The High-Mobility Group A1 Gene Up-Regulates Cyclooxygenase 2 Expression in Uterine Tumorigenesis

Abeba Tesfaye, 1,2,3 Francescopalo Di Cello, 1,2 Joelle Hillion, 1,2 Brigitte M. Ronnett, 4 Ossama Elbahliouli, 1,2,3 Raheela Ashfaq, 9 Surajit Dhara, 1,2,3 Edward Prochownik, 1,2,3 Kathryn Tworkoski, 1,2,3 Raymond Reeves, 9 Richard Roden, 9 Lora Hedrick Ellenson, 10 David L. Huso, 3, and Linda M. S. Resar 1,2,3,6

Abstract

Uterine cancer is the most common cancer of the female genital tract and is the fourth most frequent cause of cancer death in women in the U.S. Despite the high prevalence of uterine cancers, the molecular events that lead to neoplastic transformation in the uterus are poorly understood. Moreover, there are limited mouse models to study these malignancies. We generated transgenic mice with high-mobility group A1 gene (HMGA1a) expression targeted to uterine tissue and all female mice developed tumors by 9 months of age. Histopathologically, the tumors resemble human uterine adenosarcoma and are transplantable. To determine whether these findings are relevant to human disease, we evaluated primary human uterine neoplasms and found that HMGA1a mRNA and protein levels are increased in most high-grade neoplasms but not in normal uterine tissue, benign tumors, or most low-grade neoplasms. We also found that HMGA1a up-regulates cyclooxygenase 2 (COX-2) expression in transgenic tumors. Moreover, both HMGA1a and COX-2 expression are up-regulated in high-grade human leiomyosarcomas. Using chromatin immunoprecipitation, HMGA1a expression are up-regulated in high-grade human leiomyosarcoma. Using chromatin immunoprecipitation, HMGA1a expression are up-regulated in high-grade human leiomyosarcoma.

Introduction

Although high-mobility group A gene (HMGA) expression is increased in a variety of human cancers (1–9), its role in the pathogenesis of these malignancies has not been clearly delineated.

The HMGA1 gene encodes the HMGA1a and HMGA1b protein isoforms (1), which function as architectural chromatin-binding proteins involved in regulating gene expression (10–13). HMGA1a and HMGA1b proteins result from alternatively spliced mRNA and differ by 11 internal amino acids present in the HMGA1a isoform (1, 4). There are no known differences in the biological activities of these isoforms (1, 4). Both proteins contain AT hook DNA-binding domains that mediate binding to AT-rich regions in the minor groove of chromosomal DNA (10–13). After binding to DNA, the HMGA1 proteins recruit additional transcription factors, and in concert with these factors, alter gene expression (10–13). Interestingly, this gene is located on the short arm of chromosome 6, in a region known to be involved in amplifications, rearrangements, translocations, and other abnormalities correlated with human cancers (1).

We previously showed that HMGA1a transgenic mice develop aggressive, highly penetrant lymphoid malignancy (9). Here, we report that all female HMGA1a mice also developed uterine tumors resembling human uterine adenosarcomas. We show that HMGA1a is overexpressed in aggressive human uterine neoplasms, but not benign tumors or normal tissue. We also found that cyclooxygenase 2 (COX-2) is an important mediator of HMGA1a function in uterine tumorigenesis. These studies underscore the oncogenic properties of HMGA1 in a variety of tissues. Moreover, our transgenic mice should provide a useful model system to identify other molecular pathways that lead to uterine cancer.

Materials and Methods

Transgenic mice. The HMGA1a (formerly HMG-I) transgene construct and transgenic mice have been previously described (9).

Transplant experiments. Normal uterine tissue fragments from control mice or tumor fragments from transgenics (100–200 mg) were injected s.c. into female nude (nu/–/–) mice. Mice were followed for tumor formation and sacrificed after 5 weeks. Histopathologic examinations for tumors were done after necropsy.

Quantitative reverse transcription-PCR analysis. Tissues from transgenic mice and human tumors were analyzed by quantitative reverse transcription-PCR (RT-PCR) as previously described (9). The sequences for the forward and reverse primers that amplify murine HMGA1a were: 5′-GC-TGGTCGGGAGTCAGAAAG and 5′-GGTGACTTTCCGGGTCTTG, respectively, and the forward and reverse primers that amplify human HMGA1a were: 5′-AGGAAAAGGACGGCACTGAGAA and 5′-CCCCGAGGTCTCTTAGG, respectively. Forward and reverse primer sequences for murine COX-2 were: 5′-AGTGGCCTCGTGAGCTTCT and 5′-CTTCTGCAGTCCAGGG, respectively, with probe no. 11 (Roche, Universal Primer Library); forward and reverse primer sequences that amplify human COX-2 were: 5′-AGGAAAAGGACGGCACTGAGAA and 5′-CCCCGAGGTCTCTTAGG, respectively.
**Figure 1.** The HMGA1a transgenic mouse: transgene expression. A, HMGA1a mRNA and protein expression in uterine tissue of transgenic and control mice. The mRNA levels were measured by quantitative RT-PCR at 2 mo of age. Columns, relative HMGA1a mRNA in transgenic mice (n = 5) with 20 copies of the transgene compared with control littersmates (n = 5). HMGA1a mRNA was normalized to glyceraldehyde-3-phosphate dehydrogenase as a loading control and assigned a value of 1 in the controls. Columns, means from two separate experiments done in triplicate; bars, SD. HMGA1a and β-actin protein levels by Western analysis in uterus from HMGA1a transgenic mice with 20 copies of the transgene and a control littermate. B, normal mouse uterus (left) compared with uterine adenocarcinoma in a transgenic mouse (right) with immunohistochemical staining for HMGA1. C, gross necropsy findings of a typical HMGA1a transgenic mouse.

respectively. RT-PCR reactions were done in triplicate and all experiments were done at least twice.

**Western analysis.** Western analysis was done as described (9). Briefly, total cell lysates were boiled in 2× Laemmli buffer, analyzed by SDS 4% to 12% variant gradient PAGE (NuPAGE, Invitrogen), and subjected to Western analysis using a chicken polyclonal antibody raised to the NH2 terminus of HMGA1 as we previously described, but diluted 1:200 (5). To detect COX-2, the commercial COX-2 antibody (Santa Cruz Biotechnology, CAClonex CX-294; DakoCytomation) was diluted 1:1,000. The actin monoclonal antibody AC15 (Sigma Immunochemicals) was diluted 1:2,500 and used to control sample loading, also as previously described (5).

**Immunohistochemistry.** Immunohistochemistry for HMGA1 was done as described (8). Immunohistochemistry for COX-2 in the mouse tumors was done with the commercial COX-2 monoclonal antibody (Clone CX-294; DakoCytomation). Immunohistochemical staining for COX-2 in the human tumors was done as described (14).

**Cell culture.** MES-SA human high-grade uterine sarcoma cells and human BJ primary fibroblasts were grown as recommended (American Type Culture Collection).

**HMGA1a RNA interference experiments.** Small interfering RNA (siRNA; Dharmacon) was used to knock down expression of HMGA1a according to the instructions of the manufacturer. Specifically, the MES-SA cells were incubated with siRNA to HMGA1a (20 μmol/L) compared with the mock-transfected control or siCONTROL nontargeting siRNA pool which contains four negative control siRNAs without matches to human, mouse, or rat genes (20 μmol/L). After 18 to 20 h of incubation, cells were washed and placed in methylcellulose or maintained in culture dishes to harvest for protein or mRNA at the indicated time points. Protein levels were assessed for HMGA1a and control β-actin at 24, 48, 72, and 96 h after transfection with each siRNA. HMGA1a and control β-actin mRNA levels were assessed at the same time points.

**Chromatin immunoprecipitation experiments.** We did chromatin immunoprecipitation experiments as described (15) using MES-SA cells. Proteins cross-linked to chromatin were immunoprecipitated with the following antibodies: HMGA1 (16), Pol II or histone H3 (as positive controls), or IgG (as a negative control). The COX-2 promoter region with the consensus HMGA1a DNA-binding site was amplified from the immunoprecipitated protein-DNA complexes using the forward and reverse primers 5′-TAAGGGGAGAGGAGGGAAA and 5′-GACGCTCACTGCAAGTGATA, respectively. The HPR1I promoter was amplified as a negative control promoter with no HMGA1a binding sites as described (16).

**COX-2 inhibitor experiments.** The specific COX-2 inhibitor NS-398 (Sigma-Aldrich) was used at a 5 μmol/L concentration as described (17). A 20 μmol/L stock solution of NS-398 was prepared in DMSO and mixed with methylcellulose for a final concentration of 5 μmol/L.

**Methylcellulose assays.** Cells (105) were plated in six-well plates in methylcellulose medium according to the instructions of the manufacturer (StemCell Technologies, Inc.).

**Lentiviral HMGA1a construct and infection of BJ cells.** The HMGA1a coding region was amplified from the previously described HMGA1a vector containing the murine HMGA1a tagged at its 3′ end with a myc epitope (18) using the following primers: forward-CGC GCT AGC AGA ATG AGC GAG TCG GCC TCA A-3′, in which the first 15 nucleotides correspond to a “GC clamp”, an NheI restriction site, and a Kozak-ATG signal, and reverse 5′-CCG TTC TGA TTA CGC ACA AGA GTT-3′, in which the first 9 nucleotides corresponds to a GC clamp and an Xhol site and encodes the last four amino acids of human c-myc, the termination codon, and three nucleotides of the 3′ untranslated region. After amplification, the product was digested with NdeI and XhoI, and cloned directionally into the pFG12 lentiviral vector (19), which also encodes green fluorescent protein. The HMGA1a-myc coding region was sequenced within its entirety to confirm its orientation and sequence. Infections were done as previously described (18, 19).

**Transfection experiments.** Transfections were done using Lipofect-AMINE 2000 (Invitrogen) according to the instructions of the manufacturer. Specifically, pSG5-HMG-I [100 ng; see ref. (5) for vector details] was cotransfected with a plasmid containing the COX-2 promoter upstream of the luciferase gene [25–45 ng see ref. (20) for vector details] which includes the HMGA1a DNA binding site at position −60 from the transcription start site (20, 21). Cells were also transfected with the pRL-TK vector (5 ng) containing Renilla luciferase (Promega) to control for transfection efficiency. The DNA was mixed with LipofectAMINE at a ratio of 1:3 (μg/μL) in Opti-MEM (Invitrogen) and incubated with the cells for 6 h. Cells were subsequently washed and placed in DMEM with 10% fetal bovine serum. Cells were harvested for luciferase activity 24 h after transfection. Experiments were done in triplicate and repeated twice.
Results

Transgene expression. Three of the original seven informative HMGA1a transgenic founders were females and carried 1 to 28 copies of the transgene (9). The transgene was highly expressed in the uteri; both HMGA1a mRNA (Fig. 1A) and protein (Fig. 1A and B) levels were increased in the transgenic uteri compared with control uteri.

All HMGA1a transgenic mice developed uterine tumors resembling human uterine adenosarcomas. All female founders developed uterine tumors at a mean age of 4.2 ± 0.6 months (Fig. 1C; Table 1). As with the lymphoid tumors, higher copy number did not correspond to a more aggressive phenotype. The founder male with 20 copies of the transgene was successfully bred to establish a line of HMGA1a mice. The second generation transgenic was also male. All 13 informative third generation females from this line also developed uterine tumors (mean age, 8.1 ± 2.4 months; Table 1; Fig. 2A–D). All females also had leukemia and/or lymphoma at the time of death (9). Because the male transgenic mice died with lymphoid malignancy at a similar age to the females (8.3 ± 3.9 months), it is likely that the lymphoid malignancy was the primary cause of death for both the females and males.

Pathologic examination of the uteri revealed large, polypoid intracavitary endometrial tumors resembling human uterine adenosarcomas. The tumors were comprised of an overgrowth of HMGA1a transgenic founders were females and carried 1 to 28 copies of the transgene (9). The transgene was highly expressed in the uteri; both HMGA1a mRNA (Fig. 1A) and protein (Fig. 1A and B) levels were increased in the transgenic uteri compared with control uteri.

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Pathologic examination of the uteri revealed large, polypoid intracavitary endometrial tumors resembling human uterine adenosarcomas. The tumors were comprised of an overgrowth of cytologically bland endometrial stroma accompanied by benign-appearing endometrial type glands. The endometrial stroma had greatly increased volume with variable degrees of hypercellularity and edema. The epithelial component exhibited foci of glandular crowding and dilatation. One transgenic female also developed multiple nodules of benign-appearing smooth muscle in the myometrium resembling human uterine leiomyomas. There were no metastatic uterine tumors in any of the mice.

To determine the onset of the uterine tumors, female transgenics from the line with 20 copies of the transgene and age-matched controls were sacrificed at 2, 4, and 6 to 12 months. By 2 months of age, the uteri were already abnormal, with mild enlargement and the development of early adenosarcoma-like changes, including polypoid architecture and a proliferative endometrial stroma with a decrease in uterine glands (Fig. 2B and C). These abnormalities progressed and became more apparent by 4 months of age (Fig. 2B and C). By 6 to 12 months, fully developed uterine tumors were present in all female transgenics from this line (Fig. 2B and C).

Infertility in female HMGA1a mice. All female transgenic mice were infertile. Notably, the male mice with 20 copies of the transgene had normal fertility with litter sizes similar to control animals (5–10 offspring/litter). Infertility in the females is likely related to the early uterine abnormalities (Fig. 2).

The uterine tumors are transplantable. To determine if the tumors from the HMGA1a mice were malignant and transplantable, we injected uterine tumor fragments (100–200 mg) into nude mice (nu−/−; 26 total injections) and followed the mice for tumor formation. Control experiments were done by injecting normal uterine fragments from control mice (200 mg) into nude mice (4 total injections). After 5 weeks, tumors were present in 12 of 15 (80%) of the mice injected with 200 mg of uterine tumor fragments with an average tumor diameter of 0.67 ± 0.34 cm (range, 0.2–1.27 cm; Fig. 2D). Tumors were also present after 5 weeks in 5 of 11 (45%) mice injected with 100 mg of tumor fragments with an average tumor diameter of 0.78 ± 0.20 cm (range, 0.50–0.90 cm). No tumors (0 of 4) resulted from the control injections.

HMGA1a expression in human uterine neoplasms. Over-expression of HMGA1a in the uterus resulted in tumors in all female mice. To determine if this mouse model was relevant to human disease, we examined HMGA1a gene expression by quantitative RT-PCR from 17 primary human uterine neoplasms, including 8 benign or low-grade tumors, 6 high-grade tumors, and 3 with mixed high- and low-grade components. The benign tumors included leiomyoma (3), and the low-grade tumors included low-grade endometrioid carcinoma (3), low-grade stromal sarcoma (1), and low-grade adenosarcoma (1). The high-grade neoplasms

Table 1. Characteristics of HMGA1a female transgenic mice

<table>
<thead>
<tr>
<th>Generation/mouse no.</th>
<th>Age (mo)</th>
<th>Copy no.</th>
<th>Gross phenotype (activity level or death)</th>
<th>Abdominal enlargement</th>
<th>Other (prolapse, if present)</th>
<th>Microscopic (adenosarcoma-like tumor)</th>
<th>Other (leiomyoma-like tumor)</th>
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<tr>
<td>I-1</td>
<td>3.25</td>
<td>1</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>I-2</td>
<td>4.50</td>
<td>28</td>
<td>Died</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>I-3</td>
<td>4.50</td>
<td>10</td>
<td>Died</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>III-1</td>
<td>9.25</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>III-2</td>
<td>8.50</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>III-3</td>
<td>8.50</td>
<td>20</td>
<td>Decreased</td>
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<td>+</td>
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<tr>
<td>III-5</td>
<td>7.00</td>
<td>20</td>
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<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>III-8</td>
<td>8.75</td>
<td>20</td>
<td>Decreased</td>
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<td>+</td>
</tr>
<tr>
<td>III-9</td>
<td>8.25</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>III-10</td>
<td>8.75</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>III-12</td>
<td>10.00</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>III-14</td>
<td>8.00</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>III-15</td>
<td>10.25</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>III-16</td>
<td>10.50</td>
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<td>Decreased</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>III-20</td>
<td>12.50</td>
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<td>Decreased</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>III-22</td>
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<td>No change</td>
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NOTE: Independent female founders developed uterine tumors.
included serous carcinoma (3), and leiomyosarcoma (3), and the mixed high- and low-grade tumors included mixed serous/endometrioid carcinoma (3). We also studied one high-grade uterine sarcoma cell line (MES-SA cells; American Type Culture Collection). As a control, RNA from pooled, normal uteri (Stratagene) was studied. We observed striking differences in the expression levels between the benign and low-grade tumors compared with the high-grade neoplasms. All high-grade neoplasms and cell lines (seven of seven) had levels of \textit{HMGA1a} mRNA that were greater than control RNA (range, >1.5- to 21.3-fold levels; Fig. 3A). The benign tumors (three of three) and most low-grade neoplasms (four of five) had \textit{HMGA1a} mRNA levels that were lower than in controls (Fig. 3A). The benign leiomyomas had the lowest levels of expression and the aggressive leiomyosarcoma had the highest \textit{HMGA1a} expression. Interestingly, the adenosarcoma seemed to be low-grade histologically, although it had elevated \textit{HMGA1a} expression. We do not know if this tumor showed aggressive behavior in the patient. Adenosarcomas are typically low-grade but can develop high-grade components and behave aggressively.

Adequate samples were available for protein purification and Western analyses from four tumors, and we found increased HMGA1 protein levels in three of three primary high-grade uterine neoplasms compared with the normal uterine tissue (Fig. 3B). HMGA1 protein was not increased in the low-grade leiomyoma (Fig. 3B). Protein levels correlate with the mRNA in all cases.

\textit{HMGA1a} is required for transformation in human uterine tumor cells. Since we showed that overexpression of \textit{HMGA1a} was
sufficient for transformation in the transgenic mice, we sought to
determine if it was necessary for transformation. To this end, we
transfected high-grade, human uterine sarcoma (MES-SA) cells
with a siRNA oligonucleotide sequence to HMGA1a (Dharmacon).
This approach effectively silences HMGA1a, decreasing both mRNA
and protein levels (Fig. 3C; Supplementary Fig. S1). There was no
decrease in HMGA1a mRNA or protein with the siRNA controls.
We found that inhibiting HMGA1a expression blocked transfor-
mation (Fig. 3D). Specifically, cells with knocked down HMGA1a
expression had decreased anchorage-independent growth in
methylcellulose with decreased foci formation (P = 0.009, Student’s
t test). These findings indicate that HMGA1a is required for the
transformed phenotype in methylcellulose in these cells.

HMGA1a up-regulates COX-2 expression in uterine tumor-
igenesis. Next, we sought to determine how HMGA1a might
promote malignant transformation in uterine tumors. COX-2 was
previously reported to be a potential HMGA1a gene target in
vascular endothelial cells under hypoxic conditions (22). Because of
the established role of COX-2 in cancer cell growth, we investi-
gated the role of COX-2 in transformation mediated by HMGA1a. First, to
ascertain whether HMGA1a could bind to the COX-2 promoter in
human cancer cells, we did chromatin immunoprecipitation
experiments. We found that HMGA1a binds directly to the COX-2
promoter in vivo in human uterine cancer cells (Fig. 4A). In
transfection experiments, we also found that HMGA1a significantly
activates expression of the COX-2 promoter linked to a luciferase
reporter gene (20, 21) in rat fibroblasts by $2$-fold above that of the
control empty vector (P = 0.012 by Student’s t test; Supplementary
Fig. S4). To determine if COX-2 was up-regulated in the mouse and
human tumors overexpressing HMGA1a, we measured COX-2
mRNA and protein levels in these tumors. We found that COX-2
mRNA and protein (Fig. 4B) were increased in the uterine tumors
of the HMGA1a transgenic mice. In human tumors, we also found
that COX-2 mRNA was increased in the leiomyosarcomas, but not
the leiomyomas or normal uterine tissue (Fig. 4C). We also showed
that COX-2 protein was increased in the leiomyosarcomas (Fig. 4C),
but not in the benign tumors or normal tissue. Interestingly, COX-2
mRNA expression was not increased in serious tumors, suggesting

Figure 3. HMGA1 expression is increased in high-grade primary human uterine
tumors and required for transformation in high-grade human uterine cancer cells.
A, HMGA1a mRNA is increased in all (six of six) high-grade, human uterine
tumors (including a), and the carcinoma and leiomyosarcoma components
in the MES-SA high-grade uterine sarcoma cells compared with the control pooled,
normal uterine tissue mRNA, benign tumors (leiomyoma), and low-grade tumors
(endometrial carcinoma and low-grade uterine sarcoma). *, benign and
low-grade tumors. The mixed tumors with both high- and low-grade components
(mixed serous/endometrioid carcinoma) have intermediate values, which could
reflect the relative quantities of the high- and low-grade components in the
sample. The age of each patient corresponding to each tumor is as follows:
tumor no. 3 (leiomyoma), 41 y; tumor no. 4 (leiomyoma), 34 y; tumor
no. 5 (leiomyoma), 43 y; tumor no. 6 (endometrioid), 87 y; tumor no. 7
(endometrioid), 66 y; tumor no. 8 (mixed serous/endometrioid), 65 y; tumor no. 9
(mixed serous/endometrioid), 70 y; tumor no. 10 (mixed serous/endometrioid),
57 y; tumor no. 11 (mixed serous/endometrioid), 70 y; tumor no. 12 (serous),
55 y; tumor no. 13 (serous), 70 y; tumor no. 14 (serous), 58 y; tumor
no. 15 (stromal), 81 y; tumor no. 16 (adenosarcoma), 61 y; tumor no. 17
(leiomyosarcoma), 65 y; tumor no. 18 (leiomyosarcoma), 64 years; tumor
no. 19 (leiomyosarcoma), 61 years. All high-grade, aggressive tumors were
in older, postmenopausal women. Columns, averages from experiments done at
least twice in triplicate; bars, SD. HMGA1a mRNA was normalized to
glycerinaldehyde-3-phosphate dehydrogenase mRNA as a loading control.
B, HMGA1 protein was increased in the high-grade uterine tumors (three of
three), compared with the control, normal uterine tissue, or benign tumor. The
numbers above the lanes correspond to the patient samples, as identified above
in (A). C, HMGA1a mRNA and protein are decreased in the MES-SA cells
transfected with siRNA to HMGA1a (20 μmol/L) compared with the controls
(mock-transfected or the siCONTROL nontargeting siRNA pool at 20 μmol/L).
HMGA1a and control β-actin mRNA (β-actin primer/probe set; Applied
Biosystems) were assessed at 24, 48, 72, and 96 h after adding the siRNA.
HMGA1a mRNA was normalized to β-actin as the loading control. HMGA1
mRNA was decreased at all time points; only the 72-h time point is shown here
(see Supplementary Fig. S1 for mRNA at 24, 48, and 96 h). The Western blot
shows decreased HMGA1 protein in the MES-SA cells incubated with siRNA
to HMGA1 compared with the mock-transfected control or siCONTROL
nontargeting siRNA pool control. Protein was assessed for HMGA1 and β-actin
at 24, 48, 72, and 96 h after transfection with each siRNA. HMGA1 mRNA
was decreased at all time points; only the 72-h time point is shown here (see
Supplementary Data for protein at 24, 48, and 96 h). D, anchorage-independent
cell growth or foci formation are decreased in the high-grade human uterine
tumor sarcoma cells with knock-down in HMGA1 expression. The bar graph shows
the decreased number of foci in the cells incubated with siRNA to HMGA1
compared with the control siRNA. Columns, mean from two experiments done in
triplicate (41% ± 20% versus 100% ± 24%, P = 0.009, Student’s t test); bars, SD.
The photograph shows foci from the siCONTROL nontargeting controls
compared with the siRNA to HMGA1 (bar, 100 μm). Growth curves showed
that cells incubated with each siRNA had similar growth rates, demonstrating that
the decrease in foci formation was not a result of toxicity that impairs cell growth
from the HMGA1 siRNA (see Supplementary Fig. S2 for growth curves).
that this pathway was not activated in this tumor type. The mixed serous/endometrial tumors showed both increased HMGA1a and COX-2 mRNA in one sample (data not shown).

To further substantiate the link between HMGA1a and COX-2 in a different system, we infected human primary foreskin fibroblasts (BJ, CRL-2522) with a lentivirus expressing HMGA1a and green fluorescent protein or control green fluorescent protein vector alone (18, 19). We found that COX-2 mRNA and protein were significantly increased in the fibroblasts overexpressing HMGA1a, but not in the fibroblasts infected with the control vector (Supplementary Fig. S3).

To determine if COX-2 is necessary for HMGA1a-mediated transformation in human uterine cancer cells overexpressing HMGA1a, we repeated the transformation assays in methylcellulose with or without the specific COX-2 inhibitor, NS-398 (17). We found that the inhibiting COX-2 function blocked anchorage-independent cell growth (P = 0.00005, Student’s t test; Fig. 3L). These studies indicate that COX-2 was required for transformation in these high-grade uterine tumor cells that overexpress HMGA1a.

Discussion

Although the association between overexpression of HMGA1 and cancer has been well documented, there is limited evidence showing that it is responsible for malignant transformation in a living organism. We previously showed that the HMGA1a transgenic mice developed lymphoid malignancies (9). Here, we report that 100% of the female mice also developed uterine tumors. Other investigators subsequently showed that overexpression of HMGA1a from a transgene driven by the cytomegalovirus promoter developed natural killer T cell lymphomas in most mice by 24 months (23). The decreased penetrance and later onset of tumors in these mice could reflect the decreased expression of the HMGA1a gene by the CMV promoter. Taken together, these studies indicate that HMGA1a functions as an oncogene in vivo. The similarity of the transgenic tumors to human uterine adenosarcoma shows that this animal model approximates human disease. This is important because there are limited animal models of human uterine cancer and none have a high penetrance (24). Uterine adenosarcomas are uncommon, typically low-grade neoplasms in women, but other uterine cancers,
i.e., endometrial carcinomas, are the most common malignant neoplasms of the female genital tract in women in the U.S., and is a frequent cause of cancer death worldwide (24). To address the relevance of HMGA1a in the pathogenesis of more common types of human uterine cancer, we analyzed its expression in human uterine cancer tissues. Selective significant overexpression of HMGA1a in most high-grade, but not benign or most low-grade uterine neoplasms, suggests that HMGA1a may serve as a useful marker for aggressive uterine cancers and that this mouse model is relevant to study human uterine cancer. A larger, more comprehensive, study of human tumors is needed to confirm these findings. Of note, HMGA1a expression was also previously shown to be increased in cervical cancer (2).

The mechanisms involved in transformation by HMGA1a are only beginning to be elucidated. The gradual onset of tumors >6 to 9 months suggests that more than one genetic event is involved. Because HMGA1 proteins function in regulating transcription, the overexpression of these proteins could promote tumorigenesis by altering the expression of specific target genes involved in regulating cell growth (4, 7). The putative HMGA1a target genes identified to date include genes which function in cell signaling, motility, and inflammation, all of which could participate in neoplastic transformation (4, 7). In this study, we looked at the potential role of COX-2 in mediating transformation by HMGA1a. We found that HMGA1a binds directly to the COX-2 promoter in vivo and up-regulates expression of COX-2 in uterine tumorigenesis, both in mice and humans. Our studies further indicate that COX-2 is necessary for the transformed phenotype in the high-grade, human uterine sarcoma cells. Our studies indicate that this pathway is also activated in primary fibroblasts transfected to overexpress HMGA1a (Supplementary Fig. S3) and in some lymphoid tumors overexpressing HMGA1a.11 Importantly, there are several drugs that block COX-2 function, which could be used therapeutically in aggressive cancers overexpressing both HMGA1a and COX-2. Studies are under way to determine if blocking COX-2 also blocks tumorigenesis in our HMGA1a transgenic mice.

In summary, our studies indicate that HMGA1a, together with COX-2, contribute to the pathogenesis of uterine cancers. Moreover, our HMGA1a transgenic mouse will provide a useful model to elucidate additional mechanisms involved in uterine carcinogenesis.

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The High-Mobility Group A1 Gene Up-Regulates Cyclooxygenase 2 Expression in Uterine Tumorigenesis

Abeba Tesfaye, Francescopaolo Di Cello, Joelle Hillion, et al.


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