Loss of the Nucleosome-Binding Protein HMGN1 Affects the Rate of N-Nitrosodiethylamine-Induced Hepatocarcinogenesis in Mice

Yuri V. Postnikov, Takashi Furusawa, Diana C. Haines, Valentina M. Factor, and Michael Bustin

Abstract
We report that HMGN1, a nucleosome-binding protein that affects chromatin structure and function, affects the growth of N-nitrosodiethylamine (DEN)-induced liver tumors. Following a single DEN injection at 2 weeks of age, Hmgn1+/+/+ mice, lacking the nucleosome-binding domain of HMGN1, had earlier signs of liver tumorigenesis than their Hmgn1−/+ littermates. Detailed gene expression profiling revealed significant differences between DEN-injected and control saline-injected mice, but only minor differences between the injected Hmgn1+/−/+ mice and their Hmgn1−/+ littermates. Pathway analysis revealed that the most significant process affected by loss of HMGN1 involves the lipid/sterol metabolic pathway. Our study indicates that in mice, loss of HMGN1 leads to transcription changes that accelerate the progression of DEN-induced hepatocarcinogenesis, without affecting the type of tumors or the final total tumor burden of these mice.

Implications: Loss of HMGN1 leads to accelerated progression of DEN-induced hepatocarcinogenesis in mice.

Introduction
The dynamic properties of the chromatin fiber play a key role in transcriptional regulation and in the establishment and maintenance of epigenetic marks. Disruption of these processes can alter gene expression, potentially leading to various diseases including cancer. Indeed, increasing evidence links misexpression of tumor suppressors and oncogenes, and dysregulation of DNA repair processes, to alterations in chromatin structure and to changes in epigenetic marks such as covalent modification in DNA and histones (1–3). Factors that affect epigenetic processes in the genome have been shown to contribute to the progression of cancer (4) and epigenetic drugs targeting chromatin regulators show promise as anticancer agents (5). Because any factor that affects epigenetic processes could potentially contribute to tumorigenicity, it is important to examine the role of various chromatin modifiers in the etiology of cancer. Here, we investigate the potential role of the chromatin-binding protein high mobility group N1 (HMGN1) in hepatocarcinogenesis.

HMGN1 is a member of the high mobility group N (HMGN) family of chromosomal proteins. HMGN proteins are ubiquitously present in the nuclei of vertebrate cells and bind specifically to nucleosome core particles, the building blocks of the chromatin fiber (6). The chromatin residence time of all HMGNs is short; these proteins continuously roam throughout the nucleus and reside only transiently on a specific site (7). Although HMGNs can interact with all nucleosomes, their genome-wide organization is not random. Chromatin immunoprecipitation studies revealed that HMGN1 preferentially binds to gene promoters and enhancers where they tend to colocalize with DNase I hypersensitive sites, a hallmark of chromatin regulatory sites (8).

The binding of HMGN1 to nucleosomes induces structural changes in chromatin and alters the levels of posttranslational modifications of core histones raising the possibility that HMGN1 affects epigenetic regulatory processes. Significantly, the effects of HMGN1 on chromatin structure and function are contingent on the ability of the protein to bind to nucleosomes. HMGN1 mutants that do not bind to nucleosomes do not affect chromatin structure or histone modifications (9–13). We have generated the mice lacking functional HMGN1 (Hmgn1−/−/+ mice, formerly named Hmgn1−/−), that has been considered as Hmgn1 knockout mice, although the mice express heavily truncated HMGN1 that does not localize to nucleus. Studies with cells and tissues derived from genetically altered HMGN1 mice, which express HMGN1 mutant that cannot bind to nucleosomes, revealed that loss of HMGN1 function alters the cellular transcription profile (14, 15) and impairs the ability to mount a proper response to cellular stress. Hmgn1−/−
mice and cells are hypersensitive to heat shock and their ability to repair DNA damaged by either UV or ionizing radiation is impaired (16–19). Faulty repair of damaged DNA could lead to genomic instability and increased tumorigenicity. Taken together, the available information suggests that loss of HMGN1 may increase the susceptibility to tumorigenesis, a possibility that has not yet been fully examined.

Here, we examine the potential role of HMGN1 in carcinogenesis, by comparing the progression of N-nitrosodiethyamine (DEN)-induced hepatocarcinogenesis (20) in Hmgn1tm1/tm1 mice and Hmgn1+/+ littermates. DEN is a chemical carcinogen that has been extensively used for analysis of factors that affect liver cancer development, one of the most frequent human cancers (21). Young male mice are particularly susceptible to this carcinogen, as a single injection can result in hepatocellular carcinoma that is similar to that seen in humans (22). We find that loss of HMGN1 accelerates the development of liver cancers in DEN-injected mice. Transcription analysis of livers from these mice links loss of HMGN1 to alterations in several pathways including the sterol/cholesterol/lipid metabolic pathways including the sterol/cholesterol/lipid metabolic pathways.

### Materials and Methods

#### Animal studies

Hmgn1tm1tm1 (previously named Hmgn1<sup>−/−</sup>) and control Hmgn1<sup>+/+</sup> mice were generated and genotyped as described (16). In these mice, exons II, III, and IV of the Hmgn1 gene, which code for the nucleosome-binding domain of the protein, have been excised. For genotyping, tail DNA was extracted using REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich) using three primers (see Supplementary Table S1). Since female mice are less sensitive than males to DEN-induced carcinogenes, only male homozygous mice were used for the experiments. Mice received a single intraperitoneal injection of 10 μg/g body weight of N-nitrosodiethylamine (Sigma-Aldrich, Inc., Cat. 40334), or saline as a control, at 14 days of age. Mice were sacrificed at 23, 48, and 73 weeks after the injection. Animals were housed at the NCI Animal Facility and NCI-Frederick SAIC facility and cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

#### Necropsy and histopathology

All livers were harvested at necropsy, weighed, photographed, and thoroughly examined. The number of macroscopic nodules/masses ≥ 1 mm was recorded for each liver. Livers were then fixed in 10% neutral buffered formalin, routinely processed to paraffin block, and sectioned at 5 μm. Hematoxylin and eosin (H&E)-stained sections were evaluated microscopically for quantification of foci, adenomas, and carcinomas. The areas occupied by foci and neoplasmas were measured using ImageJ software (NIH, Bethesda, MD).

### Protein isolation and Western blot analysis

Liver caudate lobes were homogenized by Dounce light homogenizer in 1× PBS. The cell suspensions were washed in 1× PBS and centrifuged at 600 × g for 10 minutes. The cellular pellet was dissolved in either 0.2 mol/L sulfuric acid or 5% perchloric acid, both containing a protease inhibitor cocktail (Roche), homogenized by Dounce tight pestle homogenizer for 2 minutes, kept on ice for 5 minutes, and spun at 3,000 × g for 10 minutes. The supernatant was made 25% in TCA, incubated on ice for 15 minutes, and centrifuged at 3,000 × g for 20 minutes. The pellet was stored at −20°C overnight in 100% ethanol, air-dried, and resuspended with 50 to 100 μL of water. The preparations were reprecipitated by HCl/acetone, washed in 100% acetone, air-dried, and resuspended with 50 to 100 μL of water. Proteins were resolved on 15% Tris-glycine-SDS gels, transferred to polyvinylidene difluoride membrane, and subjected to Western blotting.

#### Immunohistochemistry staining

Immunohistochemical staining was carried out on formalin-fixed, paraffin-embedded tissue using the avidin-biotin-peroxidase complex method (Vector Laboratories) and 3'-diaminobenzidine (DAB) for staining. Proliferating cell nuclear antigen antibody (PCNA; Santa Cruz Biotechnology, sc-25280) and rabbit anti-Ki67 antibody (Abcam, ab15580) were used according to the manufacturer’s recommendation. Sections were counter-stained with hematoxylin.

#### RNA isolation

Total RNA was isolated from frozen liver tissue with TRIzol reagent (Invitrogen) followed by purification using RNeasy Kit (Qiagen) according to the manufacturer’s instructions. Reverse transcription of total RNA (2.0 μg) into first strand cDNA using oligo(dT) primers (SuperScript First Strand Synthesis System for RT-PCR; Invitrogen) has been followed by PCR using Platinum PCR SuperMix (Invitrogen) and the specific primers (see Supplementary Table S1). PCR products were resolved on 2% agarose gels and visualized by ethidium bromide staining.

#### Microarray analysis

Expression profiling was conducted for six groups of mice. Group A consisted of mice at 4 weeks of age. Two other groups consisted of 12-week-old mice injected at the age of 4 weeks either with saline (group B) or DEN (group C). Each group includes both genotypes (Hmgn1<sup>+/+</sup> and Hmgn1tm1tm1). For microarray analysis, total RNA was isolated as described above. The cDNA synthesis, fluorescent labeling of the samples, and mouse 430.2 Affymetrix expression array hybridizations were done following the suppliers’ recommendations. Arrays were scanned with Agilent scanner adjusted to achieve optimal signal intensity at both channels with <1% saturated spots and normalized to the 50th percentile of the median signal intensity. Normalized intensity values were used for further analysis.

Mouse Genome 430 2.0 array has 45,101 probe sets associated with approximately 20,000 Mouse Genome...
Molecular Cancer Research

Postnikov et al.

Informatics (MGI) gene identifiers. Probe sets were mapped to MGI identifiers using information provided by the Jackson Laboratory (http://www.informatics.jax.org/). GeneSpring GX software package (Agilent Technologies) was employed to evaluate the quality of the arrays and for analysis.

Differentially expressed genes were selected using univariate two-sample t test ($P \leq 0.01$) with a random variance model, using mean value for each of six groups (A-Hmgn1$^{+/+}$, A-Hmgn1$^{tm1/tm1}$, B-Hmgn1$^{+/+}$, B-Hmgn1$^{tm1/tm1}$, C-Hmgn1$^{+/+}$, C-Hmgn1$^{tm1/tm1}$). Unsupervised and supervised cluster analyses were done with GeneSpring GX analysis suite (version 11.5.1, Agilent Technologies, Cat. G3784AA). Functional GO and other data mining tools for sign

Quantitative real-time PCR

Selected gene expression microarray data were confirmed by quantitative real-time PCR (qRT-PCR) using Applied Biosystems 7900 HT. Sequences of primers used for qRT-PCR analyses are provided in Supplementary Table S1. GAPDH and actin primers were used for normalization of expression levels. qRT-PCR was performed using Power SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Cat. No 4391178) and iScript One-Step RT-PCR Kit with SYBR Green RNA-to-CT 1-Step Kit (Ap

Results

HMGN1 affects the latency of DEN-induced hepatocarcinogenesis

To test whether loss of HMGN1 function affects the course of DEN-induced hepatocarcinogenesis, 2-week-old male Hmgn1$^{+/+}$ and Hmgn1$^{tm1/tm1}$ littermate mice received a single intraperitoneal injection of N-nitrosodiethylamine at a dose of 10 µg/g body weight. Saline injected mice served as controls. Mice were sacrificed at ages of 25, 50, and 75 weeks and their livers examined for tumor incidence, size, multiplicity, and histologic appearance. Western blot analysis of nuclear extracts from these livers indicated that during the course of the experiment the expression of HMGN1, or of its closely related protein HMGN2, did not change and that in Hmgn1$^{tm1/tm1}$ mice, which lack nuclear HMGN1, there were no compensatory adjustment in the levels of HMGN2 (Fig. 1).

As expected, the mice injected with DEN developed tumors, whereas the saline injected mice did not. We followed the development of the tumors by sacrificing mice at ages of 25, 50, and 75 weeks after DEN injection. At 23 weeks after DEN administration, the total number of preneoplastic foci observed in Hmgn1$^{tm1/tm1}$ mice was not significantly different from that observed in their Hmgn1$^{+/+}$ littermates; however, the size of the foci was significantly larger in Hmgn1$^{tm1/tm1}$ mice (Fig. 2A). Thus, in Hmgn1$^{+/+}$ mice, 43% of the foci were smaller than 50 µm² and only 2% were larger than 500 µm², whereas in Hmgn1$^{tm1/tm1}$ mice, less than 20% of the foci were smaller than 50 µm² and almost 30% of the foci were larger than 500 µm² (Fig. 2A). Although the foci in Hmgn1$^{tm1/tm1}$ mice were larger, the rate of hepatocyte proliferation in the surrounding liver, as measured by mouse antigen Ki67 (Mki67) and PCNA immunostaining did not differ between the mice (Fig. 3A). Likewise, although qRT-PCR analyses reveal the expected upregulation of cyclin D1 (Ccnd1), Mki67, and Muc, there were no significant differences between the DEN-injected Hmgn1$^{+/+}$ and Hmgn1$^{tm1/tm1}$ littermate mice (Fig. 3B). Comparative evaluation of H&E-stained sections from the liver samples at 23 weeks after injection indicated that in both Hmgn1$^{+/+}$ and Hmgn1$^{tm1/tm1}$ mice, the early preneoplastic lesions displayed a predominant basophilic cell phenotype characteristic of the DEN-induced carcino genesis (Supplementary Fig. S1).

In contrast, at 48 weeks post-DEN, both the number and size of preneoplastic and neoplastic lesions in Hmgn1$^{tm1/tm1}$ mice were considerably larger than in Hmgn1$^{+/+}$ mice (Fig. 2B and C). The increased tumor frequency and size correlated with a higher liver-to-body weight ratio, reflecting the apparent differences in the growth rate of preneoplastic and neoplastic hepatic lesions between knockout and control mice (Fig. 2D). However, at 75 weeks of age, no significant difference between Hmgn1$^{+/+}$ and Hmgn1$^{tm1/tm1}$ mice could be detected in any of the parameters of tumor growth examined, including liver-to-body weight ratios, tumor burden, and malignancy (Fig. 2C and D). At this stage, the tumors were mostly hepatocellular adenomas and carcinomas and the type of tumors observed was essentially the same in both wild-type and Hmgn1$^{tm1/tm1}$ mice (Fig. 2E). Thus, loss of HMGN1 enhances the rate of DEN-induced hepatocarcinogenesis but does not alter the type of tumors formed.
Effects of HMGN1 on the global gene expression pattern in the livers of DEN-injected mice

To gain insights into the mechanisms whereby HMGN1 enhances the rate of tumorigenicity in the livers of DEN-injected mice, we examined the transcription profile in the livers of control and DEN-injected Hmgn1+/+ and Hmgn1tm1/tm1 littermate mice. Since changes in gene expression would precede the appearance of preneoplastic foci and tumors, we examined the hepatic expression profiles in the following 3 groups, each consisting of 6 mice. The first group A which included 4-week-old untreated Hmgn1+/+ and Hmgn1tm1/tm1 littermates served to test whether loss of HMGN1 affects the liver transcription profile before any treatment. The group B contained 12-week-old Hmgn1+/+ and Hmgn1tm1/tm1 littermates that were injected with saline at 4 weeks of age. This group served as controls for group C, which consisted of 12-week-old Hmgn1+/+ and Hmgn1tm1/tm1 littermates that were injected with DEN at 4 weeks of age. The raw data for all 18 samples can be found at GEO dataset # GSE44356.

Principal component analysis (PCA) of the transcriptomes of all livers revealed a clear separation between the 3 groups (Fig. 4A). The distinct clustering of group A from group B reflects the age-related changes in gene expression, whereas the distinct clustering of B from C reflects the DEN-induced changes in gene expression. Within each group, the Hmgn1+/+ mice formed a subcluster that was separate from their Hmgn1tm1/tm1 littermates, the largest difference between these subclusters was observed in the youngest mice (group A). Supervised comparison based on asymptotic
Hierarchical clustering defines groups of genes associated with DEN-induced altered expression patterns

Hierarchical condition tree of unsupervised clustering analysis of the differentially expressed genes (n = 520; FC ≥ 2, P ≤ 0.01) sorted groups A, B, and C (top of the heatmap) apart, without separating two genotypes in each group (Fig. 5A). Using a threshold distance of 0.25 on the y-axis dendrogram, the genes were classified into 8 major clusters, each representing a specific gene expression pattern (green boxes C1–C8, Fig. 3A). The overall pattern of clustering revealed that the differences between groups A, B, and C were larger than the differences between the $Hmgn1^{+/+}$ and $Hmgn1^{tm1/tm1}$ within each group, a further indication that loss of HMGN1 does not have a major impact on the expression levels of a specific subset of genes. Nevertheless, clusters C2, C3, C7, and C8 contained the subsets of genes affected by the loss of HMGN1. Thus, in $Hmgn1^{tm1/tm1}$ mice, the genes in clusters C2, C3, and part of C4 were downregulated, the genes in clusters C7 and C8 were upregulated. The differences between groups B and C were the most prominent in clusters C4, C5, and C6; these genes may be involved in the liver response to DEN injection.

A search for the genes linked to hyperproliferation, dysplasia, and other cancer-related genes in the various groups and clusters yielded JunB. This was the only cancer-related gene which was overexpressed in $Hmgn1^{tm1/tm1}$ mice at 4 weeks of age but not following DEN administration at 12 weeks, making it unlikely to contribute to the difference in tumor development at the later stages. Likewise, of the 65 genes associated with high risk of poor prognosis for hepatocellular carcinoma (23) only the Tbx3 transcription factor was differentially expressed in $Hmgn1^{tm1/tm1}$ livers, but its expression was downregulated. These results further support the notion that HMGN1 does not specifically regulate the expression of genes involved in liver carcinogenesis.

Functional analysis of all 520 differentially expressed genes identified the sterol/cholesterol/lipid metabolic process pathway-associated genes as the most altered ($P ≤ 4.09E-05$; Supplementary Table S3). Similar analysis of the 8 gene clusters identified by unsupervised clustering analysis (Fig. 5A) revealed that the sterol/cholesterol/lipid metabolic process pathway-associated genes were the most altered ($P ≤ 4.09E-06$).
process pathway-associated genes were most significantly over-represented ($P/C20 \geq 3.14E-12$) in cluster C2 (Fig. 5B). This cluster contained several genes belonging to this pathway including Fasn, Cyp7a1, Mvd, and Thrsp (Fig. 5B). In addition, in cluster C6, the presence of transcription factor activity pathway-oriented genes GO:0006366 is highly enriched ($P/C20 \geq 1.23E-8$). This cluster contained genes that were upregulated by DEN treatment and differed between Hmgn1$^{+/+}$ and Hmgn1tm1/tm1 littermates (Fig. 5B) and could contribute to earlier signs of DEN-induced hepatocarcinogenesis which we observed in Hmgn1tm1/tm1 mice.

Discussion

In the present study, we show that loss of HMGN1 protein in mice increases the rate of liver tumorigenesis after DEN treatment. In Hmgn1$^{+/+}$ mice, preneoplastic lesions seen at 23 weeks after DEN administration were larger than in the Hmgn1tm1/tm1 littersmates. Likewise, the number of foci and the average size of tumors seen was significantly increased in the 50-week-old mice lacking functional HMGN1 protein as compared with wild-type mice. However, at 73 weeks after DEN administration, the size and number of tumors, and their histologic appearance were similar in both Hmgn1$^{+/+}$ and Hmgn1tm1/tm1 mice. Thus, loss of HMGN1 increased the growth rate, but not the type of tumors or the tumor burden.

The results are compatible with previous findings of increased tumor incidence in Hmgn1tm1/tm1 mice suggesting that loss of HMGN1 predisposes to increased tumor susceptibility (17). Embryonic fibroblasts from Hmgn1tm1/tm1 mice were shown to proliferate faster than cells prepared from their Hmgn1$^{+/+}$ littersmates and SV40-immortalized Hmgn1tm1/tm1 cells induced more tumors than SV40-transformed Hmgn1$^{+/+}$ cells when injected into nude mice (17). In addition, HMGN1 increased the recruitment rate of PCNA to UV-damaged DNA sites (24) and loss of HMGN1 impaired the ability of Hmgn1tm1/tm1 cells to repair DNA damaged by either UV or ionizing irradiation (16, 17, 25), thereby predisposing Hmgn1tm1/tm1 mice to increased genomic instability and tumorigenicity (17, 26). In agreement with these findings (17, 26), the cancer initiation was
notably faster in Hmgn1tm1/tm1 mice treated with DEN. However, at the later stages, the differences in DEN-induced tumor development between Hmgn1+/+ and Hmgn1tm1/tm1 mice became less pronounced suggesting the differential requirements for HMGN1 function during multistep process of chemical hepatocarcinogenesis. As we have shown in the past, several proto-oncogenes, such as JunB and c-Jun, have been downregulated in the Hmgn1tm1/tm1 fibroblasts (9). Thus, HMGN1 regulates the transcription of multiple genes which may have opposing effects on the rate of tumor growth at different stages of tumor development.

Our analysis of the liver transcription profiles did not reveal significant effect of HMGN1 deletion on the expression levels of DNA damage repair factors suggesting that the accelerated hepatocarcinogenesis in Hmgn1tm1/tm1 is not due to faulty expression of these factors. In agreement with previous analyses of a variety of tissues including liver, loss of HMGN disrupted the expression of multiple genes, but only mildly, suggesting that HMGN1 fine tunes the fidelity of the cellular transcription profile (14, 15). DEN injection did not significantly increase the transcription differences between Hmgn1+/+ and Hmgn1tm1/tm1, a finding that is fully compatible with the similarity in the number and types of tumors seen in the mice. Thus, loss of HMGN1 does not fundamentally alter the cellular response to DEN.

GO analysis identified the sterol/lipid metabolic pathways as the most different category of genes between DEN-injected Hmgn1+/+ and Hmgn1tm1/tm1 mice. Emerging evidence links altered sterol/lipid metabolic profile to chronic low-grade systemic inflammation (27), which is believed to contribute to metabolic disorders, and the stagewise progression to hepatic steatosis, fibrosis, cirrhosis, and finally to carcinoma (28–30). Hepatocellular carcinoma has been also linked to nonalcoholic fatty liver disease (31). As an architectural element of chromatin, HMGN1 protein is capable to change expression profile of the cell globally, affecting several pathways simultaneously (14, 15).

Table 1. Top 10 genes, differentially expressed in livers of Hmgn1+/+ and Hmgn1tm1/tm1 mice

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<th>Gene symbol</th>
<th>Group A</th>
<th>Expression log2 ratio, Hmgn1+/+ vs. Hmgn1tm1/tm1</th>
<th>Group B</th>
<th>Expression log2 ratio, Hmgn1+/+ vs. Hmgn1tm1/tm1</th>
<th>Group C</th>
<th>Expression log2 ratio, Hmgn1+/+ vs. Hmgn1tm1/tm1</th>
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In the table, the Hmgn1tm1/tm1 genotype is indicated as Hmgn1+/−.

Mol Cancer Res; 12(1) January 2014 Molecular Cancer Research
Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Study supervision: Y. Postnikov, M. Bustin

Acknowledgments
The authors thank the NIH Library Writing Center, Bethesda, MD for editorial help.

Grant Support
The research was supported by the Center for Cancer Research, The Intramural Research Program at the NIH, National Cancer Institute, and with funds from the National Cancer Institute, NIH, under Contract No. HHSN261200800001E.

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Received July 25, 2013; revised November 6, 2013; accepted November 17, 2013; published OnlineFirst December 2, 2013.

References

HMGN1 Affects Hepatocarcinogenesis in Mice

Figure 5. Functional classification of the differentially expressed genes in livers of Hmg1 +/+ and Hmg1 tm1/tm1 mice before and after DEN or saline injection. A, heatmap describing the clustering of the expression profile of the differentially expressed genes (n = 620; FC ≥ 2, P ≤ 0.01). The conditional tree on top of the heatmap, which sorted the genes into groups A, B, and C, was generated by unsupervised clustering analysis. Groups C1 to C8 were generated using a threshold distance of 0.25 on the y-axis dendrogram. B, network analysis of genes in the two most affected GO pathways in the livers of Hmg1 -/- mice. Shown are the networks of the genes in the lipid metabolism pathway (cluster 2 of Fig. 5A) and in transcription factors pathway (cluster 6 of Fig. 5A) generated by GePS pathway analysis (Genomatix Software GmbH).
Bustin M. Chromatin unfolding and activation by HMGN1.


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