

## The High Mobility Group A1 Gene: Transforming Inflammatory Signals into Cancer?

Linda M.S. Resar

### Abstract

*High mobility group A1 (HMGA1)* is highly expressed during embryogenesis and in poorly differentiated cancers, and high levels portend a poor prognosis in some tumors. *HMGA1* induces oncogenic transformation in cultured cells and causes aggressive cancers in transgenic mice, whereas blocking it interferes with transformation in experimental models. These findings suggest a pivotal role for *HMGA1* in cancer. This review focuses on two recently described *HMGA1* transcriptional targets that mediate inflammatory signals and drive malignant transformation because they could serve as biomarkers or therapeutic targets. Further elucidation of *HMGA1* function in transformation promises to have a major impact on our war on cancer. *Cancer Res*; 70(2): 436–9. ©2010 AACR.

### Introduction

The high mobility group A1 (*HMGA1*, formerly *HMG-I/Y*) proteins participate in diverse, fundamental cellular processes, including transcriptional regulation, embryogenesis, transformation, cell cycle regulation, differentiation, senescence, viral integration, and DNA repair (1–22). They are members of a superfamily of nonhistone chromatin-binding proteins whose name reflects their rapid electrophoretic mobility in polyacrylamide gels (high mobility group). All HMG proteins share an acidic carboxyl terminus and associate with chromatin, but are distinguished by unique functional motifs that confer distinct DNA binding domains and biologic activities (1–3). The AT-hook DNA binding motif defines the *HMGA* family, which consists of *HMGA1* and *HMGA2*. Here, we focus on the *HMGA1* subfamily, comprised of *HMGA1a* and *HMGA1b* protein isoforms. These isoforms result from alternatively spliced mRNA and differ by 11 internal amino acids present only in *HMGA1a* (4). Three AT-hook domains mediate binding to the minor groove of chromosomal DNA at AT-rich regions, which enables *HMGA1* to recruit additional transcription factors and alter chromatin structure as an essential component of a higher order transcriptional complex or “enhanceosome” (3). In concert with other factors, *HMGA1* modulates gene expression. Although first described in the context of the interferon- $\beta$  promoter, the enhanceosome function likely applies to many promoters. *HMGA1* proteins also displace histones and relieve histone-

mediated repression of transcription, thereby globally facilitating transcription (5). Because *HMGA1* proteins induce conformational changes and alter gene expression, they are called “architectural transcription factors.” Although their role in transcription is well established, the repertoire of genes targeted by *HMGA1* is only beginning to emerge.

The first evidence linking *HMGA1* proteins to cancer was their discovery in cervical carcinoma cells 25 years ago (1). Subsequent studies showed high *HMGA1* expression in rapidly dividing cells, neoplastic cells (1–18), and during embryogenesis (6), but not in differentiated, adult tissues. Their evolutionary conservation and widespread occurrence in development suggested an important biologic function. *HMGA1* is induced in quiescent fibroblasts by serum or growth factors and displays delayed-early response kinetics (7). Increasing evidence shows that *HMGA1* is widely overexpressed in aggressive malignancies arising from diverse tissues of different embryonic origins. Although the list is expanding, these include cancers of the thyroid, head and neck, colon, lung, breast, pancreas, hematopoietic system, cervix, uterine corpus, prostate, and central nervous system. Moreover, large scale gene expression studies show that high expression portends a poor prognosis in some tumors (8, 18). *HMGA1* is also enriched in embryonic stem cells and high-grade (poorly differentiated) cancers, including breast, bladder, and brain cancers (18). Tissue microarray studies also show that increasing protein levels correlate with poor differentiation status and more advanced disease in diverse cancers. These findings indicate that *HMGA1* could be a useful biomarker and therapeutic target for high-grade cancers and suggest that *HMGA1* orchestrates the molecular underpinnings of a more primitive, poorly differentiated state, both in cancer and development.

### HMGA1 Function in Cancer

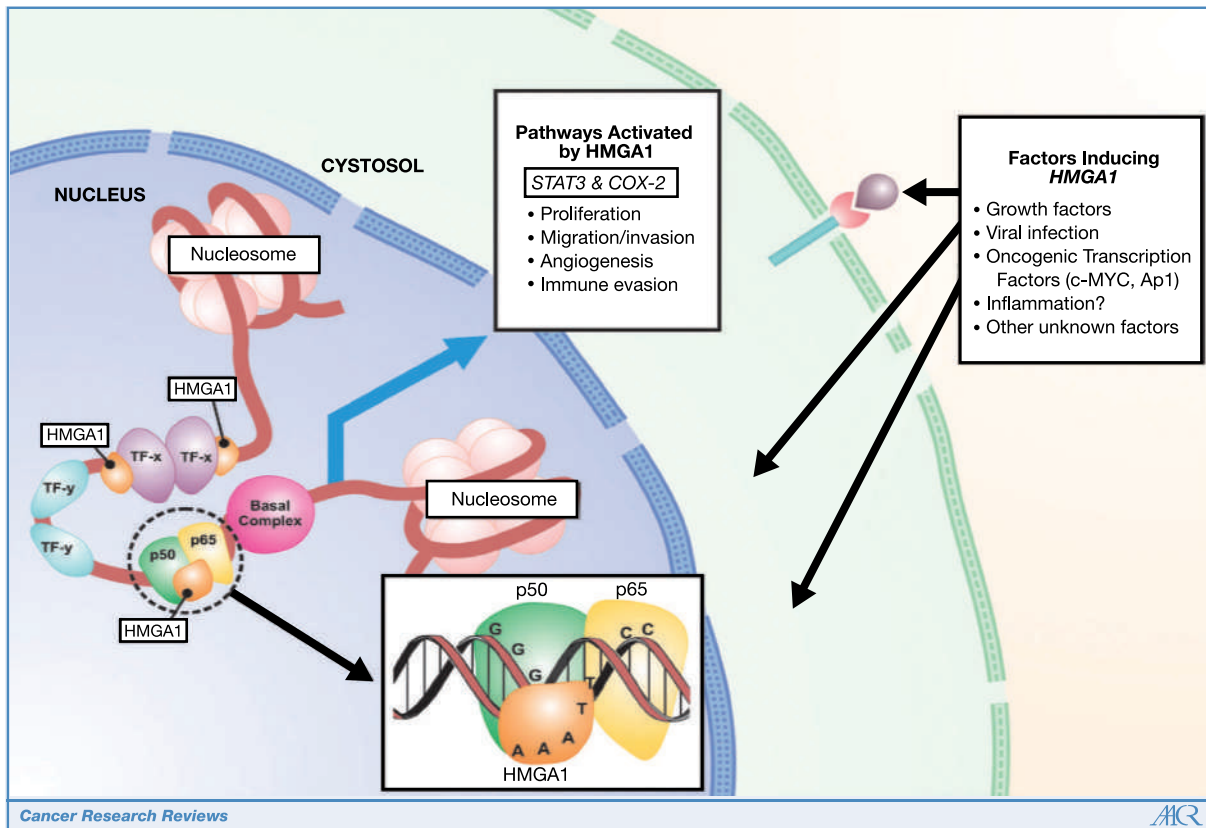
Following the discovery that *HMGA1a* is linked to cancer, investigators began to look for its function in transformation.

**Author's Affiliation:** The Johns Hopkins University School of Medicine, Baltimore, Maryland

**Corresponding Author:** Linda M.S. Resar, The Johns Hopkins University School of Medicine, Ross Research Building, Room 1025, 720 Rutland Avenue, Baltimore, MD 21205-2109. Phone: 410-614-0712; Fax: 410-955-0185; E-mail: lresar@jhmi.edu.

doi: 10.1158/0008-5472.CAN-09-1212

©2010 American Association for Cancer Research.



**Figure 1.** Induction of *HMGA1* results in binding to the minor groove at AT-rich regions in chromatin and recruitment of transcriptional regulators. NF- $\kappa$ B (p65/p50) and other transcription factors (TF-x, TF-y) are shown, which lead to the formation of the enhanceosome and activation of oncogenic pathways.

Our laboratory provided early evidence that *HMGA1* contributes directly to neoplastic transformation (9–11). Forced overexpression of *HMGA1a* or *HMGA1b* induces a transformed phenotype with anchorage-independent cell growth and xenograft tumorigenesis in immortalized cells. *HMGA1a* is also directly activated by the c-Myc proto-oncoprotein and up-regulated in Burkitt's lymphoma, an aggressive tumor with constitutive *c-myc* expression. Moreover, inhibiting *HMGA1* expression in Burkitt's and other cancer cells blocks anchorage-independent cell growth and proliferation. Knock-down of *HMGA1* also causes apoptosis in lung, breast, and thyroid cancer cells, indicating its central role in diverse malignancies (12). In MCF-7 breast cancer cells, *HMGA1b* induces an epithelial-to-mesenchymal transition (13), further linking these proteins to a primitive, de-differentiated state in cancer.

The subsequent development of mouse models misexpressing *HMGA1* provided the most compelling evidence that *HMGA1* causes cancer. As reported here 5 years ago, *HMGA1a* transgenic mice succumb to aggressive T-cell lymphoid malignancy by 2 to 10 months (11). The females also develop uterine sarcomas (15). In this model, murine *HMGA1a* is driven by the H-2K promoter and immunoglobulin  $\mu$  enhancer with expression in major histocompatibility complex class I-expressing cells and B cells (11). Another group reported a transgenic

model with murine *HMGA1b* driven by a CMV promoter, and these mice develop natural killer lymphomas and pituitary adenomas (14). These tumors occur later and with a decreased penetrance compared with the *HMGA1a* mouse model. The variation in phenotypes could relate to the different isoforms expressed or to different levels of tissue-specific transgene expression. More recently, *HMGA1* knock-out mice were made and both homozygous and heterozygous null mice develop a decrease in T cells with a relative increase in B cells, erythroid cells, and myeloid cells, and a phenotype described as lymphomyeloproliferative disease (19). The proliferative disease progresses to frank malignancy in some cases, which led to the speculation that *HMGA1* has tumor suppressor activity. The hematologic findings also resemble lymphoproliferative disease seen in humans with T-cell immunosuppression, suggesting that T-cell deficiency is the cause of the hematologic abnormalities.

Despite recent progress, the molecular mechanisms that mediate *HMGA1* function are only beginning to emerge. Its diverse activities have been attributed to its role as a chromatin remodeling transcription factor, which could lead to induction of different molecular pathways depending on the cellular context. Accordingly, promoter analyses and gene expression profile studies have uncovered downstream gene targets and an

Downloaded from <http://aacrjournals.org/cancerres/article-pdf/70/2/436/2637971436.pdf> by guest on 13 September 2024

HMGA1 “oncogenic transcriptome” is emerging. These studies suggest that HMGA1 orchestrates diverse cellular processes through modulating gene expression, including pathways involved in inflammation, proliferation, transformation, metastatic progression, angiogenesis, and DNA repair (Fig. 1). Although only about 50 HMGA1 transcriptional targets have been reported, most include nuclear factor- $\kappa$ B (NF- $\kappa$ B) regulatory elements in their promoter regions, and many participate in mediating inflammatory pathways. Given the increasing evidence that inflammation is a precursor lesion in some cancers, the link between HMGA1 and NF- $\kappa$ B suggests that these proteins cooperate to induce inflammatory signals and drive transformation. Here, we highlight two recently identified gene targets related to inflammation and oncogenesis because their functions could be blocked in cancer therapy.

### HMGA1a in Cancer: New Insights

**Cyclooxygenase-2.** Cyclooxygenase-2 (*COX-2*) was originally identified as a putative HMGA1 target gene in hypoxic vascular endothelial cells (20). Under low oxygen tension, HMGA1 binds to an AT-rich region near an NF- $\kappa$ B binding site in the *COX-2* promoter *in vitro* and up-regulates *COX-2* expression in transfection experiments. We also found that HMGA1 activates *COX-2* expression during tumorigenesis (15, 16). Uterine sarcomas from the *HMGA1a* transgenics have increased *COX-2* expression, and treatment with sulindac (a COX-1/COX-2 inhibitor) inhibits tumor growth (15). Both *HMGA1* and *COX-2* are also overexpressed in leiomyosarcomas, a highly lethal, human uterine sarcoma. Sulindac or celecoxib (a more selective COX-2 inhibitor) results in blockade of anchorage-independent cell growth and xenograft tumorigenesis in human uterine sarcoma cells, but only in cells with high *HMGA1a* levels (16). This finding indicates that targeting COX-2 is effective in selected tumors with dysregulation of the HMGA1-COX-2 pathway. *COX-2* is induced by inflammatory signals, and, like *HMGA1*, is thought to elicit a number of cellular pathways involved in neoplastic transformation, including proliferation, angiogenesis, metastasis, and inhibition of apoptosis. It is not clear if hypoxia is a prerequisite for *COX-2* induction by HMGA1, and further studies are needed to elucidate the role of this pathway in other cancers.

**Signal transducer and activator of transcription 3.** Recent studies also uncovered signal transducer and activator of transcription 3 (*STAT3*) as a critical downstream target of HMGA1 (17). *STAT3* is up-regulated in cells and transgenic mice engineered to overexpress *HMGA1a*. Similar to the *COX-2* promoter, HMGA1 binds to an AT-rich region near an NF- $\kappa$ B binding site and activates *STAT3* expression. HMGA1 occupies this promoter in cultured cells from hematopoietic malignancies. Moreover, a *STAT3* small molecule inhibitor induces apoptosis in leukemic cells from *HMGA1* transgenics, but not in normal lymphoid cells, indicating that this pathway could be a rational therapeutic target in some malignancies. In ongoing studies, we are testing additional *STAT3* small molecule inhibitors in our transgenic models

*in vivo*, and preliminary data show decreases in the tumor burdens.<sup>1</sup> The HMGA1-*STAT3* pathway is of interest because *STAT3* is a key mediator of inflammatory signals and molecular pathways that contribute to cancer initiation and progression, including proliferation, angiogenesis, metastatic progression, survival, and immune evasion.

### Future Directions

Although recent studies have identified downstream gene targets, it is likely that these results provide only a glimpse of HMGA1 transcriptional networks in cancer (Fig. 1). A global investigation of both mRNAs and microRNAs should lead to the discovery of rational therapeutic targets relevant to diverse cancers. Interestingly, both *COX-2* and *STAT3* are important transducers of inflammatory signals with NF- $\kappa$ B regulatory elements in their promoter regions near the HMGA1 binding sites. The presence of NF- $\kappa$ B sites in HMGA1 transcriptional targets suggests that these proteins function together as reported in the regulation of *interferon- $\beta$*  expression. Although not directly proven, HMGA1, together with NF- $\kappa$ B and other transcription factors, could form an enhanceosome that constitutes a major “hub” activated by inflammation. This hub, in turn, could induce cellular pathways that lead to cancer initiation and progression. In some settings, overexpression of *HMGA1* could activate inflammatory pathways and circumvent the need for inflammation to promote oncogenic transformation. Our preliminary data from gene expression analysis support the role of HMGA1 in inducing multiple inflammatory pathways in transformation (Resar L.M.S. Unpublished data). Further dissection of these pathways will reveal how HMGA1 promotes transformation and tumor progression, and could identify rational interventions to treat, or even prevent, cancer. Our studies have focused on pathways downstream of HMGA1 because the ability to target nuclear oncogenes therapeutically is only emerging. Additional studies to identify molecular circuitry induced by HMGA1 as well as functional analysis in preclinical mouse models are needed before these findings can be translated to the clinic.

Although it is clear that growth factor signaling, oncogenic transcription factors, viruses, hypoxia, and inflammatory signals induce *HMGA1* expression, the molecular mechanisms mediating *HMGA1* overexpression in most tumors is unknown. Amplification has not been detected to date. Epigenetic changes or microRNA alterations could play a role and studies to uncover the molecular mechanisms inducing *HMGA1* should advance our ability to block its activity in cancer therapy. Recent studies also implicate *HMGA1* in maintaining “stemness” and understanding its role in this setting should elucidate therapeutic targets relevant to cancer and cancer stem cells (18). Emerging evidence also indicate that HMGA proteins participate in cellular senescence

<sup>1</sup> Resar L.M.S. Unpublished data.

(21), and this activity could potentially be exploited in therapy. Finally, HMGA1 activity in DNA repair could play a central role in transformation and is worthy of further study (22). It is likely that there are, as yet, undiscovered HMGA1 cellular activities that will provide insight for novel therapeutic approaches in cancer, given their importance in embryogenesis and cancer. Further investigation of HMGA1 proteins in development and malignant transformation promises to bolster our war on cancer—clearly a war worth waging.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### References

- Lund T, Holtman J, Frederiksen M, Laland SG. On the presence of two new high mobility group-like proteins in HeLa S3 cells. *FEBS Lett* 1983;152:163–7.
- Bustin M. Regulation of DNA-dependent activities by the functional motifs of the high-obility-group chromosomal proteins. *Mol Cell Biol* 1999;19:5237–46.
- Du W, Thanos D, Maniatis T. Mechanisms of transcriptional synergism between distinct virus-inducible enhancer elements. *Cell* 1993;74:887–98.
- Johnson KR, Lehn DA, Elton TS, Reeves R. Complete murine cDNA sequence, genomic structure, and tissue expression of the high mobility group protein HMG-I(Y). *J Biol Chem* 1988;263:18338–42.
- Zhao K, Kas E, Gonzalez E, Laemmli UK. SAR-dependent mobilization of histone H1 by HMG-I/Y *in vivo*: HMG-I/Y is enriched in H1-depleted chromatin. *EMBO J* 1993;12:3237–47.
- Chiappetta G, Avantiaggiato V, Visconti R, et al. High level expression of the HMGI(Y) gene during embryonic development. *Oncogene* 1996;13:2439–46.
- Lanahan A, Williams JB, Sanders LK, Nathans D. Growth-factor induced delayed early response genes. *Mol Cell Biol* 1992;12:3919–29.
- Pomeroy SL, Tamayo P, Gaasenbeek M, et al. Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature* 2002;415:436–42.
- Wood LJ, Mukherjee M, Dolde CE, et al. HMG-I/Y: A new c-Myc target gene and potential oncogene. *Mol Cell Biol* 2000;20:5490–2.
- Wood LJ, Maher JF, Bunton TE, Resar LMS. The oncogenic properties of the HMG-I gene family. *Cancer Res* 2000;60:4256–61.
- Xu Y, Felder TS, Bhattacharya R, et al. The HMG-I oncogene causes highly penetrant, metastatic lymphoid malignancy in transgenic mice and is overexpressed in human lymphoid malignancy. *Cancer Res* 2004;64:3371–5.
- Scala S, Portella G, Fedele M, Chiappetta G, Fusco A. Adenovirus-mediated suppression of HMGI(Y) protein synthesis as potential therapy of human malignant neoplasias. *Proc Natl Acad Sci U S A* 2000;97:4256–61.
- Reeves R, Ederg DD, Li Y. Architectural transcription factor HMGI(Y) promotes tumor progression and mesenchymal transition of human epithelial cells. *Mol Cell Biol* 2001;21:575–94.
- Fedele M, Pentimalli F, Baldassarre G, et al. Transgenic mice overexpressing the wild-type form of the HMGA1 gene develop mixed growth hormone/prolactin cell pituitary adenomas and natural killer cell lymphomas. *Cancer Res* 2005;24:3427–35.
- Tesfaye A, Di Cello F, Hillion J, et al. HMGA1a Up-regulates Cox-2 in uterine tumorigenesis. *Cancer Res* 2007;67:3998–4004.
- Di Cello F, Hillion J, Aderinto A, et al. COX-2 inhibitors block uterine tumorigenesis in HMGA1a transgenic mice and human uterine cancer xenografts. *Mol Cancer Ther* 2008;7:2090–5.
- Hillion J, Dhara S, Sumter TF, et al. The HMGA1a-STAT3 axis: an “Achilles heel” for acute leukemia? *Cancer Res* 2008;68:10121–7.
- Ben-Porath I, Thomson MW, Carey VJ, et al. An embryonic stem cell-like signature in poorly differentiated aggressive tumors. *Nat Genet* 2008;40:499–507.
- Fedele M, Fidanza V, Battista S, et al. Haploinsufficiency of the Hmga1 gene causes cardiac hypertrophy and myelo-lymphoproliferative disorders in mice. *Cancer Res* 2006;66:2536–43.
- Ji Y-S, Xu Q, Schmedtje JF. Hypoxia induces high mobility-group protein I(Y) and transcription of cycooxygenase vascular endothelium. *Circ Res* 1998;83:295–304.
- Narita M, Narita M, Krizhanovsky V, et al. A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. *Cell* 2006;126:503–14.
- Adair JE, Maloney SC, Dement GA, Wertzler KJ, Smerdon MJ, Reeves R. High-mobility group A1 proteins inhibit expression of nucleotide excision repair factor xeroderma pigmentosum group A. *Cancer Res* 2007;67:6044–52.

### Acknowledgments

This work is dedicated to Dr. Donald Coffey, the consummate teacher, mentor, and scientist.

### Grant Support

Grants R01 CA092339, R03 CA139331, R21 CA18343, and P50 CA096888 from the National Institutes of Health, the Leukemia & Lymphoma Scholar Award, Alex's Lemonade Stand Foundation Innovation Award, and the J.P. McCarthy Foundation.

Received 4/6/09; revised 10/2/09; accepted 10/5/09; published OnlineFirst 1/12/10.