Identification of the Benign Mesenchymal Tumor Gene *HMGA2* in Lymphangiomyomatosis

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Abstract

The normal expression pattern of *HMGA2*, an architectural transcription factor, is primarily restricted to cells of the developing mesenchyme before their overt differentiation during organogenesis. A detailed *in situ* hybridization analysis showed that the undifferentiated mesoderm of the embryonic lung expressed *Hmg2a* but it was not expressed in the newborn or adult lung. Previously, *HMGA2* was shown to be misexpressed in a number of benign, differentiated mesenchymal tumors including lipomas, uterine leiomyomas, and pulmonary chondroid hamartomas. Here, we show that *HMGA2* is misexpressed in pulmonary lymphangiomyomatosis (LAM), a severe disorder of unknown etiology consisting of lymphatic smooth muscle cell proliferation that results in the obstruction of airways, lymphatics, and vessels. Immunohistochemistry was done with antibodies to *HMGA2* and revealed expression in lung tissue samples obtained from 21 patients with LAM. In contrast, *HMGA2* was not expressed in sections of normal adult lung or other proliferative interstitial lung diseases, indicating that the expression of *HMGA2* in LAM represents aberrant gene activation and is not due solely to an increase in cellular proliferation. *In vivo* studies in transgenic mice show that misexpression of *HMGA2* in smooth muscle cells resulted in increased proliferation of these cells in the lung surrounding the epithelial cells. Therefore, similar to the other mesenchymal neoplasms, *HMGA2* misexpression in the smooth muscle cell leads to abnormal proliferation and LAM tumorigenesis. These results suggest that *HMGA2* plays a central role in the pathogenesis of LAM and is a potential candidate as a therapeutic target. [Cancer Res 2007;67(5):1902–9]

Introduction

The high mobility group (HMG) of proteins is a heterogeneous collection of small, acidic, architectural transcription factors (1, 2). Based on homology, these HMG proteins are further classified into three distinct families: HMBG (formerly HMG-1/2), HMGN (formerly HMG-14/17), and HMGA (formerly HMG1; ref. 1). There are three members of the HMGA family, HMGA1a (formerly HMGI) and HMGA1b (formerly HMGY), two proteins resulting from alternate splicing of the same gene, and *HMGA2* (formerly HMGIC). HMGA2 is encoded by a separate gene (3). Humans and mice have single functional copies of the *HMGA2* genes, which are highly conserved between the two species (2). Each of the HMG proteins contains three DNA binding domains, termed AT hooks, because of their affinity for AT-rich sequences of DNA. The HMGA family members also possess a highly acidic “tail” at their COOH terminus (2, 4). Whereas they themselves do not possess transcriptional activities per se, the HMGA proteins have been shown to enhance the transcription of several genes by coordinately binding in the minor groove of DNA and facilitating interactions with and among transcription factors (5, 6).

HMGA2 has been shown to play a major role in mammalian growth and development. The homozygous null mouse mutation results in the pygmy phenotype (7, 8) and most organs are equally retarded in size, but histologically normal, with all the various cell types present within an individual organ (8). The normal temporal expression pattern of *HMGA2* in both mice and humans is restricted to early embryogenesis, predominantly in developing mesenchyme (8–10). Therefore, the reduced stature of the null mouse is a direct result of decreased proliferation of undifferentiated mesenchymal cells during the development of individual organs.

Human studies have strongly implicated *HMGA2* as responsible for aberrant states of proliferation. Disruption and expression of the gene has been found in a number of benign mesenchymal tumors including lipomas, uterine leiomyomas, endometrial polyps, adenomas of the salivary gland, and pulmonary chondroid hamartomas (11–14). In one study, 70% of examined cases of pulmonary chondroid hamartomas had rearrangements affecting the *HMGA2* gene (13). In lipomas and uterine leiomyomas (fibroids), disruptions were shown to result in the generation of novel *HMGA2* fusion transcripts consisting of the DNA binding domains of *HMGA2* linked to novel sequences that encoded for transcriptional regulatory domains (11, 15). Subsequent studies, however, revealed that there were lipomas and uterine leiomyomas that developed without the generation of fusion transcripts because normal or truncated forms of the *HMGI* genes were found to be expressed (14, 16). Therefore, the structure of the *HMGI* transcript does not seem to be the primary determining factor in the formation of these benign mesenchymal tumors. Instead, because *HMGA2* is mainly expressed in undifferentiated cells, it has been hypothesized that it is the inappropriate activation or misexpression of an *HMGI* gene in a terminally differentiated mesenchymal cell that activates the tumorigenic pathway and leads to a benign mesenchymal tumor (16). For example, *HMGA2* is not expressed in normal adult fat tissue (10) but is misexpressed in the terminally differentiated fat cells (17) that constitute lipomas.
(11, 15). These studies further emphasize the critical role of HMGA2 in mesenchymal proliferation and differentiation.

Lymphangioleiomyomatosis (LAM) is a rare, progressive disease characterized by the abnormal proliferation of smooth muscle cells in the airways, vasculature, and lymphatics of the lung (18, 19). The smooth muscle cell population found in LAM is phenotypically heterogeneous, with spindle cells containing round nuclei alongside large epithelioid cells with clear cytoplasm (20). The LAM cells are considered smooth muscle cells based on their reactivity with antibodies to α-smooth muscle actin (18, 19) and often express vimentin, which is a mesenchyme-specific intermediate filament (18). Interestingly, LAM patients are often afflicted with extra-thoracic tumors, most notably renal angiomyolipomas (18, 19). Because LAM manifests as a benign, abnormal proliferation of mesenchymal cells (18), a possible role for HMGA2 was investigated in the pathogenesis of the disease.

Materials and Methods

In situ hybridization. Pregnant female mice of desired gestation times (11.5–14.5 days post coitum) were sacrificed by cervical dislocation and embryos were dissected and fixed overnight in fresh 4% paraformaldehyde in PBS. After 24 h, embryos were dehydrated through increasing concentrations of ethanol and cleared in 1:1 ethanol/ethylene glycol followed by 100% ethanol. The paraffin-embedded tissue was then sectioned (9 μm thick) and mounted on 3-aminopropyl triethoxysilane–treated slides (Sigma, St. Louis, MO). Slides were then baked at 37 °C overnight and desiccated at 4 °C (21). Single-stranded sense and antisense mRNA probes corresponding to exons 2 and 3 of mouse Hmga2 were prepared and used for the in situ hybridization analysis as previously described (21).

Reverse transcription-PCR analysis. Reverse transcription was done on 1 μg of total RNA extracted from LAM tissue, normal lung tissue, or mouse embryonic tissue at different developmental stages. The RNA was mixed with 50 pmol of the Hmga2 exon 5–specific antisense (reverse) primer (5′-CTTCAAAAGATCCACTGCTGCTGAGG-3′), heated to 70 °C for 10 min, and placed on ice. The annealed RNA-primer solution was used with 1 μL (200 units) of SuperScript II Rnasenase H− Reverse Transcriptase (Life Technologies, Inc., Carlsbad, CA) in a 20-μL first-strand synthesis reaction mixture [50 mmol/L Tris-HCl, 75 mmol/L KCl, 3 mmol/L MgCl2, 10 mmol/L DTT, 0.5 mmol/L each deoxynucleotide triphosphate (dNTP)] placed at 94 °C for 15 min. One microliter of first-strand cDNA was then used in a 50-μL PCR reaction mixture (10 mmol/L Tris-HCl, 1.5 mmol/L MgCl2, 50 mmol/L KCl, 0.4 mmol/L each dNTP) with 2.5 units Taq DNA polymerase (Roche Diagnostics, Mannheim, Germany) with 50 pmol of the Hmga2-specific primers (reverse and forward from exon 2: 5′-CGGTGAGCCCTCTTCTAAAGAGACC-3′). After 5 min denaturation at 94 °C, 35 cycles of amplification (94 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min) were done, followed by a 7-min final extension at 72 °C. The RNA samples were also tested for the presence of the GAPDH transcript. Reverse transcription and PCR reaction conditions were exactly the same as they were for the Hmga2 protocol, except the GAPDH-specific reverse primer (5′-CTAAGCACTTGGTGTGAGGG-3′; from exon 7) and forward primer (5′-CGTATGGGCGCTTGTCAC-3′; from exon 2) primers were used with a 60 °C annealing temperature during the 35 cycles of amplification. DNA fragments were resolved by gel electrophoresis in a 2% agarose gel and visualized by ethidium bromide.

Immunohistochemical studies. Normal, interstitial lung disease, and LAM tissue samples were fixed in neutral buffered formalin (18 h) and embedded in paraﬃn for sectioning (see above). Formalin-fixed, paraﬃn-embedded sections of normal and diseased human lung tissues were analyzed for their reactivity to the following antibodies: HMGA2, HMB45, α-smooth muscle actin, heavy caldesmon, and vimentin. Alkaline phosphatase complexes were detected with Vector Red (Vector Laboratories) yielding a red reaction product. 3′,3′-Diaminobenzidine (Vector Laboratories) was used as a substrate for peroxidase complexes yielding a brown reaction product at the site of the target antigen. Sections were counterstained with hematoxylin (blue) or methyl green. Antibodies (HMB45, α-smooth muscle actin, heavy caldesmon, and vimentin) were obtained from DAKO (Glostrup, Denmark). For confocal analysis, double immunostaining was done. The primary antibodies were diluted (HMB45 1:400, HMB45 1:50 in PBS with 10% serum) and incubated in a wet chamber with the sections overnight at 4 °C. Detection was done by using a FITC-conjugated polyclonal goat anti-rabbit immunoglobulin G (IgG; Abcam, Cambridge, MA) secondary antibody for HMGA2 as well as a TRITC-conjugated goat anti-mouse (Sigma-Aldrich) secondary antibody for HMB45. The HMGA2-specific antibody was raised in a female New Zealand white rabbit and recognizes a single 12-kDa band in

Figure 1. In situ hybridization analysis for Hmga2 in developing mouse lung. Photomicrographs of sections of 11.5 (A, B, and H), 12.5 (C and D), 13.5 (E and F), and 14.5 d post coitum (gpc; G) in the developing lung. Sections were hybridized with the antisense (A–G) or sense (H) Hmga2 riboprobe. A, C, E, G, and H, dark-field; B, D, and F, phase-contrast views of (A), (C), and (E), respectively. E, endoderm; M, mesenchyme; t, tubule; d, distal tubules (identified by their characteristic cuboidal epithelium); p, proximal tubules (identified by their columnar epitheilum). Magnification, × 100.
Investigations into the expression pattern for analysis revealed that the gene is temporally restricted to normal adult human lung but was detected in RNA isolated from LAM lung tissue.

Western blots (8) that corresponds to the expected size for HMGA2. Sections were stained or counterstained with H&E by standard procedures.

**Generation of transgenic mice.** The 3.7-kb mouse smooth muscle cell α-actin promoter fragment (23) was subcloned into Bluescript (Stratagene, La Jolla, CA) and the BamHI site was modified to an XbaI site by filling in with Klenow and adding XbaI linkers. After excision with XbaI, the promoter was subcloned into a vector containing the HMGA2 gene linked to β-globin polyadenylation sequence. The resulting plasmid was analyzed by restriction enzyme digest and sequenced across the ligation sites to ensure the correct orientation of the construct. Mice were initially screened after birth with PCR analysis on the tail preparation. After it was determined that the transgenic founders did not survive, pregnant females were sacrificed at 14 days post coitum. The embryos were then genotyped and immediately placed in formalin for histologic analysis. Lung tissues from transgenic and wild-type animals were fixed, embedded, and sectioned as described above. Formalin-fixed, paraffin-embedded sections were analyzed for their reactivity to the following antibodies as described above: HMGA2 (1:400), α-smooth muscle actin (1:200). In addition, Ki67 immunostaining was done with antibodies against Ki67 (1:40 in PBS with 3% serum). Detection was by an ABC-based method (Vector Laboratories) as described above.

**Results**

Expression of Hmga2 in the developing mouse lung. Investigations into the expression pattern for Hmga2 have previously revealed that the gene is temporally restricted to embryogenesis in both humans and mice (8, 10). A more detailed analysis has been done in mice by Northern and Western blot analysis and revealed that Hmga2 RNA and protein can be detected in the whole embryo as early as 10.5 days post coitum, with levels of both decreasing sharply at 13.5 days post coitum and undetectable after 15.5 days post coitum (8). The temporal expression of Hmga2 is specific to each tissue (9) and a cell type–specific expression pattern of Hmga2 has not been done during lung development. In situ hybridization analysis revealed that expression was mainly observed in the mesenchyme of the developing lung, before its overt differentiation. At 11.5 days post coitum, the lung bud reveals Hmga2 expression within the sphere of mesoderm, into which the elongating bronchus is branching (Fig. 1A and B), and no expression in the bronchial endoderm. This pattern of Hmga2 expression continues as lung development progresses, as shown at 12.5 days post coitum (Fig. 1C and D). At this developmental stage, branching morphogenesis of the lung has been initiated and the tubules derived from the endoderm do not express Hmga2 whereas the surrounding mesoderm retains expression of the gene (Fig. 1C and D). Interestingly, it is this mesoderm that will form the mesenchymal lung components of the adult (24), including the smooth muscle cells proliferating in LAM.

During lung morphogenesis, there is a gradient of differentiation such that the proximal tubules are more differentiated than the distal tubules (25). Interestingly, the in situ hybridization analysis revealed that Hmga2 expression was restricted to the less differentiated distal tubules and absent in the more differentiated proximal tubules (Fig. 1E and F). Thus, at 13.5 days post coitum, a transition in Hmga2 expression is observed concomitant with the

**Table 1. HMGA2 immunohistochemical analysis of various lung tissue samples**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pathology</th>
<th>HMGI-C immunoreactivity</th>
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<tr>
<td>LAM1</td>
<td>Lymphangiomyomatosis</td>
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<tr>
<td>LAM2</td>
<td>Lymphangiomyomatosis</td>
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<td>LAM3</td>
<td>Lymphangiomyomatosis</td>
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<td>LAM4</td>
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<td>LAM6</td>
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<td>LAM8</td>
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<td>LAM21</td>
<td>Lymphangiomyomatosis</td>
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<tr>
<td>NSIP1</td>
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<td>NSIP2</td>
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<td>NSIP3</td>
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<td>EG1</td>
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<td>EG2</td>
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<td>EG3</td>
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NOTE: Nineteen cases of LAM, eight cases of interstitial lung disease, and two normal lung samples were examined. Renal angiomyolipomas were present in LAM11, LAM12, and LAM16 but not in LAM1 and LAM17. Angiomyolipoma status of the other patients was not available. LAM15 was obtained from a TSC patient.
state of differentiation of the bronchial tubules. Transient expression of Hmga2 was observed in the distal tubule epithelium at 13.5 days post coitum; however, by 14.5 days post coitum, the in situ hybridization analysis shows no expression throughout the lung (Fig. 1G). Thus, in the mesenchyme, Hmga2 expression within the developing mouse lung is restricted to the undifferentiated, proliferating cells during organogenesis and is extinguished by 14.5 days post coitum.

**HMGA2 expression in LAM.** HMGA2 is mainly expressed in undifferentiated, mesenchymal cells (see above). However, when HMGA2 is misexpressed in a differentiated mesenchymal cell, a tumorigenic pathway is activated, which leads to a benign mesenchymal tumor (11, 15). Because the smooth muscle cells in LAM are differentiated and benign (20), HMGA2 expression was investigated in this tumor type. As expected, HMGA2 expression was not observed in RNA isolated from wild-type 16.5 and 18.5 days post coitum embryonic and newborn lungs, as well as in the mouse and human adult lung (Fig. 2). Intriguingly, expression was observed in the RNA sample extracted from a diseased biopsy specimen of a patient diagnosed with pulmonary LAM (Fig. 2).

To extend the above studies, HMGA2 expression was investigated at the cellular level in a number of lung tissue samples. LAM samples were obtained from patients with varying severity of the disease. Immunohistochemical studies were done with the specific anti-HMGA2 serum (8). Nineteen of 19 lung samples (Table 1) from patients diagnosed with LAM exhibited nuclear staining in the LAM smooth muscle cells (Fig. 3A and B). Similar intensity of staining was observed in all LAM samples independent of the clinical stage of the disease. Consistent with the diagnosis of LAM, the cells exhibited a positive reaction with antibodies to both caldesmon (Fig. 3D) and α-smooth muscle actin (Fig. 3C). HMGA2 is not detectable in adult tissues (10) and, as expected, the antibody did not react with cells in sections of normal lung (Fig. 3E). Significantly, eight separate samples from patients with other interstitial lung diseases were also assayed, and none stained positively with the anti-HMGA2 antibody (Fig. 3F; Table 1).

It has been reported (26) that LAM cells differ from normal smooth muscle cells in that they react with the monoclonal antibody HMB45, which recognizes a melanocyte antigen (27). However, the percentage of LAM cells that react with HMB45 varies between tumors (18) and is said to range from 17% to 67% of the α-smooth muscle actin–positive cells in the diseased tissue (18). Similar results were obtained for the majority of the LAM samples under investigation (Fig. 4A). In contrast, the HMGA2 antibody reacted with >90% of the LAM cells within each tumor (Figs. 3B and 4B). Double immunostaining of the LAM sample with HMGA2 and HMB45 showed that there were cells reacting with both antibodies (Fig. 4C). Occasional HMB45-positive cells are HMGA2

Figure 3. Immunohistochemical analysis of LAM tissue. Pulmonary explant stained with H&E (A) showing expansion of interalveolar septa (arrows) and emphysematous dilation of alveolar spaces. The LAM smooth muscle cells are positive for HMGA2 (B) and heavy caldesmon (arrows, brown reaction product in C). The immunoperoxidase stain for smooth muscle actin (D) shows bundles of smooth muscle cells (arrows, brown reaction product) within expanded alveolar spaces characteristic for LAM. HMGA2 immunostaining is present in most smooth muscle cells populating the thickened interalveolar septa (B, arrows). Both normal lung samples (E) and a sample from a patient with interstitial pulmonary fibrosis (F) did not react with the HMGA2 antibodies. Magnification, ×50 (A, B, E, and F); ×375 (C and D).
negative (Fig. 4C) but ~50% of HMGA2-positive cells in the thickened alveolar septa of LAM exhibited no staining for HMB45 (Fig. 4C). Confocal microscopy identified several cells of varying sizes that stained for HMGA2 but did not stain for HMB45 (Fig. 4D).

**Fluorescence in situ hybridization analysis of LAM patient tissue samples.** Misexpression of HMGA2 in benign mesenchymal tumors arises through multiple mechanisms that sometimes include translocations at the locus. Therefore, we examined a subset of patients from our study for chromosomal disruption at the HMGA2 locus using fluorescence in situ hybridization (FISH) analysis. The representative sample shows that individual cells consistently contain only two positive signals for each probe and suggests against selective HMGA2 amplification (Fig. 5). Therefore, HMGA2 misexpression in LAM cells does not arise due to translocations at the locus and is similar to the situation observed in uterine leiomyomatosis and other benign mesenchymal tumors.

**Expression of HMGA2 in smooth muscle cells.** To examine whether misexpression of HMGA2 could lead to abnormal proliferation of lung smooth muscle cells, transgenic mice were generated that expressed the human HMGA2 gene in their lungs under the direction of the α-actin promoter. This promoter has been used in several other studies and directs transgene expression to the airway smooth muscle cells (23,28). Fertilized F1 (C57BL/6 × CBA/J) eggs were microinjected and 98 mice were born. None of these animals were found to harbor the transgene by PCR analysis. The lack of founders suggested that the expression of the construct caused lethality and the death of transgenic mice in utero. Additional microinjection experiments were done, and foster mothers were sacrificed for embryos at 14 days post coitum. Dissection of the foster mothers revealed viable embryos with no masses identified. Four of the 22 embryos were positive for the transgene. The embryos were fixed in neutral buffered formalin and the lung tissues analyzed histologically. Histologic analysis of the lungs of mice showed an increase in the proliferation of mesenchymal cells surrounding the alveoli of the transgenic mice when compared with control lungs (Fig. 6A and B). The increase in mesenchymal cells seems to infringe on the development of the airways (Fig. 6B). Ki67 staining (a marker of proliferation) revealed a significant increase of staining in the lungs of the transgenic mice when compared with the lungs of control mice (Fig. 6C and D). The proliferation of smooth muscle cells is a prominent pathologic feature seen in the lung tissues of human patients with LAM.

**Discussion**

This study has shown that 21 of 21 pulmonary LAM tissue samples exhibited the coordinate misexpression of the developmentally regulated architectural factor HMGA2. None of the samples examined exhibited cytogenetic abnormalities on FISH analysis. Furthermore, the results allow for a classification of LAM with other benign mesenchymal proliferations known to be associated with HMGA2 misexpression (2).

Investigations of spatial and temporal expression patterns throughout normal mammalian embryogenesis have led to the understanding that Hmga2 expression is highest in the developing mesenchyme of a number of organs (5, 7). This pattern of expression was confirmed in the lung in this study when Hmga2 expression was primarily observed in the undifferentiated mesoderm of the developing lung bud. As development progresses and these cells differentiate, Hmga2 was no longer expressed in the lung. This cell type-specific expression pattern of Hmga2 in undifferentiated mesenchymal cells has led to the hypothesis that HMGA2 misexpression in differentiated mesenchymal cells plays a central role in the pathogenesis of an array of benign mesenchymal neoplasms (2). The misexpression of HMGA2 in the differentiated smooth muscle cells of the lung in all the investigated LAM tumor samples implies that a similar mechanism occurs in LAM as for the previously described human benign mesenchymal tumors.

As expected, HMGA2 was not detected in the terminally differentiated smooth muscle cells of the normal adult lung. Interestingly, expression was also not detectable in lung tissues from all eight patients suffering from other interstitial lung diseases. These lung diseases are characterized by the presence of proliferating fibroblasts (29–31). This reinforces the idea that HMGA2 expression does not result simply from mesenchymal cell proliferation per se, but HMGA2 misexpression defines a change in the biological state of the cell that is related to transformation. The evidence
from the other benign mesenchymal neoplasms (11–16) strongly suggests that HMGA2 misexpression is a pivotal event in the pathogenesis of these tumors and leads to a deregulation of HMGA2 target genes in a differentiated cell. A similar mechanism is proposed for the proliferating smooth muscle cells of the lung that ultimately results in LAM.

The HMB45 monoclonal antibody reacts with gp100, a melanocyte lineage–specific antigen involved in the later stages of eumelanin biosynthesis (27), which is also expressed in melanomas (20). HMB45 has been found to react with the nonproliferating subpopulation of cells (32) in the LAM tumor, and, although unlikely to be involved in the pathogenesis of the tumor (18, 32), the monoclonal antibody is used as a diagnostic marker for LAM (26). However, there is a wide variation in the number of LAM tumor cells that express the antigen (18). Interestingly, the majority (>90%) of LAM cells in the 21 tumor samples investigated in the present study stain with the antibody raised against HMGA2. Additionally, there was not an apparent discrimination between the two morphologic cell types present. Therefore, we propose that utilization of the HMGA2 antibody could aid in the pathologic diagnosis of LAM.

There are two distinct groups of patients that suffer from LAM. The first group of patients has a sporadic form of LAM that is often accompanied by solitary and asymptomatic renal angiomyolipomas (18). The second group that develops symptoms of LAM consists of patients with the autosomal dominant inherited disorder, tuberous sclerosis (TSC; ref. 19). TSC is a tumor suppressor gene syndrome that is associated with seizures, mental retardation, and benign tumors of the brain, heart, kidney, lung, and skin (33). The tumors in the kidney are also renal angiomyolipomas that tend to be multiple and bilateral (18, 34). In comparison with the incidence of LAM in the general population of one in a million, 1% to 5% of TSC patients may develop symptomatic LAM (35, 36). The higher incidence of LAM in TSC patients and the common presence of angiomyolipomas (although their clinical presentation is different) have led to the hypothesis that the genes responsible for TSC (TSC1 and TSC2) cause both the noninheritable (sporadic LAM) and TSC-associated forms of the disease. This has been extended to the concept that LAM could be a forme fruste of TSC (37).

Molecular confirmation of this hypothesis is conflicting. Studies on LAM cells have suggested that patients with sporadic LAM have a loss of heterozygosity at the TSC2 locus with an accompanying nonsense mutation in the other allele (38, 39). However, investigators have identified expression of the TSC2 gene product tuberin in the smooth muscle cells of sporadic LAM patients by immunohistochemistry (19). This same antibody, in stark contrast, did not stain cerebral or renal hamartomas of tuberous sclerosis complex with tuberin mutations from TSC patients (40).

Clearly, TSC is involved in the pathogenesis of LAM because of the high incidence of LAM in TSC patients. However, the
loss of the wild-type allele may not be necessary for cellular proliferation in LAM (41), which has also been shown to be the case for other tumor lesions from TSC patients (42). Therefore, this implies that alternative etiologies need to be considered other than a genetic mechanism at the TSC locus. For example, it has recently been shown that activation of the extracellular signal–regulated kinase (Erk) pathway can lead to posttranslational inactivation of TSC in tissue culture cell lines (43). Interestingly, the activation of the Erk pathway has been shown to induce the expression of HMGA2 (44). Future studies will elucidate whether the HMGA2 pathway mediates its action in a TSC-dependent manner or through a novel pathway mediating the proliferation of smooth muscle cells in LAM. The transgenic expression studies of HMGA2 combined with the human immunohistochemistry studies presented in this article provide an exciting alternative mechanism (in conjunction with a haploinsufficiency in the TSC genes) for proliferation of the smooth muscle cells in LAM. These studies provide data that would classify LAM with other benign mesenchymal tumors in which misexpression of HMGA2 is the primary determining factor (11, 13–16). Supporting this is the fact that every sample of LAM analyzed in the present study exhibited HMGA2 expression. Furthermore, it is important to note that the panel of LAM samples were obtained from both sporadic and TSC patients with or without angiomylipomas. We propose that the TSC genes act as modifiers to HMGA2 misexpression, causing an increased susceptibility to the development of LAM in TSC patients.

The lack of an effective treatment for LAM (45–49) emphasizes the need for a mechanistic understanding of the pathogenesis of the disease so that rational therapies can be developed. Here we have shown for the first time that the disorder develops with the coordinate misexpression of HMGA2 in an adult cell population. The fact that all LAM samples were identified with significant HMGA2 expression strongly suggests that the gene is playing a critical role in the pathogenesis of this disease. The results suggest an additional target towards which treatment strategies for LAM can be directed. Additionally, the involvement of HMGA2 in LAM allows for the classification of the disease with the other mesenchymal tumors known to be associated with HMGA2. Therefore, therapeutic efforts directed at these more common tumors by targeting HMGA2 can additionally be expected to aid in the treatment of LAM.

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References


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