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

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

Hematology Division, Departments of Medicine, Pediatrics, Pathology and Gynecology and Obstetrics; 2Molecular and Comparative Pathobiology; and 3Oncology, The Johns Hopkins University School of Medicine, Baltimore, Maryland; 4The University of Texas Southwestern Medical Center, Dallas, Texas; 5Children’s Hospital of Pittsburgh, Pittsburgh, Pennsylvania; 6Washington University, Pullman, Washington; and 3Gynecologic Pathology, Cornell University, New York, New York

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 leiomyosarcomas. Moreover, both HMGA1a and COX-2 are over-expressed in in vivo promoter in human uterine cancer cells and activates its expression in transfection experiments. We also show that blocking either HMGA1a or COX-2 in high-grade human uterine cancer cells blocks anchorage-independent cell growth in methylcellulose. These findings show that HMGA1a functions as an oncogene when overexpressed in the uterus and contributes to the pathogenesis of human uterine cancer by activating COX-2 expression. Although a larger study is needed to confirm these results, HMGA1a may be a useful marker for aggressive human uterine cancers. [Cancer Res 2007;67(9):3998–4004]

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 HMGA1a 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 HM-G1) 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 human HMGA1a were: 5’-GC-TGGTGCCGGATGAGAA and 5’-GGTGACCTTCCGGTCTTG, respectively, and the forward and reverse primers that amplify human HMGA1b were: 5’-AGGAAAAGGCCGCTGAGAA and 5’-CCCGAGGTTCTTCTTGGT, respectively. Forward and reverse primer sequences for murine COX-2 were: 5’-AGTGGCGCAGTGGCTCTCT and 5’-CTTCTGAGTCGCCAGTTCCA, respectively, with probe no. 11 (Roche, Universal Primer Library); forward and reverse primer sequences that amplify human COX-2 were: 5’-GATGAGCCTTGCGAGCTCT and 5’-CGAACGATCCACAGTCCCT.
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× Laemml 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 HMGA1a mRNA and protein expression in uterine tissue of transgenic and control mice. The mRNAs 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 littermates (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 uteri from HMGA1a transgenic mice with 20 copies of the transgene and a control littermate. B, normal mouse uterus (left) compared with uterine adenosarcoma in a transgenic mouse (right) with immunohistochemical staining for HMGA1. C, gross necropsy findings of a typical HMGA1a transgenic mouse.

**Immunohistochemistry.** Immunohistochemistry for HMGA1a was done as described (8). Immunohistochemistry for COX-2 in the mouse tumors was done with the commercial COX-2 antibody (Santa Cruz Biotechnology, CAClone 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).

**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 plated in methylocellulose 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: HMGA1a (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'-TAAGGGGAGAGGGGAAA and 5'-GACGCTCCTGCAAGTCGTA, 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 mmol/L stock solution of NS-398 was prepared in DMSO and mixed with methylocellulose for a final concentration of 5 μmol/L.

**Methylocellulose assays.** Cells (105) were plated in six-well plates in methylocellulose 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 5' end with a myc epitope (18) using the following primers: forward-CGC GCT AGC AGA ATG AGC GAG GTA, respectively. The HMGA1a-myc coding region was sequenced in 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 \textit{HMGA1a} transgenic founders were females and carried 1 to 28 copies of the transgene (9). The transgene was highly expressed in the uteri; both \textit{HMGA1a} mRNA (Fig. 1A) and protein (Fig. 1A and B) levels were increased in the transgenic uteri compared with control uteri.

All \textit{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 \textit{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 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 dilation. 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 \textit{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 \textit{HMGA1a} mice were malignant and transplantable, we injected uterine tumor fragments (100–200 mg) into nude mice (\textit{nu/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.

\textit{HMGA1a} expression in human uterine neoplasms. Over-expression of \textit{HMGA1a} in the uterus resulted in tumors in all female mice. To determine if this mouse model was relevant to human disease, we examined \textit{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>
<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>
</tr>
</thead>
<tbody>
<tr>
<td>I-1</td>
<td>3.25</td>
<td>1</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<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>
<td>+</td>
<td></td>
</tr>
<tr>
<td>III-2</td>
<td>8.50</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>III-3</td>
<td>8.50</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>III-5</td>
<td>7.00</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>Cervical</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-8</td>
<td>8.75</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-9</td>
<td>8.25</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-10</td>
<td>8.75</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-12</td>
<td>10.00</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-14</td>
<td>8.00</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-15</td>
<td>10.25</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-18</td>
<td>10.50</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-20</td>
<td>12.50</td>
<td>20</td>
<td>Decreased</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>III-22</td>
<td>6.25</td>
<td>20</td>
<td>No change</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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 transformation (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 tumorigenesis.** 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 investigated 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 ($P = 0.21$ 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 in 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 serous tumors, suggesting...
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,

Figure 4. HMGA1a directly up-regulates COX-2 in uterine tumorigenesis. A, chromatin immunoprecipitation experiments with sheared chromatin from MES-SA cells after cross-linking proteins bound to DNA with formaldehyde (15). The bar graph shows the quantity of DNA immunoprecipitated with the following antibodies (all from Upstate, excluding the HMGA1a antibody): HMGA1a (16), Polymerase II (Pol II or histone H3—both as positive controls), or rabbit IgG (as a negative control). Additional negative controls included no chromation and no DNA. The HPRT promoter sequence was also used as a negative control because there are no HMGA1 DNA-binding sites in the region amplified, and previous chromatin immunoprecipitation experiments also showed no binding by HMGA1 to the amplified region (16). The gel shows total input DNA compared with DNA immunoprecipitated with the same antibodies. B, COX-2 mRNA and protein are increased in the uteri of the transgenic mice compared with controls. COX-2 mRNA was measured in transgenics ($n = 5$) compared with controls ($n = 5$), all taken in the metestrus stage of the estrous cycle in mice at 2 mo of age. The metestrous cycle was identified by vaginal swabs because this stage has the lowest infiltration of polymorphonuclear or other inflammatory cells, and inflammatory cell infiltrates could alter the baseline uterine tissue COX-2 expression. Columns, mean from two separate experiments done in triplicate; bars, SD. COX-2 protein levels were increased in the uteri of the transgenic mice (right) compared with controls (bar, 10 μm). C, COX-2 mRNA and protein are increased in the human leiomyosarcoma tumors. COX-2 mRNA from leiomyosarcomas ($n = 3$) was compared with benign leiomyomas ($n = 3$) or pooled, normal uteri. Columns, mean from two separate experiments done in triplicate; bars, SD. Using immunohistochemical staining, COX-2 protein is increased in human leiomyosarcoma compared with leiomyoma or normal uterine myometrium. D, blocking COX-2 function with the NS-398–specific COX-2 inhibitor (5 μmol/L) resulted in decreased anchorage-independent cell growth and foci formation in the human uterine sarcoma MES-SA cells. The bar graph shows the decrease in the percentage of foci formation from two separate experiments done in triplicate (100% = 11% versus 38% ± 20%; $P = 0.00005$, Student’s $t$ test) and foci from MES-SA cells with the NS-398 inhibitor or control vehicle (bar, 100 μm). Growth curves of MES-SA cells with or without NS-398 displayed similar growth rates, indicating that the NS-398 at 5 μmol/L concentrations was not toxic to cell growth at the concentrations used (see Supplementary Fig. S2 for growth curves).
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. 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.

Acknowledgments

Received 5/17/2005; revised 2/24/2007; accepted 3/20/2007.

Grant support: The American Cancer Society Scholar Award 5244 (J. Hillion). NIH PO1 CA87657 (L.M.S. Resar), NIH R01 CA092339, Leukemia & Lymphoma Scholar Award 1694-06, and R21SG108797R (L.M.S. Resar), NIH T32 CA60441 (partial support for O. Elbahloul and A. Tesfaye), and the Gynecologic Cancer Foundation Susan A. Olde Endometrial Cancer Research Grant (J. Hillion).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Susan MacDonald and Don Coffey for insightful discussions, encouragement, and advice. We also thank the Johns Hopkins Transgenic Core Facility. The Gynecology Tumor Procurement Bank provided the uterine tumor samples. The COX-2 promoter construct was a kind gift from Ray DuBois. Lastly, we would like to acknowledge the numerous authors of articles we could not reference secondary to space limitations.

11 F. Di Cello and L.M.S. Resar, unpublished data.

References


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

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


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/67/9/3998

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2008/01/21/67.9.3998.DC1

Cited articles
This article cites 23 articles, 9 of which you can access for free at:
http://cancerres.aacrjournals.org/content/67/9/3998.full.html#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
/content/67/9/3998.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.