The Pathogenesis of Renal Angiomyolipoma: Alternatives to the Perivascular Epithelioid Cell

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Abstract

Although the perivascular epithelioid cell has been proposed and widely accepted as the precursor to renal angiomyolipoma, a normal perivascular epithelioid cell has never been identified, casting doubt on this concept. Nine archived cases of renal angiomyolipoma were examined with routine and immunohistochemical stains. Particular attention was given to small microscopic lesions of angiomyolipoma which were found in association with larger macroscopic tumor nodules. Evaluation of these cases and review of the literature led to the proposal of 4 pathogenetic routes of renal angiomyolipoma development with 4 well described and recognizable precursor cells. It is noted that these are not mutually exclusive routes of pathogenesis and more than one may occur concurrently. The 4 proposed precursor cells are: 1) the renal capsular smooth muscle cells, particularly in cases referred to as renal “capsuloma”, 2) the glomerular mesangial cell in cases of intraglomerular angiomyolipoma, 3) renal tubular epithelial cells via epithelial to mesenchymal transition, and in particular epithelial to pericytic transition, and 4) the renal interstitial vascular associated pericyte with a proliferative phase and transition to angiomyolipoma.

Keywords: Renal angiomyolipoma, Pathogenesis, Epithelial to mesenchymal transition, Epithelial to pericytic transition, Vascular associated pericytes

Introduction

Renal angiomyolipoma (AML) has been included in what has been described as the perivascular epithelioid cell family of tumors (PEComas). The putative cell underlying this family of tumors, the perivascular epithelioid cell, was first described and subsequently named as such due to the similar morphologic, ultrastructural, and immunologic appearances of the neoplastic cells comprising disparate lesions including clear cell tumor of the lung and renal angiomyolipoma [1, 2]. The family was subsequently expanded to include a variety of other neoplasms, including pulmonary lymphangioleiomyomatosis [3]. Despite early skepticism [4], the concept of PEComa has become widely accepted and has been incorporated into the current WHO classification of tumors of the urinary system [5]. Although the originators of this term and review articles of this family of tumors do not explicitly state that the perivascular epithelioid cell is the cell of origin for these diverse neoplasms, an early diagram suggest this concept [1-3, 6]. In addition, there is fairly widespread misconception that there is a perivascular epithelioid cell which gives rise to this family of neoplasms [7, 8]. This is somewhat surprising given that all descriptions of this family of tumors and reviews of this family of tumors clearly note that no normal precursor perivascular epithelioid cell has ever been discovered. Furthermore, there is some evidence for a pericyte origin for this tumor [9]. In this study I examined renal angiomyolipomas with a variety of morphologic patterns to evaluate for morphologic and immunohistochemical evidence to suggest alternative cells of origin for renal angiomyolipoma other than the heretofore undiscovered perivascular epithelioid cell.

Materials and Methods

The pathology archive files of El Camino hospital were searched for cases of renal AML. 66 cases were identified, 49 (74%) in women and 17 (26%) in men. 6 cases had concomitant renal cell carcinoma (2 women, 4 men). Partial nephrectomy was performed in 41 (62%), total nephrectomy was performed in 17 (26%) and needle biopsies were performed in 8 (12%). 9 cases where there was a total nephrectomy and there were histology paraffin blocks available for further studies were selected for review to optimize the amount of non-tumor renal parenchyma available for histologic evaluation. H/E stained slides were reviewed with particular attention to possible precursor lesions. In one of the selected cases (case 4) there was a clinical diagnosis of the tuberous sclerosis complex (TSC). None of the other 8 cases had a clinical diagnosis of TSC. For this study, because the focus was on possible precursor...
lesions, small nodules of AML were defined as areas of AML removed from main tumor masses and measuring ≤ 3mm in size. For selected slides from each case, IHC stains for a variety of antigens were performed: Cathepsin K, smooth muscle actin, caldesmon, HMB-45, CD 31 and D2-40. All IHC stains were performed on the Leica Bond instrument. Source of antibody, and retrieval conditions are as follows: Cathepsin K: Cell Marque antibody retrieval condition ER2 at 20 minutes, smooth muscle actin: Leica antibody, no antigen retrieval, caldesmon Agilent antibody, retrieval condition ER2 at 20 minutes, beta-catenin Leica antibody, retrieval condition ER1 at 20 minutes, HMB-45 Leic antibody, retrieval condition Enzyme 1 at 5 minutes CD 31 Agilent antibody, retrieval condition ER2 at 10 minutes, and D2-40 Leica antibody; retrieval condition ER1 at 30 minutes. All antibodies are ready to use antibodies and do not require dilution.

Results

Pathologic features of the 9 cases selected for evaluation are shown in Table 1. IHC stains were performed on macroscopic renal AML, selected small nodules of AML, and on areas of capsular AML. There were 2 cases (cases 1, 2) where the AML lesions clearly appeared to arise from capsular smooth muscle. Both cases were associated with renal cell carcinoma. In one case there was a single small (2 mm) capsuloma identified without parenchymal involvement (Figure 1). In the second case, the lesional AML cells appeared predominantly capsular (Figure 2) with parenchymal areas confined to the areas of myomatous hyperplasia associated with the renal cell carcinoma. The AML lesions in these 2 cases were purely spindle cell lesions, without associated fatty or epithelioid areas. The IHC staining characteristics of these capsular proliferations are shown in Table 2. Small nodules of renal AML were not identified in the renal parenchyma of either of these cases.

Small nodules of AML were identified in 4 cases (cases 4-7). In 3 of these cases there were also multiple macroscopic nodules of tumor. In 3 cases the largest nodule of renal AML was located in the renal pelvis. The sizes of the small nodules of AML ranged from 0.3 mm to 2.7 mm in size. The lesions were located in the renal cortex without lesions identified in the medullary portion of the kidney. The composition of cells in these small nodules varied (Figure 3 & Table 3). In the smallest nodules there were admixed renal tubules that could be identified. However, as the size of these small nodules of AML increased the areas with admixed renal tubular epithelium decreased. The IHC staining characteristics of the small nodules are shown in Table 2. In some of the small AML nodules one could find renal tubules with strong Cathepsin K staining, but without HMB-45, caldesmon, or muscle specific actin (Figure 4).

All cases were examined for the presence of intraglomerular lesions of AML and none were identified. In 2 cases there were areas of putative pericytic hyperplasia surrounded by a thick basal lamina material (Figure 5). In some areas the pericytic proliferation showed changes suggestive of a transition to an epithelioid renal AML (Figure 6). These areas were found in macroscopic nodules of AML with epithelioid differentiation.

There were 2 cases (cases 8, 9) where there were neither capsular nor small AML lesions identified. Both of these cases had a limited amount of non-tumoral renal parenchyma sampled. One of these cases had multifocal

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Macroscopic AML Lesion(S)</th>
<th>Macroscopic Multifocality</th>
<th>Microscopic Appearance AML Lesion(S)</th>
<th>Small Nodules ≤ 3mm</th>
<th>Associated Lesions</th>
<th>#Sections</th>
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<tr>
<td>1</td>
<td>62M</td>
<td>71F</td>
<td>Renal capsuloma 2mm</td>
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<td>No</td>
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<td>2</td>
<td>67M</td>
<td>1.7 cm</td>
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<td>No</td>
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</tr>
<tr>
<td>3</td>
<td>52M</td>
<td>3-5 cm</td>
<td>Cortical lesions; 3-5 cm</td>
<td>Yes Not enumerated Up to 3 cm</td>
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<td>No</td>
<td>TSC, PLAM, LN + with AML RCC, Hemangioma</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>4F</td>
<td>6.4 cm</td>
<td>Renal pelvic mass 6.4 cm</td>
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<td>No</td>
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<td>7</td>
<td>6F</td>
<td>7 cm</td>
<td>Cortical nodule; 7 cm</td>
<td>Yes 2 additional 5, 7 mm</td>
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<td>No</td>
<td>Renal vein invasion</td>
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</tr>
<tr>
<td>8</td>
<td>7F</td>
<td>9.6 cm</td>
<td>Cortical nodule; 9.6 cm</td>
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<td>Typical</td>
<td>No</td>
<td>No</td>
<td>5</td>
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Table 1: Pathologic features of cases of renal AML

#Sections indicates the number of histologic sections where there was a variable amount of non-tumor renal parenchyma available for histologic evaluation. (RCC = renal cell carcinoma; PLAM = pulmonary lymphangioleiomyomatosis; TSC = tuberous sclerosis complex)
Figure 1: Renal capsuloma.
A. Spindle cell proliferation of cells essentially confined to the renal capsule. (H/E stain, 40 x magnification)
B. Cathepsin K IHC stain shows strong positive staining of the spindle cell proliferation. (Cathepsin K IHC, 200 x magnifications)
C. HMB-45 IHC stain shows patchy positive staining of spindle cell proliferation. (HMB-45 IHC, 200 x magnifications)
D. Casdesmon IHC stain shows strong positive staining of the spindle cell proliferation. (Caldesmon IHC, 200 x magnifications)

Figure 2: Capsular renal AML lesion.
A. Proliferation of spindle shaped cells surrounding a clear cell renal cell carcinoma. (H/E stain, 40 x magnifications)
B. Cathepsin K IHC stain showing distribution of cathepsin K positive proliferation of spindle shaped cells surrounding the renal cell carcinoma. (Cathepsin K IHC, 40 x magnifications)
C. Cytologic features of the capsular spindle cell proliferation. H/E stain, 200 x magnifications)
D. Cathepsin K IHC stain shows positive staining of the capsular spindle cell proliferation. (Cathepsin K IHC, 200 x magnifications)
Table 2: Staining patterns for macroscopic nodules of renal AML and for microscopic nodules
+ indicates weak positive staining, ++ indicate strong positive staining, and f+ indicates focal staining. For CD 31 and D2-40 NP indicates no increased proliferation of small capillaries or lymphatics as compared to surrounding renal parenchyma.

Table 3: Small nodules of renal angiomyolipoma, Distribution and sized of the morphologic appearances of the nodules.
Spindle = predominant spindle cell morphology.
Mixed = mixed spindle and fatty morphology.
Fatty = predominantly fatty morphology.
Epithelioid = predominant epithelioid morphology.
Figure 4: Microscopic nodule of renal AML.
A. Microscopic nodule of renal AML, predominant spindle cell type. Note admixed renal tubules. (H/E stain, 200 x magnifications)
B. Cathepsin K IHC stain shows positive staining of the spindle cell proliferation. Note positive staining of renal tubule (upper right). (Cathepsin K IHC, 200 x magnifications)
C. HMB-45 IHC stain shows positive staining of the spindle cell proliferation. (HMB-45 IHC, 200 x magnifications)
D. Caldesmon IHC shows positive staining of the spindle cell proliferation. (Caldesmon IHC, 200 x magnifications)

Figure 5: Putative pericytic precursor proliferation (vascular spindle cell proliferation)
A. Proliferation of vascular spindle shaped cells surrounded by basal lamina-like material. (H/E stain, 200 x magnifications)
B. CD 31 IHC shows positive staining of vascular endothelium without staining of the vascular spindle cell proliferation. (CD 31 IHC, 200 x magnifications)
C. Actin IHC shows positive staining of both vascular spindle cell proliferation and renal AML. (Actin IHC, 200 x magnifications)
D. Cathepsin K IHC shows absence of staining of vascular spindle cell proliferation and strong positive staining of the surrounding renal AML. (Cathepsin K IHC, 200 x magnifications)
Figure 6: Putative pericytic precursor proliferation with transition to renal AML.
A. Vascular structure surrounded by thick basal lamina-like material and with putative pericytic precursor proliferation and area suggestive of transition to renal AML. (H/E stain, 200 x magnifications)
B. CD 31 IHC showing positive staining of vascular endothelium without staining of pericytic precursor proliferation or renal AML. (H/E stain, 200 x magnifications)
C. Actin IHC showing positive staining of pericytic precursor proliferation and renal AML. (Actin IHC, 200 x magnifications)
D. Cathepsin K IHC showing positive staining of renal AML but not the pericytic precursor proliferation. (Cathepsin K IHC, 200 x magnifications)

Figure 7: Proposed pathogenesis of perivascular distribution of renal AML via EMT/EPT. On the right is an example of the perivascular accentuation of the renal AML infiltrate. (H/E stain, 200 x magnifications). On the left is a diagrammatic sketch of a proposed pathogenesis invoking EMT/EPT of renal tubular epithelium resulting in tumor cells with pericytic-like properties including the propensity to migrate to and support vascular structures.
tumor with a large 7 cm dominant intraparenchymal tumor mass. The other had a large single 9.6 cm parenchymal mass. In addition to the 4 cases with an associated renal cell carcinoma, there were other associated findings. In one case of multifocal renal AML, there was also an associated anastomosing hemangioma identified. The hemangioma stained for CD 31, but not for D2-40. In one case a small medullary fibroma was noted.

Discussion

There is a spectrum of morphologic patterns by which AML involves the kidney [10]. These include 1) typical renal AML with or without capsular involvement and with or without multifocal involvement of the renal parenchyma, 2) renal capsular AML also referred to as “renal capsulomas”, and 3) the rare intraglomerular AML. It has also been noted that renal AML may occur concurrently with renal cortical carcinomas and oncocytomas [11]. Renal AML cases with associated renal cell carcinoma are enriched in this series because radical nephrectomies were selected for review to enhance the non-tumor renal parenchyma available for pathologic evaluation. All of these morphologic patterns have been described in patients with the tuberous sclerosis complex as well as in sporadic cases. Renal AML has been grouped into the “family of PEComas”. It has been implied that this family of tumors arises from a common progenitor cell. However, it has been rightly noted that there has never been a “normal” perivascular epithelioid cell identified which, in my opinion, casts considerable doubt on this concept. Furthermore, there is evidence that the uterine LAM lesions, which are included in the PEComa family of tumors, arise from Mullerian smooth muscle cells [12]. The current study of renal AML casts further doubt on this concept of a “PEComa family of tumors”. Herein, I propose that renal AML arises from known and well described precursor cells. I propose that there are multiple pathogenetic routes from which renal AML may arise and it is important to note that in some cases multiple routes may occur concurrently (Table 4).

First, I propose that some cases of renal AML arise from the smooth muscle in the renal capsule. In this study there were 2 cases including 1 case which is perhaps best regarded as a renal “capsuloma” where both grossly and microscopically, the neoplasms appear to arise from the renal capsule. Smooth muscle has been well described in the renal capsule where it is usually histologically inconspicuous [13]. However, it is easily seen in cases where the capsule becomes distended by renal cell carcinoma and there is a secondary myomatous hyperplasia as seen in cases 2, 3. The capsular AML proliferations appear spindle shaped and express the typical markers of renal AML with positive cathepsin K, HMB-45, actin, and caldesmon. The tumors arising in the capsule may extend into the perinephric fat or may extend into the renal parenchyma. In some cases the tumor is confined to the renal capsule. In such cases it is hard to argue the tumor arose elsewhere. Although the proliferations that appear to arise in the renal capsule are morphologically of the spindle cell type of AML, as they extend either into the renal parenchyma or into the perinephric tissues, they undergo transition to a typical mixed spindled and fatty morphology. They may also show epithelioid differentiation.

Second, I propose that the intraglomerular lesions of AML arise from the mesangial cells of the glomerulus. I was unable to identify any intraglomerular lesions in this series of cases and this discussion will rely on the well described cases reported in the literature, where the intraglomerular AML lesions have been described in TSC associated cases, as well as in sporadic cases [14, 15]. They have also been described in renal AML associated with TSC2/PKD1 continuous gene syndrome [16]. These reports have included IHC and EM studies of the glomerulus which demonstrate these lesions to be confined to the glomerulus. Perhaps no other structure has been examined more extensively with electron microscopy than the glomerulus. It is important to note that no perivascular epithelioid cell has ever been described in the glomerulus. This essentially excludes that cell as the cell of origin for this lesion. The mesangial cell has been suggested as being “related” to these intraglomerular AML lesions [14]. Mesangial cells are smooth muscle-like microvascular pericytes [17]. They contain a contractile apparatus similar to smooth muscle, including actin, myosin and tropomyosin. In addition, it is interesting to note that the normal mesangial cell secretes matrix metalloproteinase and VEGF, similar to the LAM cell [18]. Although the mesangial cell is proposed as the progenitor cell for the intraglomerular lesions of AML, it is unlikely that this is the cell of origin for most cases of renal AML. The intraglomerular lesions are rare and there has never been a documented case of these lesions extending outside of the glomerulus to form a macroscopic lesion. Nevertheless, the recognition of this cell as a progenitor cell in some cases, provides insight into renal AML pathogenesis and also important supportive evidence for following proposed pathogenetic mechanisms.

In this study, a careful examination of the cases for small microscopic renal AML lesions was performed in an attempt to shed light on the pathogenesis of this tumor. Although there was one case of macroscopic invasion of the renal vein, there was no evidence of microscopic lymphovascular space involvement in any of these cases. The presence of multiple small microscopic nodules, well removed from the dominant

<table>
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<tr>
<th>Proposed Cells of Origin for Renal Angiomyolipoma</th>
<th>Type of renal angiomyolipoma</th>
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<tbody>
<tr>
<td>Renal capsular smooth muscle cells</td>
<td>Renal Capsulomas</td>
</tr>
<tr>
<td>Glomerular mesangial cells</td>
<td>Intraglomerular angiomyolipoma</td>
</tr>
<tr>
<td>Renal tubular epithelial cells via EMT/EPT</td>
<td>Typical/epithelioid renal angiomyolipoma</td>
</tr>
<tr>
<td>Renal endothelial associated pericytes</td>
<td>Epithelioid renal angiomyolipoma</td>
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Table 4: Proposed cells of origin for renal angiomyolipoma
tumor masses, in my opinion, suggests that these small nodules of renal AML are precursor lesions for renal AML, similar to the multifocal presence of precursor LAM lesions described in the uterus [12]. The smallest of these microscopic lesions contained intimately admixed renal tubules with the AML cells. There were no identifiable pericytic proliferative lesions noted in the 36 small renal AML nodules examined. In some of these small nodules, there were admixed tubules which strongly expressed cathepsin K. I propose that the third route for the pathogenesis of renal AML involves epithelial to mesenchymal transition (EMT) in these small microscopic foci. There is good evidence that EMT occurs in the kidney with a spectrum of renal cell carcinomas with an associated mesenchymal component having been described [19]. In addition, there is some debate regarding the ability of renal epithelial cells to undergo EMT in the pathogenesis of renal fibrosis [20, 21]. EMT is best described and accepted in neoplastic conditions [22]. Of particular interest for renal AML is the description of epithelial to pericytic transition (EPT) in experimental studies on cancer cells whereby the transition to mesenchymal cells confers pericytic properties on the transformed cells [23]. Pericytic properties include the propensity for these cells to migrate to perivascular sites in an attempt to stabilize blood vessels and improve perfusion and growth of the tumor. This would explain the peculiar perivascular aggregations of tumor cells seen in renal AML. Rather than arising there from an imaginary perivascular epithelioid cell and spreading out from that site, it is more likely that these transformed cells with pericytic properties are migrating to these perivascular sites (Figure 7). Further theoretical support for EMT in the pathogenesis of renal AML comes from the experimental documentation that EMT derived cells have multilineage differentiation potential [24], and in particular are able to differentiate into mature adipocytes [25], as are found in renal AML lesions.

The final pathogenetic route and cell of origin for renal AML lesions has been previously proposed. It has been proposed that renal AML may arise from the endothelial associated pericytes in the renal interstitium [9]. The published evidence includes the documentation that renal AML cells express common pericyte markers including angiotensin II type 1 receptors. In the series of cases I report, there are 2 cases where potential pericytic precursor lesions were identified. The putative pericytic proliferations were associated with a luminal endothelial lining. The pericytes stained with contractile filament markers smooth muscle actin and caldesmon. They were cathepsin K and HMB-45 negative. There were also associated with increased basal lamina-like matrix surrounding them. The production of basal lamina is thought to be a collaborative effort between endothelial cells and pericytes [26]. The presence of an abundance of basal lamina surrounding these areas adds some evidence to the interpretation that these represent proliferating pericytes. Unfortunately, there is no specific marker for the pericyte and it is not possible to exclude the possibility that these proliferating cells represent vascular smooth muscle cells. However, the proliferations appear more cellular and the cells have less cytoplasm than typical vascular smooth muscle cells. The pathogenesis of intraglomerular lesions from the modified pericytic mesangial cell would add some support for this concept of the renal interstitial pericyte as a potential cell of origin for renal AML.

The lesions with the putative precursor pericytic proliferations were identified in macroscopic nodules of renal AML showing epithelioid differentiation and I was unable to find precursor pericytic lesions in any of the small nodules of renal AML that I examined. The pericytic nature of the renal AML cells could also be well explained by the EMT/EPT route of pathogenesis with the added advantage of having the fatty differentiation explained by the multilineage differentiation potential of EMT derived cells. EPT would also explain a previous suggestion that pericytic antigen expression by renal AML is more likely a manifestation of “pericytic mimicry” by tumor cells of another origin [27].

Conclusion

In this study, I have focused on examining renal AML lesions for possible precursor lesions. I have proposed 4 pathogenetic routes for this tumor to evolve: 1) from renal capsular smooth muscle; 2) from the glomerular mesangial cell; 3) from renal tubule epithelial cells via EMT and in particular via EPT; and 4) from interstitial endothelial associated pericytic cells. None of these pathogenetic routes are mutually exclusive.

While the first 2 of these proposals are strongly supported by morphologic evidence, the proposal of EMT/EPT in the pathogenesis of renal AML as well as the origin of renal AML from endothelial associated pericytic cell remain theoretical and without conclusive proof. It has been noted that there are no morphologic/immunophenotypic features that can conclusively prove EMT [21]. Nevertheless, I believe there is sufficient evidence to support further investigation into these models of the pathogenesis of renal AML.

References


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