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Original contribution

# Expression of MDM2 and p16 in angiomyolipoma☆

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**Summary** Angiomyolipoma (AML) arises primarily from the kidney but may grow into the retroperitoneal space mimicking a primary retroperitoneal tumor. Fine needle aspiration (FNA) and core needle biopsy of AML, particularly the fat-predominant variant, may be difficult to distinguish from retroperitoneal well-differentiated liposarcoma (WDLS) or lipoma. Commonly used immunomarkers, MDM2 and p16, have proven useful in diagnosing WDLS and dedifferentiated liposarcoma (DDLS), while HMB45 and Melan-A are melanocyte-related markers characteristically expressed in AML. In this study, we investigated the utility of MDM2 and p16 along with HMB45 and Melan-A immunohistochemical analysis in distinguishing AML from WDL/DDLS or lipoma. Immunohistochemically, AMLs demonstrated focal MDM2 expression (40% of cases) and focal/diffuse expression of p16 (60%). AMLs marked focally or diffusely with HMB45 (76% of cases) and Melan-A (96%). These latter two immunomarkers were not expressed in any of the WDLS/DDLSs or lipomas tested. WDLS/DDLSs showed focal/diffuse expression of MDM2 (91% of cases) and p16 (97%). While focal expression of MDM2 and p16 was observed in 14% and 67% of lipomas, respectively, no lipoma exhibited diffuse MDM2 positivity. In our hands, MDM2 expression by itself cannot exclude the diagnosis of AML or lipoma, and p16 alone is not helpful in separating AML and conventional lipoma from WDLS/DDLS. However, along with morphology, an immunohistochemical battery including HMB45, Melan-A, MDM2 and p16 are useful in distinguishing AML from WDLS/DDLS or lipoma. For equivocal cases, fluorescence in situ hybridization for MDM2 should be performed.


## 1. Introduction

Perivascular epithelioid cell tumors (PEComas), collectively named after the enigmatic perivascular epithelioid cell from which they presumably arise, constitute a family of mesenchymal tumors that exhibit overlapping histologic features and a dual myogenic and melanocytic immunophenotype [1,2]. Angiomyolipoma (AML), a prototypic PEComa, is a triphasic neoplastic process composed of a variable admixture of lipid-laden cells resembling adipocytes, spindled and epithelioid myoid cells, and abnormal thick-walled blood vessels [2]. AML arises primarily within the kidney, but can grow into the retroperitoneal space, or rarely present in retroperitoneum without renal attachment [3], thereby mimicking a primary retroperitoneal tumor.

Although well-differentiated liposarcoma (WDLS) represents the single most common primary sarcoma encountered

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in the retroperitoneum, lipoma and AML also occur in this region [3], and may be difficult to separate on CT imaging [4]. Compounding matters further, all three lesions are composed at least in part of lipid-laden cells that have the potential to exhibit cytologic atypia. In addition, the dedifferentiated component of some examples of WDLS (DDLS) mimics the spindle cell element of AML. These ambiguous cytologic and histologic features are often magnified in the setting of small tissue samples such as fine needle aspiration (FNA) and core needle biopsies (CNB), and may result in diagnostic confusion.

Important differences in clinical course and treatment of these two entities further dictate correct diagnosis. Retroperitoneal WDLS/DDLS requires surgery, and has a high rate of recurrence due to difficulties in completely excising the tumor in this region. Patients with uncomplicated AML can be initially managed with initial active surveillance [5], whereas symptomatic lesions are treated by radical and partial nephrectomy [6,7], selective arterial embolization [7] or ablative therapies, including cryoablation and radio frequency ablation [7]. In addition, patients with tuberous sclerosis complex–associated angiomyolipomas have shown promising results with mTOR inhibitors [7]; therefore, a correct diagnosis is imperative.

Immunohistochemistry is oftentimes used to assist in the diagnosis of AML and WDLS/DDLS. The former characteristically expresses melanocyte-related immunomarkers, HMB45 and Melan-A, and less often, TFE3 and microphthalmia transcription factor, and myogenic markers, smooth muscle actin, calponin and occasionally, h-caldesmon and desmin [8-10]. The hallmark molecular event in the pathogenesis of WDLS/DDLS is amplification of chromosome 12q13-15 resulting in increased copy numbers of MDM2 (considered the "gold standard" of diagnosis) [11] and consequent overexpression of MDM2 [12,13]. Expression of p16 has proven a sensitive and specific marker for separating WDLS (atypical lipomatous tumor)/DDLS from other adipocytic tumors [14,15] and has been touted as especially helpful in differentiating WDLS (atypical lipomatous tumor) from deep lipoma [14].

To date, only a few studies have attempted to differentiate WDLS/DDLS from AML and lipoma with immunohistochemistry [13,16]. As this differential has some relevance in the retroperitoneum [13,16], we decided to evaluate the ability of commonly used immunoreagents MDM2, p16, HMB45, and Melan-A to accomplish this task.

## 2. Materials and methods

### 2.1. Case selection

After approval by our institutional review board (STU #82702), we retrieved 25 cases of AML (16 surgically resected and 9 CNB cases), 33 retroperitoneal liposarcomas (27 WDLS and 6 DDLS) of which 12 were initially diagnosed by CNB (9 WDLS and 3 DDLS), and 21 resected lipomas (4 with prior CNB) from the Pathology Department of Northwestern Memorial Hospital. Data collected included age and sex of the patient, and the surgical and cytopathology diagnoses.

### 2.2. Core needle biopsy

Percutaneous CNBs were performed under guidance of ultrasound (US) or computed tomography (CT) imaging using a 20-gauge core biopsy device. One to four CNB passes with corresponding touch preparations (TP) were obtained. The touch preparation slides were air-dried and stained with modified Giemsa (Diff-Quik) stain for on-site evaluation for specimen adequacy and interpretation by a board-certified cytopathologist.

### 2.3. Histology and immunohistochemistry

CNBs and representative sections from partial nephrectomy specimens and resected retroperitoneal tumors were formalin-fixed, paraffin-embedded (FFPE), microtome processed, placed on glass slides, and stained with hematoxylin and eosin (H&E) for histomorphology examination.

Immunohistochemical stains were performed on the sections of the FFPE tissue with appropriate positive and negative controls (ie, positive controls expressed the immunoreagent and negative controls showed no expression). Antibodies against MDM2 (M41-113, Invitrogen, Carlson, CA), p16 (705-4713, Roche Diagnostics, Indianapolis, IN), HMB45 (M0634, DakoCytomation, Carpinteria, CA), and Melan-A (M7196, DakoCytomation) were used. Positive result for MDM2 and p16 required nuclear staining in viable tumor cells. Immunohistochemical staining was graded in a semiquantitative manner and scored as "diffuse" if >50% of tumor cells were positive, "focal" if between 5% and 50% of tumor cells expressed the protein, and "rare" if <5% of tumor cells were positive. When less than 5% of tumor cells were positive, a negative score was assigned.

### 2.4. Fluorescence in situ hybridization

Four-micrometer slides were prepared from FFPE. Fluorescence in situ hybridization (FISH) was performed using a dual-color MDM2 probe set with the chromosome 12 centromere labeled as spectrum green serving as the control locus and the MDM2 gene located at 12q15 labeled as spectrum orange (Leica Biosystems, Buffalo Grove, IL). Standard laboratory procedure and the instructions from the manufacturer's protocol were followed in conducting FISH analysis.

The normal signal pattern consists of two MDM2 (orange) and CEP12 (green) signals. Cells with gains in copy numbers demonstrate a total of 3 to 5 copies of both CEP12 and MDM2 signals and are considered as exhibiting aneuploidy. The signal patterns are considered as positive for MDM2 amplification when (1) the ratio of MDM2/CEP12 is $\geq$3.0, (2) there are at least six or more MDM2 signals/per cell, or (3) MDM2 signals are clustered [17].