Epidermal Growth Factor Receptor Positivity in Angiomyolipoma Contiguous to Renal Cell Carcinoma: Report of Two Cases with Immunohistochemical Analysis

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Abstract. Renal cell carcinoma (RCC) and angiomyolipoma (AML), usually unassociated, have occasionally been reported to coexist in the same person, usually in patients with tuberous sclerosis. We report two patients without tuberous sclerosis whose nephrectomy specimens contained renal cell carcinoma directly contiguous to AML in the same kidney. When immunohistochemical staining for epidermal growth factor receptor (EGFR) was performed on the RCCs, an interesting observation was made. The contiguous AMLs demonstrated strong positivity for EGFR, a feature not observed in isolated AMLs. The significance of this finding is unclear. Paracrine regulation may exist between these two closely adjacent tumors leading to synchronous high expression of EGFR in the AML adjacent to RCC, which may in turn affect the biologic behavior of these AMLs, compared to AMLs not associated with RCC.

Introduction

Angiomyolipoma (AML) is a benign renal neoplasm with a reported incidence of 0.1% in males and 0.2% in females in a population without tuberous sclerosis [1]. AML is a mixed mesenchymal tumor composed of smooth muscle, thick-walled blood vessels, and mature adipose tissue. AML was originally thought to be a hamartoma, but cytogenetic and molecular data indicate that it indeed is a neoplasm [2,3]. AML and RCC have occasionally been reported to coexist in the same individual, but separately in the opposite or same kidney, and often in patients with tuberous sclerosis [4,5]. An AML that is directly contiguous to a RCC is very rare, with only six reported cases [6,7]. We report two cases of AMLs in direct contiguity to concurrent RCCs. An interesting observation in these tumors following immunohistochemical staining for epidermal growth factor receptor (EGFR) will also be described.

Case Reports

Case #1. A 74-yr-old man with chronic azotemia and proteinuria was found to have a solid kidney mass with calcification, demonstrated during a duplex venous ultrasonogram of his right lower extremity for possible deep venous thrombosis. A CT scan confirmed a bilobate exophytic hypoechogenic mass with calcification in the interpolar region of the right kidney. The hypoechogenic solid component measured 2.7 x 2.6 x 2.3 cm. Inferior to this hypoechogenic mass was a 1.4 x 1.4 x 1.1 cm echogenic focus, possibly representing an AML. Right nephrectomy was performed.

Case #2. A 65-yr-old woman was found by CT scan to have a mass in the right kidney on PET scan. The patient had multiple endocrine neoplasia type I syndrome, with prior adrenalectomy for pheochromocytoma and thyroidectomy for thyroid carcinoma. A CT scan showed a large exophytic enhancing right renal mass measuring 6.4 x 5.5 cm; just medial to this mass was a 1.0 x 0.8 cm low-density lesion. Laparoscopic right nephrectomy was performed.

Control case #1. A 49-yr-old woman was found by CT scan to have a 14 x 12 x 11 cm left renal mass with fatty elements. Laparoscopic left nephrectomy was performed.

Control case #2. A 56-yr-old woman presenting with gross hematuria was found to have a 3 cm mass in the lower pole of the left kidney. When a left open partial nephrectomy was performed, the mass measured approximately 4.5 cm on CT scan with some fat density.

Control case #3. A 46-yr-old woman with a history of a large left renal AML presented with a 3.8 cm right lower pole renal mass on CT scan.
When a right partial nephrectomy was performed, the mass measured approximately 4.5 cm.

Materials and Methods

Immunohistochemical stains for EGFR were performed on the two cases of RCC with AML and the three control cases of isolated renal AMLs. Formalin fixed, paraffin embedded tissue sections (4-5 μm) were immunostained on a Dako Autostainer (Dako Corp., Carpinteria, CA). The procedure involved blocking endogenous peroxidase with 3% hydrogen peroxide for 10 min followed by a 10-min enzyme treatment using Proteinase K (Dako). The primary antibody, mouse anti-EGFR, Clone 31G7, 1:20 (Zymed Labs, San Francisco, CA) was then applied for 30 min. Detection was performed using LSAB+ and DAB+ substrate (Dako). A solution of 2% cobalt chloride was applied after the DAB+ chromogen for 5 min as an enhancing step. All incubations were performed at room temperature and the rinses between all automated steps were done with TBST. The slides were counterstained with Mayer’s hematoxylin. EGFR was considered positive when there was strong cell membrane positivity. Pan-melanoma immunohistochemistry (cocktail of Mart-1 and HMB-45, CM078B, Biocore, CA) with primary antibodies at 1:500 dilution was also carried out, using the above described procedure, to confirm the diagnosis of AML.

EGFR expression in the tissues was measured using ChromaVision Automated Cellular Imaging System (ACIS, San Juan Capistrano, CA). This system uses automated brightfield microscopy imaging to detect, classify, and count stained cellular objects based on predetermined color and morphology. On each scanned slide, multiple areas of high intensity staining were selected. Several (4 to 10) regions were selected for each case. Using the ACIS intensity scoring platform, the staining intensity in each selected region was determined and the mean percent positivity was determined.

EGFR expression was also determined semi-quantitatively for RCCs and for each component of AMLs (ie, vessels, smooth muscle, and adipose tissue), by two pathologists, who assigned a staining intensity score, ranging from 0 to 3 (0 = No staining, 1 = Weak, 2 = Moderate, 3 = Intense) along with the approximate percentage of cells with a particular score. Based on data, a semi-quantitative immunoreactivity score was derived from the product of percentage of tumor cells staining for EGFR and the average intensity of that staining using the formula: Weighted staining intensity = Σ Intensity score x percentage of cells. In addition, the membranous and cytoplasmic staining was scored independently and the mean of both was considered as the final score. The maximum weighted staining intensity score was 300.

Results

Pathologic findings. Case #1. The main resected specimen consisted of an 8.6 x 2.5 cm right kidney. There was a circumscribed tan-pink and yellow mass measuring 4.2 x 4.2 x 2.5 cm. The mass was composed of a 2.6 x 2.6 x 1.2 cm focally necrotic tumor and an adjacent discrete 1.6 x 1.4 x 1.3 cm homogeneous tumor. Microscopically, the larger tumor was a papillary RCC, with focal necrosis, cystic change, hemorrhage, and calcification. The smaller homogeneous tumor was a mixture of adipose tissue, smooth muscle, and blood vessels, characteristic of AML. The RCC and AML were directly contiguous but distinctly separate (Fig. 1A).

Case #2. The resected kidney measured 8.6 x 6.1 x 3.6 cm and bisection revealed a 7.1 x 4.7 x 4.6 cm yellow mass with focal necrosis and hemorrhage. Adjacent to the mass was a white firm nodule measuring 0.7 x 0.6 x 0.5 cm. Microscopically, the large mass was a chromophobe RCC. The closely approximating, 0.7 cm mass showed a mixture of adipose tissue, smooth muscle, and blood vessels, characteristic of AML. The RCC and AML were directly contiguous but distinctly separate as in Case #1.

Control case #1. The resected kidney measured 25 x 16 x 8 cm. Bisection revealed a large fatty tumor occupying most of the cortex, medulla, and pelvis with approximately 3 cm of normal kidney remaining at the upper pole. Microscopically, the tumor was composed of adipose tissue, smooth muscle, and blood vessels characteristic of AML.

Control case #2. The specimen consisted of multiple fragments of tan-red fatty tissue measuring 12.9 x 9.8 x 3.5 cm in aggregate. Microscopically, it was composed of adipose tissue, smooth muscle, and blood vessels characteristic of AML.

Control case #3. The specimen was received as multiple pieces of macerated and friable tan-grey tissue grossly resembling fibroadipose tissue along with small portion of renal parenchymal tissue measuring 9 x 7 x 2 cm in aggregate. Microscopically, it was composed of adipose tissue, smooth muscle, and blood vessels characteristic of AML.

Immunohistochemistry. The results of EGFR staining intensity scores obtained by ACIS-assisted analysis are listed in Table 1. The papillary renal cell carcinoma showed strong EGFR expression. Surprisingly, the adjacent AML cases also showed strong positivity (Figs. 1B, 1C, 2B, 2C). In contrast, the expression of EGFR in the control cases of AML not associated with RCC was weak (Fig. 2D). The percentage of cells positive for EGFR in the two AMLs...
Fig. 1. Composite papillary RCC and AML. (A) This image shows an AML (top) and a papillary RCC (lower half) with extensive degeneration and focal calcification (H&E, scale bar = 500 µm). (B) EGFR immunostain shows strong positivity in both the AML and a viable focus (arrow) of papillary RCC (EGFR, scale bar = 1000 µm). (C) High magnification composite image showing AML in the lower half and contiguous papillary RCC in upper half (EGFR, scale bar = 200 µm).

Fig. 2. EGFR positivity in isolated (control) and contiguous AMLs. (A) AML directly contiguous to a RCC (H&E, scale bar = 100 µm). (B) Smooth muscle and adipose tissue components of this contiguous AML exhibit strong membranous and cytoplasmic EGFR positivity. In comparison (inset image) perirenal fat from the same case shows minimal EGFR expression (EGFR, scale bar = 100 µm). (C) The vascular component (arrows) of the contiguous AML shows moderate EGFR staining compared to strong EGFR expression, both cytoplasmic and membranous (inset), in surrounding smooth muscle elements (EGFR, scale bar = 100 µm). (D) An isolated AML shows weaker EGFR expression throughout the tumor (EGFR, scale bar = 100 µm).
Discussion

We report AMLs directly contiguous to RCCs in two patients without tuberous sclerosis. In addition to the relative rarity of the coincidental occurrence of these two lesions, an incidental discovery was made when immunohistochemical staining for epidermal growth factor receptor (EGFR) was performed on the RCCs. It was noted that the contiguous AMLs showed strong cytoplasmic and membrane positivity for EGFR, which was similar to that of the RCCs. In contrast, AMLs occurring as solitary kidney lesions showed low EGFR positivity. To exclude the possibility of renal cell carcinomas associated with prominent angioleiomyoma-like proliferation [8], immunohistochemical staining of melanocytic markers (pan-melanoma cocktail) was performed on the AMLs associated with RCCs. In a review of concurrent AMLs and RCCs by Jimenez et al [4], all 22 RCCs were negative and all 25 AMLs were positive for melanocytic markers. Our two cases of AML associated with RCC showed positivity for melanocytic markers, confirming the diagnosis of AML, while the adjacent RCCs were negative.

Epidermal growth factor receptor (EGFR) is a tyrosine kinase and a member of the ErbB/HER family of receptor proteins. It is involved in a signaling cascade that influences proliferation and other tumor-promoting activities as well as responsiveness to chemotherapy [9,10]. EGFR is frequently over-expressed or is abnormally activated in tumors [9,11]. There is evidence that increased EGFR expression correlates with poor clinical outcome [10,12]. Numerous reports

Table 1. Image analysis system (ACIS)-assisted EGFR staining intensity scores.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Score (%)</th>
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<tbody>
<tr>
<td>AML Controls</td>
<td></td>
</tr>
<tr>
<td>Control #1</td>
<td>34</td>
</tr>
<tr>
<td>Control #2</td>
<td>37</td>
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<tr>
<td>Control #3</td>
<td>33</td>
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<tr>
<td>Mean</td>
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<tr>
<td>RCC-associated AML</td>
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<td>Case #2</td>
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<tr>
<td>Mean</td>
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</tr>
<tr>
<td>Case #2</td>
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<tr>
<td>Mean</td>
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AML = angiomyolipoma
RCC = renal cell carcinoma

associated with contiguous or proximate renal cell carcinomas was 96% and 99%, each with 2+ to 3+ positivity. The percentage of EGFR positive tumor cells in the three AMLs not associated with RCCs was 34%, 37%, and 33%, respectively, with 1+ positivity.

The results of the semi-quantitative, weighted EGFR staining intensity scores are listed in Table 2. The RCCs showed the highest weighted EGFR staining intensity score (mean score, 270), followed by the contiguous AMLs (mean score 210). The isolated control angiomyolipomas exhibited the weakest EGFR staining (mean score 135). This pattern of EGFR expression was similar to that observed with the image analysis system. Overall the membranous staining was stronger than the cytoplasmic staining in all tumors.

When EGFR expression of each component of AMLs was compared, some differences were observed between the control cases and the AMLs contiguous to RCCs. In control/isolated AMLs, the highest EGFR expression was seen in smooth muscle, followed by vessels, with the least in the adipose tissue component, whereas in AMLs associated with RCCs, highest EGFR expression was seen in the adipose tissue, followed by smooth muscle, with lowest expression in the vascular component. Perirenal adipose tissue in all specimens was negative for EGFR.

The two contiguous AMLs showed strong positivity with pan-melanoma cocktail immunohistochemical staining, confirming the diagnosis, while the two contiguous RCCs were negative.

Epidermal growth factor receptor (EGFR) is a tyrosine kinase and a member of the ErbB/HER family of receptor proteins. It is involved in a signaling cascade that influences proliferation and other tumor-promoting activities as well as responsiveness to chemotherapy [9,10]. EGFR is frequently over-expressed or is abnormally activated in tumors [9,11]. There is evidence that increased EGFR expression correlates with poor clinical outcome [10,12]. Numerous reports
indicate that EGFR is a promising target for cancer therapy. Anti-EGFR monoclonal antibodies have shown antitumor activity in colorectal carcinoma, squamous cell carcinoma of the head and neck, non-small cell lung cancer, and renal cell carcinomas [8]. In renal cell carcinomas, overexpression of EGFR is a possible cause of increased tumor cell proliferation. Moch et al [13] demonstrated an association between a high Ki-67 and EGFR positivity. Atlas et al [14] demonstrated that epidermal growth factor can stimulate renal cancer cells in vivo and some antibodies against EGFR can inhibit growth.

In this study, we made the interesting observation of EGFR expression in AMLs directly associated with RCCs. EGFR was strongly expressed in AMLs contiguous to RCCs, but not in the AMLs not associated with RCCs. The significance of this finding is not clear. A previous study on two cases of composite RCC and AML suggested that some RCCs develop from the same precursor cell as AML or from a component of AML [15]. Our finding may also represent a previously not described paracrine regulation between these two adjacent and unrelated tumors. Synchronous expression of EGFR may influence the biologic behavior of the AML. More research is needed to elucidate the mechanism of this intriguing phenomenon.

References