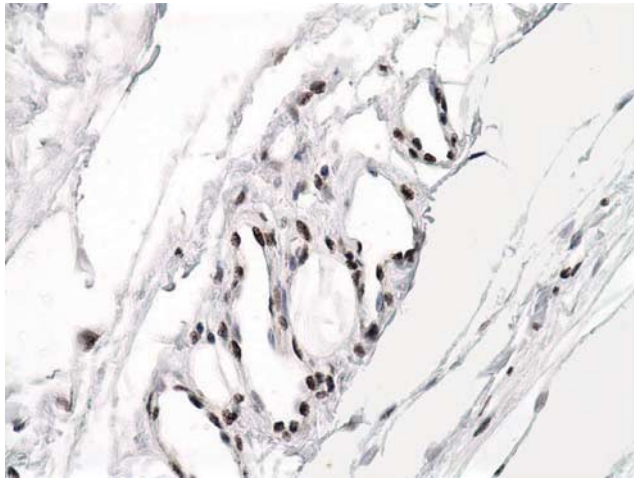


Figure 2 ER β expression in normal capillaries.

Table 2 ER β expression as a function of gender

Gender	0	1+	2+	3+
Male (24)	0	2	4	18
Female (29)	1	1	4	23

and ER β . Today, virtually all ER determinations are performed with an antibody-based method, typically using a monoclonal antibody against the ER α protein which, due to the specificity of the antibody, only detects ER α . Therefore, any tissue expressing only ER β will result in a false negative, accounting for the historical discrepancy in the literature regarding ER in vascular tumors.

The influence of hormones in vascular neoplasia has long been suspected due to an increased incidence in women and a tendency for rapid growth during pregnancy. Ligand binding assays of steroid hormone receptors in vascular tumors gave variable results, with only two studies reporting the presence of ER. (Table 3). Subsequently, Kumagami²¹ used antibodies to steroid hormones to detect bound hormone in tissue sections and discovered evidence of bound estradiol and progesterone in 5/5, testosterone in 2/5, and dihydrotestosterone in 3/5 cases. Later, immunohistochemical studies were uniformly negative for ER, including a large series by Hwang *et al*²² and Liang *et al*.²³ These studies employed commercially available anti-ER α antibodies and, consequently, would be expected to fail to detect ER β . The antibody against ER β used in the current study was raised against a peptide sequence from the carboxy terminus of ER β , a region of the protein that lacks homology to the corresponding area of ER α .²⁴ Lack of cross reactivity with ER α was confirmed via Western blot (Biogenex, personal communication). The data described here clarify the earlier errors promoted through the use of antibodies raised against ER α , and establish that ER β is strongly expressed in vascular neoplasms.

Although the spectrum of mesenchymal neoplasms remains to be evaluated, ER β expression has been characterized in a number of adenocarcinomas including those of the breast,²⁵ ovary,^{26–28} endometrium,²⁹ esophagus,³⁰ stomach³¹ and colon.^{31,32} Interestingly, in breast,²⁵ ovary^{25–28} and endometrium,²⁹ malignancy is associated with loss of ER β expression while in adenocarcinoma of the esophagus³⁰ increased expression is seen. One study has shown decreased ER β expression in colonic adenocarcinoma³¹ while another study³² suggests that, as

Table 3 ER expression in vascular neoplasia from historical literature

Author	Year	Tumor type	Site	Cases positive/total cases	Methodology
Chaudhuri <i>et al</i> ⁴¹	1980	Angiosarcoma	Soft tissue	3/5	Ligand binding
Chaudhuri <i>et al</i> ⁴²	1981	Angiosarcoma	Soft tissue	2/10	Ligand binding
Bracaglia <i>et al</i> ⁴³	1982	Angiosarcoma	Breast	0/1	Ligand binding
Brentani <i>et al</i> ²	1983	Angiosarcoma	Breast	2/2	Ligand binding
Sasaki <i>et al</i> ³	1984	Strawberry hemangioma	Skin	9/9	Ligand binding
Baker <i>et al</i> ¹	1985	Hemangioma, cavernous	Skin	0/1	Ligand binding
Weiss and Enzinger ⁴⁴	1986	Spindle cell hemangioendothelioma	Soft tissue	0/2	Ligand binding
Ohuri <i>et al</i> ⁴	1991	Epithelioid hemangioendothelioma	Lung	0/5	IHC
Anderson <i>et al</i> ⁵	1991	Pseudoangiomatous hyperplasia	Breast	0/5	IHC
Nichols <i>et al</i> ⁶	1992	Lobular capillary hemangioma	Soft tissue	0/21	IHC
Bollinger <i>et al</i> ⁷	1994	Epithelioid hemangioendothelioma	Multifocal	0/1	IHC
Saegusa <i>et al</i> ⁴⁵	1995	Cavernous hemangioma	Liver	0/4	Ligand binding
Powell <i>et al</i> ⁴⁶	1995	Pseudoangiomatous hyperplasia	Breast	4/14	IHC
Singh <i>et al</i> ⁴⁷	1996	Angiosarcoma	Breast	0/1	IHC
Rivasi <i>et al</i> ⁴⁸	1996	Hemangioma	Ovary	0/3	IHC
Lamovec and Bracho ⁴⁹	1996	Epithelioid hemangioma	Bone	0/3	IHC
Schammel and Tarassoli ⁵⁰	1998	Angiosarcoma	Uterus	0/4	IHC
Jurkovic <i>et al</i> ⁵¹	1999	Hemangioma	Ovary	0/1	IHC
Schwartz <i>et al</i> ⁵²	2000	Hemangioma	Bone	0/1	IHC
DiTomaso <i>et al</i> ⁵³	2000	Cavernous hemangioma	Skin	0/10	IHC
Miliaras <i>et al</i> ⁵⁴	2001	Hemangioma, capillary	Ovary	1/1	IHC

is seen in gastric adenocarcinoma,³⁰ there is no significant difference between normal and dysplastic mucosa. The situation is complicated by the recent discovery of additional splice variants and isoforms of ER β in normal tissue^{33–35} as well as evidence that the interaction of ER α and ER β levels may play a role in carcinogenesis.²⁸

The demonstration of ER β in vascular neoplasms suggests a role for estrogen antagonists in the therapy of the most aggressive vascular tumors: angiosarcomas. Although rare, angiosarcomas have a uniformly poor prognosis, which correlates with tumor site and size.^{36,37} Moreover, perhaps due to their association with the systemic vasculature, angiosarcomas metastasize widely and often present initially with multifocal disease, rendering them particularly refractory to surgical therapy. A new generation of antiestrogenic compounds and selective ER modulators (SERM) are in development, some of which preferentially target ER β .^{38–40} As yet, none of these SERM has been tested in angiosarcomas and it remains uncertain whether the effect on normal endothelial cells would preclude systemic therapy. Nonetheless, further study is warranted.

We have demonstrated that, despite previous immunohistochemical evidence to the contrary, a broad range of benign and malignant vascular neoplasms as well as normal blood vessels express ER, specifically ER β . Furthermore, we have showed that the previous inconsistencies in the ER status of vascular neoplasms—and perhaps other entities as well—are attributable to the unexpected existence of a second ER which remains to be fully classified. It is perhaps worth contemplating the existence of a third ER, as yet undetected by our more modern methods but fully present to the biochemists of before.

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