

HIF Transcription Factor Expression and Induction of Hypoxic Response Genes in a Retroperitoneal Angiosarcoma

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Abstract. *Angiosarcoma is a rare and highly aggressive tumor of endothelial origin. The molecular mechanisms driving angiosarcoma growth have not been fully elucidated, although autocrine stimulation by vascular endothelial growth factor (VEGF) secretion may play a role in the pathogenesis of this tumor. We identified a patient with a very rare form of angiosarcoma arising from the retroperitoneum. Immunohistochemical analysis demonstrated widespread up-regulation of the hypoxic response pathway as a mechanism of enhanced VEGF expression. Disordered regulation of the hypoxic response pathway can result in the expression of factors such as VEGF and erythropoietin, which may promote autocrine tumor growth in angiosarcoma.*

Angiosarcoma is a rare sarcoma of purely endothelial origin, which has seldom been reported to arise in the retroperitoneum or kidney (1-4). These tumors are composed of poorly-differentiated endothelial cells, which form diffuse tubular networks, that are highly vascular and often hemorrhagic. Retroperitoneal angiosarcoma tends to be aggressive and rapidly fatal if not amenable to primary surgical management. Angiosarcomas, as endothelial cells, express the receptors for vascular endothelial growth factor (VEGF), flt-1 (VEGF-R1) and KDR/flk-1 (VEGF-R2). Previous studies have demonstrated that these tumors can also produce VEGF, which provides a potential mechanism

of autocrine-mediated growth stimulation (5,6). VEGF is primarily expressed and secreted from cells of epithelial origin, but the mechanism promoting VEGF expression in these tumors has not been identified.

A potential mechanism for the enhanced expression of VEGF is the activation of the hypoxic response pathway. The cellular hypoxic response includes the transcriptional activation of genes involved in angiogenesis, erythropoiesis and anaerobic metabolism. This response is mediated by the transcription factors HIF1 α (hypoxia inducible factor) and HIF2 α , which activate the hypoxic response by promoting the transcription of a large number of genes involved in angiogenesis (VEGF, angiopoietin 1 and angiopoietin 2), oxygen delivery (erythropoietin) and glycolysis (glut-1, lactate dehydrogenase, phosphofructokinase, carbonic anhydrases) (7-9). Under normal oxygen tension, HIF (this term designates either HIF1 α or HIF2 α) is rapidly targeted for proteasomal degradation through an interaction with the von Hippel-Lindau (VHL) tumor suppressor (10-12). When oxygen levels become limiting, this interaction is disrupted and HIF accumulates in local regions of hypoxia. HIF can also be found at high levels in tumors independent of hypoxic stimulation (13). Clear cell renal cell carcinomas associated with VHL mutations demonstrate HIF expression, as well as expression of HIF target genes, including VEGF (14). HIF regulation can also be mediated by transcriptional activation *via* loss of the TSC2 tumor suppressor gene, which causes HIF accumulation as well as VEGF accumulation as a result of loss of inhibition of mTOR (15). Additionally, HIF expression may be modulated by the PTEN/AKT pathway (16). Although many potential mechanisms of HIF activation have been postulated, tumors which express HIF and VEGF have been demonstrated to correlate with both tumor vascularity and growth rate (17).

We report an analysis of one patient presenting with a primary retroperitoneal angiosarcoma, identifying constitutive HIF overexpression in this tumor as a mechanism for aberrant

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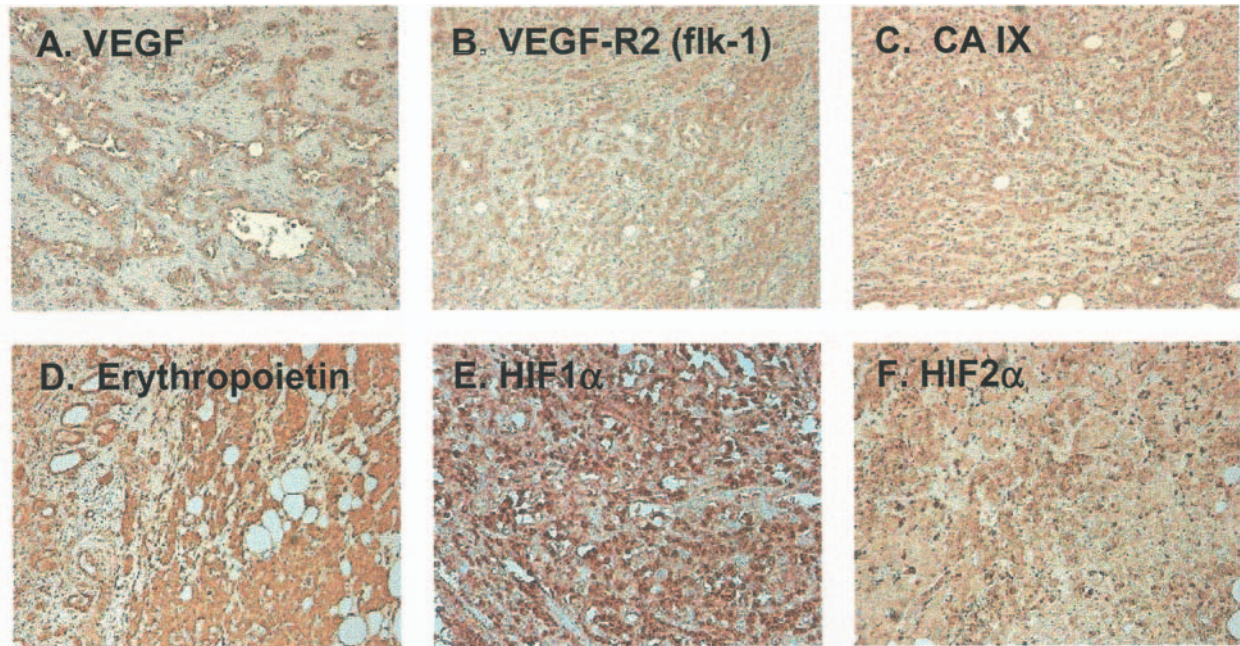


Figure 1. Expression of hypoxic response targets in angiosarcoma. Immunohistochemistry detected diffuse strong staining with antibodies targeting (A) VEGF, (B) VEGF-R2 (*flk-1*), (C) carbonic anhydrase, (D) erythropoietin, (E) HIF1 α , (F) HIF2 α . 20X resolution.

VEGF expression. Furthermore, HIF expression promotes transcription of other growth factors including erythropoietin, suggesting multiple modes of autocrine-stimulated tumor growth.

Materials and Methods

Immunohistochemistry. The tumor tissue was fixed immediately following resection and paraffin embedded according to routine clinical protocols. Immunohistochemical stains were performed as described previously (18). Primary antibodies included HIF1 α (Novus), HIF2 α (Novus), VEGF (NeoMarkers), VEGFR2 (Dako), Flk1 (Novus), erythropoietin (Genzyme) and carbonic anhydrase IX (Santa Cruz). Secondary antibody detection was performed with appropriate biotin-conjugated antibodies (Vector Laboratories). Detection was enhanced with ABC enhancement kit (Vectashield) and detected with DAB reagent (Vector Laboratories). Appropriate positive and negative controls were performed (data not shown).

Results

A 49-year-old male with a history of cadaveric renal transplant for glomerulonephritis-related renal failure was found to have a mass in the lower pole of the native left kidney without associated lymphadenopathy. A radical nephrectomy was performed, which revealed a primary retroperitoneal angiosarcoma with invasion and replacement of the native left kidney. We performed immunohistochemistry on this tumor specimen and found high levels of expression of both VEGF and the VEGF receptor, *flk-1* (Figure 1, A and B).

Tumor sections within this angiosarcoma demonstrated high level expression of other genes transcriptionally activated by the hypoxic response pathway, including carbonic anhydrase IX and erythropoietin (Figure 1, C and D), not normally expressed in endothelial cells. Endothelial cells in general and cells from this angiosarcoma, however, do express the erythropoietin receptor (data not shown), suggesting another autocrine mechanism supporting the proliferation of this tumor. Finally, we observed that both HIF1 α and HIF2 α were expressed at high levels throughout the tumor, but limited to the tumor cells and sparing the surrounding stroma, an uncommon expression pattern for normal hypoxic regulation (Figure 1, E and F).

Discussion

Endothelial cell growth is an important mechanism for processes of both tissue development and tumorigenesis. While endothelial cells in general share many common features, subsets of endothelial cells, in particular endothelial cells of the glomerulus, express a unique footprint of endothelial specific growth promoting genes (19). Understanding mechanisms of disordered endothelial cell growth, especially in unique locations such as the kidney, is an important first step to unraveling the activities of angiogenesis and vasculogenesis which play an important part in the development of highly vascular tumors in the kidney or other organs.

Taken together, our observations suggest that, in this angiosarcoma, the hypoxic response pathway was aberrantly activated as demonstrated by widespread high level expression of both HIF1 α and HIF2 α . This mechanism for the promotion of angiosarcoma is also supported by recent studies of mouse models of tumorigenesis. In a mosaic conditional knockout of VHL, the animals developed angiosarcomas of the liver in addition to hemangiomas and angiectasis in multiple tissues (20). Additionally, in the TSC2+/- mouse, angiosarcomas developed on the tail, paws and mouth (21). In this angiosarcoma, two autocrine feedback mechanisms (both the VEGF and erythropoietin signaling pathways), which could support tumor growth, were activated in coordination with the HIF transcriptional response pathway. While this correlation requires a more thorough evaluation, it provides a unique potential pathway for tumorigenesis which may provide targets for drug therapy in this difficult to treat population.

Acknowledgements

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