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IMMUNOHISTOCHEMICAL BASIS FOR ADJUVANT ANTI- ANGIOGENIC TARGETED THERAPY FOR RENAL CARCINOID: INITIAL CASE REPORT

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Abstract

Objectives—To evaluate the immunohistochemical profile of a carcinoid (low grade neuroendocrine tumor of the kidney) from a patient with lymph node positive disease who remains disease free for 31 months after radical nephrectomy, lymph node dissection and adjuvant therapy with sunitinib malate.

Methods—Immunohistochemical staining was performed for chromogranin, synaptophysin, CD31, VEGF, HIF-1 α , HIF-2, and Glut-1. Staining was evaluated in 3 high-power fields and samples scored as strongly positive (3+), moderately positive (2+), weakly positive (1+) or negative (0). A clear cell renal cell carcinoma was used as positive control.

Results—Immunohistochemical staining was strongly positive VEGF, weak to moderately positive for HIF-2 and negative for HIF-1 α and Glut-1.

Conclusions—Our case of primary renal carcinoid stained intensely for VEGF and HIF-2 consistent with a VHL-HIF1-HIF2-Glut1 independent pathway for VEGF activation. These data suggest that like other neuroendocrine tumors, primary renal carcinoid is a potential target for anti-angiogenic therapy with sunitinib.

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Keywords

renal carcinoid; neuroendocrine carcinoma; anti-angiogenic; sunitinib

INTRODUCTION

Renal neuroendocrine tumors include carcinoid, atypical carcinoid, neuroendocrine carcinoma (small cell carcinoma of the kidney/oat cell carcinoma), primitive neuroectodermal tumor, neuroblastoma, pheochromocytoma, and paraganglioma [1–2]. The World Health Organization classifies neuroendocrine tumors along a continuum of disease: carcinoids are categorized on the low grade end of the spectrum while neuroendocrine carcinomas are categorized on the high grade end. Although primary renal carcinoid tumors are generally thought to be associated with a favorable prognosis, several reports have documented metastatic disease in a significant portion of patients [3–5].

Because most of the literature on these tumors is based on case reports and small case series, their management and prognosis is unclear. No clear adjuvant therapy has been established for cases associated with metastatic disease. Herein we describe novel immunohistochemical features of a renal carcinoid and discuss the rationale for treatment with targeted anti-angiogenic therapy.

CASE REPORT: A 35 year old healthy female of Indian (South Asian) descent with presented in February of 2006 with severe abdominal pain prompting an abdominal ultrasound. Imaging revealed a left renal mass which on follow-up contrast-enhanced CT scan (Fig. 1a–d) demonstrated an enhancing (average HUFU 70) well-circumscribed central hilar left renal mass measuring $3.5 \times 4.0 \times 3.5$ cm without adenopathy. Head, chest CT, abdomen and pelvis CT were negative for other lesions. Ureteroscopy was negative for a renal pelvis mass. She subsequently underwent a percutaneous biopsy which was consistent with papillary renal cell carcinoma. An uneventful laparoscopic transperitoneal radical nephrectomy and extended lymph node dissection (crus of the diaphragm to the inferior mesenteric artery) was completed on 4/6/06 and the patient was discharged on post-operative day 2. Final pathology revealed a 3.2cm low-grade primary renal carcinoid with two of four lymph nodes involved with metastatic disease (pT1aN2M0). She was subsequently started on 50mg of sunitinib (SU11248) and experienced severe hand-foot syndrome and diarrhea. The dose was then reduced to 25mg with a modest improvement in symptoms. She currently tolerates an alternating dose of 25mg with 12.5mg every other day with mild, tolerable symptoms. Of note, she also takes oral contraceptives which may effect the dosing of sunitinib. She has completed CT scans every 6 months and currently remains disease free after 31 months.

MATERIALS AND METHODS**Immunohistochemistry**

Five-micron slides were cut from formalin-fixed paraffin embedded tissue samples for immunohistochemistry. Slides were deparaffinized in 3 changes of xylene for 5 min each

followed by rehydration in graded alcohols. Antigen retrieval was achieved by heating the slides in Tris-EDTA buffer pH 9.0 in a microwave oven at 95°C for 20 min. Endogenous peroxidase activity was inhibited by incubation in 3 per cent hydrogen peroxide in methanol for 10 min. Sections were then incubated overnight at 4°C with the following primary antibodies: mouse anti-HIF-1 α (1:100, BD Biosciences, San Diego, CA), rabbit anti-HIF-2 α (1:200, Novusbio, Littleton, CO), mouse anti-VEGF (1:250, Chemicon, Temecula, CA) and rabbit anti-Glut1 (1:200; Dako, Carpinteria, CA). Isotype-matched negative controls were also included. The immunoperoxidase polymer method (EnvisionTM PO System; Dako, Carpinteria, CA) with 3,3-diaminobenzidine as chromogen was used to develop staining. The sections were counterstained with Mayer's haematoxylin and permanently mounted. Staining was evaluated in 3 high-power fields and samples scored as strongly positive (3+), moderately positive (2+), weakly positive (1+) or negative (0). Staining pattern (membranous, cytoplasmic or nuclear) was also recorded. A clear cell renal cell carcinoma tissue sample removed surgically from a patient with von Hippel Lindau who was found to have a G>A, NT 713, Arg 167GLN (exon 3, missense) germline mutation of the VHL gene was used as positive control.

RESULTS

Gross and Histologic Features

The morcellated nephrectomy specimen weighed 154g. The tumor measured 3.2cm in maximum diameter and appeared grossly as a gray-tan nodule with a central scar. H&E staining was consistent with a renal carcinoid. The tumor cells were uniform and polygonal and arranged in a trabecular pattern with a rich capillary network. The cells demonstrated a moderate amount of eosinophilic cytoplasm. No mitoses, atypia or necrosis was seen. A total of two of four periaortic and infrarenal lymph nodes measuring 0.3cm – 2cm were positive for metastatic disease. The tumor in the lymph nodes was also low grade with no mitoses, cellular pleomorphism or necrosis present.

Immunohistochemistry

The tumor demonstrated patchy staining for chromogranin, weak positive staining for synaptophysin, and abundant vessels that stained strongly for CD31. For the neuroendocrine tumor, although it was strongly positive for VEGF, the staining for HIF-2 was weak-to-moderately positive and consistently negative for HIF-1 α and Glut-1 (Fig. 2). For the latter, positive staining of erythrocytes (internal positive control) was observed. Strongly positive cytoplasmic staining for HIF-2 α , VEGF and Glut1 was obtained in the clear cell carcinoma used as positive control, whereas for HIF-1 α nuclear accumulation was observed (Fig.2). In both cases, incubation of the samples with isotype-matched IgG (negative control) resulted in no staining. Table 1 shows a summary of the staining results.

DISCUSSION

Primary renal carcinoid tumors are low grade, well-differentiated malignant neuroendocrine neoplasms that have histologic features identical to carcinoids found in other locations such as the gastrointestinal tract [1,3]. To date, only about 70 cases have been reported in the

literature [1,3,4] Carcinoids are usually unifocal lesions, presenting at a median age of 50–52 years with no gender predilection [1,3]. Renal carcinoids arise in association with horseshoe kidneys in approximately 25% of cases [4,6,7]. Most patients present with pain or hematuria; carcinoid syndrome is seen in 0–15% of cases [3,4,6]. Somatostatin receptor scintigraphy with 111-Indium-labeled octreotide may be of adjunctive value in diagnosis [4].

Histologically, PRCs are characterized by tightly packed trabeculae and cords of cells with so called granular (“salt-and-pepper”) chromatin creating a “ribbon-like” appearance [3]. Most cases have 0–2 mitoses per high-power field and lack necrosis. Immunostains are generally positive for cytokeratins, synaptophysin, chromogranin, neuron specific enolase, bombesin and CD56 [5]. We found strong positive staining for chromogranin, and weak positive staining for synaptophysin consistent with primary renal carcinoid; the diagnosis was confirmed by two senior GU pathologists at different centers (NN and MM). Although the tumor architecture was well preserved in the tumor fragments we examined, the effect of morcellation on immunohistochemistry due to the admixture of tumor and normal tissues is unknown. We found strong positive staining for CD31 indicative of a highly vascular tumor. This prompted staining for VHL associated markers which was positive for VEGF and HIF-2 but not for HIF-1 α and Glut-1 suggesting a VHL-HIF1-HIF2-Glut-1 independent pathway for VEGF activation. This staining pattern suggests that PRC may be a potential target for anti-angiogenic therapy.

Although PRC are generally thought of as low grade tumors associated with a favorable prognosis, in fact about one third of carcinoid tumors metastasize [2]. In the largest assembled series, Hansel and Epstein obtained follow-up in 12 of 13 patients with metastatic disease identified at the time of surgery [3]. Seven patients underwent additional therapy including systemic treatment with a variety of agents (5-fluorouracil, carboplatin, doxorubicin, capecitabine, and temozolomide). Although follow-up was incomplete and a definitive median survival for patients with synchronous metastases could not be determined, many patients with positive lymph nodes were alive at follow-up ranging from 1–5 years. In another large review of 10 cases, Shurtleff and coworkers identified the development of metastatic disease in 23% of patients [4]. Three of 10 patients died secondary to renal carcinoid over a mean follow-up of 27.6 months: one patient had metastatic disease at presentation while the other two had lymph node involvement found on final pathology; the other 7 patients who were free of metastasis survived over a 26 month follow-up.

A common observation in these series is the tendency for a protracted clinical course despite metastatic disease. Traditionally, radical nephrectomy or partial nephrectomy has been the treatment of choice. To date, the use of chemotherapy for renal carcinoid has not been reported. Like clear cell renal cell carcinoma, neuroendocrine tumors of the pancreas and intestinal carcinoids have been shown to overexpress both VEGF and VEGF receptor subtypes suggesting a possible target for anti-angiogenic therapy [8–11]. Sunitinib malate is a small molecule tyrosine kinase inhibitor with antiangiogenic and antitumoral activity that inhibits several tyrosine kinases involved in tumor growth, angiogenesis and metastatic progression including VEGF receptor and PDGF receptor. While several studies have documented its efficacy in metastatic clear cell renal cell carcinoma, limited data exist

however on adjuvant use for PRC. In a phase II study by Kulke et al, sunitinib was administered to patients with advanced intestinal carcinoids and pancreatic neuroendocrine tumors [12]. Of patients with carcinoids, the objective response rate was 2.4% (1 of 41 patients) and 83% (34 of 41 patients) had stable disease over the study period. The median time to tumor progression was 10.2 months in carcinoid patients with a one year survival of 83.4%. A more recent report demonstrated salvage activity of sunitinib against advanced rectal carcinoid [13]. Our index patient remains disease free on adjuvant sunitinib treatment at 31 months after nephrectomy despite node positive disease. Follow-up over the next several years will be essential given the prolonged clinical course associated with these tumors.

Of note, the low dose of sunitinib on which our patient was maintained (e.g. 12.5mg/25mg alternating days) may be viewed as unconventionally low. However, our patient was a thin female (BMI 17.4) who was also continuing to take oral contraceptives which contained ethinyl estradiol (which is a putative inhibitor of sunitinib metabolism). Because sunitinib is primarily metabolized by liver enzymes, in particular CYP3A4, the ethinyl estradiol could well compete for the CYP3A4 binding site, leading to higher sunitinib blood levels (14). Thus, at standard doses she may have suffered from increased toxicity due to higher blood levels. Reducing the dose led to tolerable side effects, suggesting that the current dosing strategy was titrated properly while the patient was on her oral contraceptive.

The need for adjuvant therapy in this setting is debatable, since the patient was grossly free of disease after surgery and the role of adjuvant therapy for this relatively rare cancer remains unknown. Targeted agents are expensive and can have multiple side effects which can be difficult to tolerate on a long-term basis. Observation with salvage treatment as necessary would have certainly been a reasonable option and was offered to our patient. However she and her physician husband elected to proceed with the aforescribed adjuvant therapy.

CONCLUSION

The present study represents to the best of our knowledge the first demonstration of VEGF staining in a renal carcinoid tumor. Whether or not treatment with adjuvant sunitinib has a long-term impact on her disease status awaits further follow-up; however at nearly 3 years, this patient remains disease free.

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Figure 1.

a–d. A: Contrast enhanced CT, arterial phase (axial view) showing an enhancing $3.5 \times 4.0 \times 3.5$ cm central hilar mass without adenopathy. B: 10 minute delayed phase, axial view. C: Arterial phase, coronal view D: Arterial phase, coronal view.

Figure 2.
H&E and immunostains of the neuroendocrine carcinoma and a clear cell renal cell carcinoma (positive control) demonstrating mild/moderate positive staining HIF2 (B) and strong positive staining for VEGF (D).

Table 1

Immunohistochemistry results.

	HIF1-α	HIF2-α	VEGF	Glut-1
Neuroendocrine tumor				
Intensity	0	1–2+	3+	0
Staining Pattern	-	Cytoplasmic	Cytoplasmic	-
Clear Cell RCC				
Intensity	3+	3+	3+	3+
Staining Pattern	Nuclear	Cytoplasmic	Cytoplasmic	Cytoplasmic, membrane