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The *High Mobility Group A1* (*HMGA1*) gene is highly overexpressed in human uterine serous carcinomas and carcinosarcomas and drives *Matrix Metalloproteinase-2* (*MMP-2*) in a subset of tumors☆

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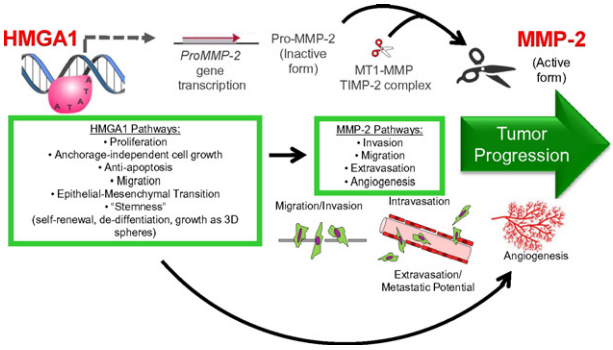
HIGHLIGHTS

- Deficiency of *Mmp-2* impairs uterine tumorigenesis in *Hmga1* transgenic mice
- *HMGA1* is overexpressed in aggressive human uterine carcinosarcomas and serous carcinomas
- *HMGA1* and *MMP-2* are positively correlated in a subset of human carcinosarcomas
- *HMGA1* occupies the *MMP-2* promoter in human carcinosarcoma cells
- Targeting the *HMGA1* pathways could disrupt progression of aggressive uterine tumors

GRAPHICAL ABSTRACT



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ABSTRACT

Objectives. Although uterine cancer is the fourth most common cause for cancer death in women worldwide, the molecular underpinnings of tumor progression remain poorly understood. The *High Mobility Group A1* (*HMGA1*) gene is overexpressed in aggressive cancers and high levels portend adverse outcomes in diverse tumors. We previously reported that *Hmga1a* transgenic mice develop uterine tumors with complete penetrance. Because *HMGA1* drives tumor progression by inducing *Matrix Metalloproteinase* (*MMP*) and other genes involved in invasion, we explored the *HMGA1-MMP-2* pathway in uterine cancer.

Abbreviations: HMGA1a, High Mobility Group A1 gene; *MMP-2, Matrix Metalloproteinase-2*; RT-PCR, real time polymerase chain reaction.

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Methods. To investigate *MMP-2* in uterine tumors driven by *HMGA1*, we used a genetic approach with mouse models. Next, we assessed *HMGA1* and *MMP-2* expression in primary human uterine tumors, including low-grade carcinomas (endometrial endometrioid) and more aggressive tumors (endometrial serous carcinomas, uterine carcinosarcomas/malignant mesodermal mixed tumors).

Results. Here, we report for the first time that uterine tumor growth is impaired in *Hmga1a* transgenic mice crossed on to an *Mmp-2* deficient background. In human tumors, we discovered that *HMGA1* is highest in aggressive carcinosarcomas and serous carcinomas, with lower levels in the more indolent endometrioid carcinomas. Moreover, *HMGA1* and *MMP-2* were positively correlated, but only in a subset of carcinosarcomas. *HMGA1* also occupies the *MMP-2* promoter in human carcinosarcoma cells.

Conclusions. Together, our studies define a novel *HMGA1-MMP-2* pathway involved in a subset of human carcinosarcomas and tumor progression in murine models. Our work also suggests that targeting *HMGA1* could be effective adjuvant therapy for more aggressive uterine cancers and provides compelling data for further preclinical studies.

1. Introduction

Uterine cancer is the most frequently diagnosed gynecologic malignancy in the United States and the fourth most common cancer in American women. In addition, it is the sixth most common cancer in women worldwide [1–3]. Moreover, the incidence of uterine cancer has been increasing over the last two decades, possibly due to increasing obesity rates, and the death rate for uterine corpus cancer has been rising since 2000 [2–3]. The five year survival rate for women with distant metastases is <20% reflecting the limited treatment options available [1–3]. Thus, further study has the potential to significantly benefit women's health. Carcinomas are the most frequent type of uterine cancers, with endometrioid carcinomas being the most common subtype. The majority of endometrioid carcinomas are low-grade, with indolent behavior in most cases [1–2]. They are generally treated with surgery alone and have favorable outcomes. In contrast, uterine serous carcinomas are less common and are, by definition, high-grade, with generally aggressive behavior even when present at lower stages. Uterine sarcomas are relatively uncommon, comprising only ~5% of all uterine cancers. They occur in older women, behave aggressively, and are often associated with poor outcomes. Carcinosarcomas are high-grade uterine cancers with both malignant epithelial (carcinomatous) and mesenchymal (sarcomatous) components. The 5-year survival for carcinosarcomas is 24–50% for all stages [3]. Adenosarcomas are even less common, but have significantly better survival rates than carcinosarcomas [3]. Despite the high overall prevalence of uterine cancers, the molecular events that lead to the distinct subtypes are poorly understood.

The *High Mobility Group A1* (*HMGA1*) gene is overexpressed in diverse, refractory tumors [4–31], including uterine carcinomas and sarcomas [4–5]. The *HMGA1* gene encodes the HMGA1a and HMGA1b chromatin remodeling proteins, which function in modulating gene expression [6,30–31]. HMGA1 proteins are members of the HMGA family of AT-hook DNA binding proteins that consists of HMGA1a, HMGA1b, and HMGA2 [30–32]. *HMGA1* is enriched in aggressive cancers and embryonic stem cells [4–31,33–34]. In a previously published pilot study of 19 primary tumors, we found that *HMGA1* is overexpressed in high-grade uterine cancers, but not in normal uterine tissue, benign tumors, or most low-grade neoplasms of the uterus [4]. We also discovered that *Hmga1a* transgenic mice develop aggressive lymphoid tumors and uterine sarcomas by 9 months of age with complete penetrance [4,10]. Together, these findings highlight a central role for *HMGA1* in diverse high-grade tumors.

Matrix metalloproteinases (MMPs) are a family of over 20 zinc-dependent proteinases important to the homeostasis of the extracellular matrix [35–40]. They were originally characterized based on their ability to degrade the extracellular matrix and basement membrane, which facilitates tumor cell invasion, migration, intravasation into the circulation, extravasation out of the bloodstream, and ultimately metastasis. In some tumors, MMP activity correlates with cellular invasiveness and metastatic potential [35–36,40]. More recently, MMPs were shown to exert other important biologic effects relevant to cancer including the processing of critical proteins involved in angiogenesis, apoptosis, chemotaxis, cell migration, and cell proliferation [35–39]. Surprisingly, tumor suppressor functions have also been identified for *MMP* family members [37,40]. We previously found that HMGA1 up-regulates expression of *MMP-2* in lung cancer cells, but only in poorly differentiated tumors, indicating that this pathway could drive tumor progression and anaplasia in a subset of poorly differentiated, stem-like lung cancers [23]. HMGA1 also up-regulates expression of *MMP-2* in prostate cancer [11]. In addition, HMGA1 induces *MMP-9* in pancreatic cancer [35] and *MMP-13* in breast cancer tumor models [30], suggesting that the *HMGA1-MMP* axis is important in diverse human cancers.

Here, we discovered that the *Hmga1-Mmp-2* pathway is important in uterine tumorigenesis using a genetic approach in mice. In primary human tumor samples, we also found that *HMGA1* is up-regulated in most tumors, with highest levels in the more aggressive, high-grade carcinosarcomas and serous tumors. We also found that the *HMGA1-MMP-2* pathway was up-regulated, in a subset of carcinosarcomas. These findings indicate that *HMGA1* could serve as a therapeutic target in aggressive uterine carcinomas and sarcomas. Although further studies are needed, our findings also suggest that the *HMGA1-MMP-2* pathway may be a rational target for cancer therapy in a subset of carcinosarcomas, thus highlighting the role for personalized therapy in these aggressive tumors.

2. Materials and methods

2.1. Primary tumor samples, RNA preparation and quantification, and quantitative RT-PCR

A total of 76 primary uterine tumor samples (29 endometrioid carcinomas, 30 carcinosarcomas and 17 serous carcinomas) were obtained from de-identified patient samples. Sufficient RNA was generated from 24 endometrioid carcinomas, 23 carcinosarcomas, and 14 serous carcinomas using Trizol as we previously described [4–5]. From the RNA, cDNA was prepared, and *HMGA1a* and *MMP-2* mRNA levels were assessed by quantitative RT-PCR (qRT-PCR) as we previously described [4–5].

To expand our sample size of primary uterine tumors, we also compared gene expression in the Cancer Genome Atlas (TCGA) database from uterine corpus and endometrial carcinoma (UCEC), which included 546 primary uterine tumors and 35 normal uterine tissue controls for expression of *HMGA1* and *MMP-2*. TCGA UCEC expression data, version 2015_11_01, was downloaded using the Broad Institute's firehose application (https://gdac.broadinstitute.org/). Clinical data, including histology, was downloaded from the TCGA data portal (https://tcga-data.nci.nih.gov./tcga/).