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Transgenic Mice Expressing a Truncated Form of the High Mobility Group I-C Protein Develop Adiposity and an Abnormally High Prevalence of Lipomas*

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Chromosomal translocations in human lipomas frequently create fusion transcripts encoding high mobility group (HMG) I-C DNA-binding domains and C-terminal sequences from different presumed transcription factors, suggesting a potential role for HMG I-C in the development of lipomas. To evaluate the role of the HMG I-C component, the three DNA-binding domains of HMG I-C have now been expressed in transgenic mice. Despite the ubiquitous expression of the truncated HMG I-C protein, the transgenic mice develop a selective abundance of fat tissue early in life, show marked adipose tissue inflammation, and have an abnormally high incidence of lipomas. These findings demonstrate that the DNA-binding domains of HMG I-C, in the absence of a C-terminal fusion partner, are sufficient to perturb adipogenesis and predispose to lipomas. We provide data supporting the central utility of this animal model as a tool to understand the molecular mechanisms underlying the development of one of the most common kind of human benign tumors.

The high mobility group (HMG)[1] I family of proteins consists of three low molecular weight proteins, which are preferentially associated with active chromatin (1). Two of the members, HMG I and HMG Y are encoded by one gene, with the isoforms resulting from alternative splicing (2). The third protein, HMG I-C, is encoded by a unique gene (3, 4). All three proteins are highly homologous, interacting with A/T-rich stretches of DNA via three DNA-binding domains (termed A-T hooks), which wrap around the minor groove of the double helix (5–8). The interaction of HMG I proteins with the minor groove has been shown to alter the conformation of target DNA, either bending straight DNA or reversing intrinsic bends at the site of interaction (9, 10). The proteins also have a conserved acidic tail, which appears to control sequence specificity of DNA binding; removal of this tail results in high affinity, promiscuous binding of the truncated protein to DNA (11).

In addition to their role in chromatin structure, HMG I proteins appear to be important in eukaryotic gene regulation. HMG I proteins are essential for inducible expression of several genes including the interferon-β, the HLA-DRA class II major histocompatibility complex, and E-selectin genes (12–15). HMG I proteins could participate in gene activation by inducing conformational changes in a *cis*-element (16) through interaction with a transcription factor (8, 12, 17, 18), by bridging two neighboring factors (19, 20), or by displacing histone H1 from nucleosomes (21, 22).

A role of HMG I proteins in facilitating inducible gene expression is compatible with the developmental and temporal expression of the HMG I proteins. All three proteins are preferentially expressed during embryonic and fetal stages of development, when dynamic changes in gene expression occur. The HMG I-C gene is completely silent, and HMG I(Y) expression is either absent or 200-fold reduced in adult tissues (23–25). Indeed, mice with both HMG I-C alleles disrupted exhibit the *pygmy* phenotype (small size and drastic reduction of body fat content), providing *in vivo* evidence for a role for the protein and its target genes in embryonic cell growth or differentiation (23). Expression of the HMG I(Y) proteins is also transiently induced during lymphocyte activation or inflammatory responses, when genes of the immune system are expressed *de novo* (26, 27).

Circumstantial evidence supports the view that alterations in HMG I protein expression may contribute to abnormal cell growth. First, expression of both the HMG I(Y) and I-C genes is frequently derepressed in a variety of tumor tissues (28–30). Second, reduction of HMG I-C expression in a panel of thyroid tumors cell lines was shown to decrease the *in vitro* growth rate and *in vivo* tumorigenicity of these cells (31, 32). Third, ectopic expression of truncated HMG I-C in NIH 3T3 cells allows these cells to grow in reduced serum and without contact inhibition (33). Finally, chromosomal rearrangements within the HMG I-C and I(Y) genes have been observed in several benign tumor types, especially in mesenchymal tumors and particularly in lipomas. In many of these tumors, aberrant transcripts containing exons 1–3 of HMG I-C (which encode the three DNA-

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This work is dedicated to Jack L. Strominger and Tom Maniatis.

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[1] The abbreviations used are: HMG, high mobility group; AP, anchored oligo(dT) primer(s); ARP, arbitrary primer; CAP, Cbl-associated protein.

binding domains) have been found fused to sequences from other genes, such as LIM domains or activation domains from known transactivators (34–40). Expression of a truncated HMG I-C protein (lacking its C-terminal domain) or the inappropriate expression of fusion partners (or both) could therefore have a role in tumorigenesis.

To understand the role of HMG I-C in the etiology of such tumors, we have generated a transgenic mouse model where a truncated form of the HMG I-C protein is expressed in most somatic cells. The protein expressed contains all of the N-terminal sequences of the human HMG I-C protein, including the three DNA-binding domains, but lacks any amino acid sequence downstream of the third DNA-binding domain.

EXPERIMENTAL PROCEDURES

Transgenic Construct—The H-2K^b-HMG I-C (1–3) expression vector (see Fig. 1*A*) was constructed by inserting the first 249 nucleotides of the human HMG I-C cDNA downstream of the H-2K^b promoter and upstream of an in frame stop codon. H-2K^b polyadenylation sequences are cloned downstream of the stop codon. Specifically, an *Nru*I-*Bsa*BI 4-kilobase fragment was removed from a 9.5-kilobase *Sal*I-*Sal*I DNA fragment containing the wild type H-2K^b gene, deleting all of the coding region of the H-2K^b gene, and an *Eco*RI site was created at the site of deletion. A fragment containing 16 nucleotides from the mouse HMG I-C 5′-untranslated domain and 249 bases spanning the first three exons of the human HMG I-C cDNA was subsequently cloned into the *Eco*RI site. A stop codon was created after nucleotide 249 of the HMGI-C coding sequence.

Generation of Transgenic Mice—The 6-kilobase DNA fragment containing the transgene was purified by sucrose gradient centrifugation and was injected into the male pronuclei of single-cell stage fertilized eggs from superovulated FVB/N females (41).

Screening of Transgenic Mice and Expression Analysis—Transgenic mice were identified by Southern blot analysis. Tail DNAs (20 μg) were digested with *Eco*RI and fractionated on 0.8% agarose gels. Southern blots were prepared with Hybond N+ nylon membranes (Amersham Pharmacia Biotech) and hybridized with a ^{32}P-labeled probe spanning the first three exons of the human HMG I-C cDNA. Expression of the transgene was confirmed by Western blot analysis. HMG I proteins and histone H1 were extracted by homogenization in a 5% perchloric acid solution and precipitated with acetone (28). Approximately 5 μg of protein extract was electrophoresed in a 12% polyacrylamide-SDS gel and transferred to polyvinylidene difluoride filters (Amersham Pharmacia Biotech) using a Bio-Rad Semi-dry trans-blot system. Recombinant HMG Y and HMG I-C proteins were used as controls. The filter was incubated with a polyclonal antibody against the N-terminal region of HMG I-C, with no cross-reactivity with HMG Y. The Western light kit (Tropix) was used for signal detection.

Pathological Analysis—All transgenic and control mice were monitored on a daily basis for any evidence of physical abnormalities. Early observations indicated that the HMG I-C transgenic mice were larger than nontransgenic littermates, prompting us to weigh subsequent mice at the indicated time points. Mice were sacrificed for autopsy at 10–12 months of age. Animals were anesthetized prior to sacrifice by cardiac puncture, and the entire mouse fixed in Bouin's medium (Fisher). Incisions were made in the skull and abdomen to ensure that the fixative bathed all tissues. An exhaustive histologic analysis of control and transgenic mice was then performed to detect not only lipomas but any other evidence of abnormal tissue architecture.

Fluorescent Differential Display Analysis—Differential display experiments were carried out using the FluoroDD kit (Beckman Coulters, Inc.). In brief, equal amounts of total RNA from samples to be compared (fat tissue from a nontransgenic mouse, fat tissue from a HMG I-C transgenic littermate, and lipoma tissue from a HMG I-C transgenic mouse) were reverse-transcribed using a two-base anchored oligo(dT) primers (AP) to synthesize first strand cDNA. Use of the two bases at the 3′ end of AP subdivides the mRNA pool into 12 fractions. This subset of first strand cDNA was subjected to polymerase chain reaction amplification using a FluoroDD-tagged version of the original APs with a second (unlabeled) upstream arbitrary primer (ARP), which contains a 10-base primer sequence. Twenty ARPs were used to cover the entire mouse genome. 17-nucleotide (T7 promoter) and 16-nucleotide (M13 reverse) sequences were incorporated into AP and ARP, respectively, for subsequent reamplification and direct sequencing of excised differential display bands. After gel electrophoresis (Genomyx LR) and image scanning (Genomyx SC), the expression profile of the three RNAs were compared, and bands specific to each starting RNA were excised from the gel and stored in TE buffer at −20 °C. The differential display bands were reamplified with a full-length T7 promoter 22-mer (5′-GTAATACGACTCACTATAGGGC-3′) and a M13 reverse (-48) 24-mer (5′-AGCGGATAACAATTTCACACAGGA-3′) for 25 cycles. The amplified DNA was purified and sequenced using the M13 reverse primer to obtain the maximum sequence from the 5′ end. Sequences were compared with the GenBank™ data base using the BLAST software (National Center for Biotechnology Information).

RESULTS

Generation and Screening of Transgenic Mice—To assess whether the truncated HMG I-C protein affects normal cell growth and contributes to the genesis of lipomas, we expressed the protein in most somatic cells of the mice using a well characterized class I major histocompatibility complex promoter/enhancer combination (42, 43). The class I major histocompatibility complex promoter/enhancer is transcriptionally active in virtually every cell type, and it is not considered a strong control element. As indicated under "Experimental Procedures," a complementary DNA fragment containing the first 249 nucleotides of the human HMG I-C mRNA and encoding the N-terminal portion of the protein (including the three DNA-binding domains) was generated by polymerase chain reaction and sequence confirmed. This fragment was then subcloned downstream of the H-2K^b promoter/enhancer as shown in Fig. 1*A*. Purified transgene was microinjected into the male pronuclei of FVB/N oocytes and transferred into pseudo-pregnant recipient mice.

Four independent founders were identified by Southern blot analysis of tail-derived DNA. Fig. 1*B* illustrates a typical Southern blot showing the variable copy numbers of the transgene detected in the different founder mice. Although founder mice 1-c and 1-d did not breed, founder mice 1-a and 1-f produced litters that were viable into adulthood and fertile. The comparison of the phenotypes observed in lines 1-a and 1-f thus allowed us to exclude the probability of artifacts because of the site of integration of the transgene into the genome. The fairly ubiquitous expression of the truncated HMG I-C polypeptide was confirmed by Western blot analysis using HMG I-C-specific polyclonal antiserum (Fig. 1*C*). Although a few tissues (mesentery, heart, testes, and brain) expressed little or no detectable transgene-directed protein, the vast majority of tissues did express the truncated HMG I-C polypeptide. Most tissues did not express the endogenous HMG I-C gene. This is the first demonstration that HMG I-C truncated transcripts give rise to stable proteins, because all other studies have analyzed either genomic DNA or mRNA extracted from tumor tissues or cell lines.

Overabundance of Adipose Tissue in Transgenic Mice—Because HMG I-C-deficient mice exhibit a *pygmy* phenotype (they are small and lean because of decreases in adipose tissue) and because aberrant transcripts encoding a truncated HMG I-C polypeptide are observed in human lipomas, there was an *a priori* reason to suspect that our transgenic mice would present with effects on adipocyte growth. We therefore measured the body weight of both male and female transgenic and nontransgenic mice. At least five mice were weighed for each age category, and a Student's t test was used to confirm that the observed differences were statistically significant. p values are included in the legend of Fig. 2. Both male and female transgenic mice weighed approximately 15–28% more than nontransgenic control mice, up to 3 months of age (Fig. 2). All transgenic mice exhibited this phenotype relative to nontransgenic controls. The increased body weight presents early in mouse development and becomes less significant as the mice age. Gross examination and histologic analysis of transgenic and nontransgenic mice indicated that the increased body

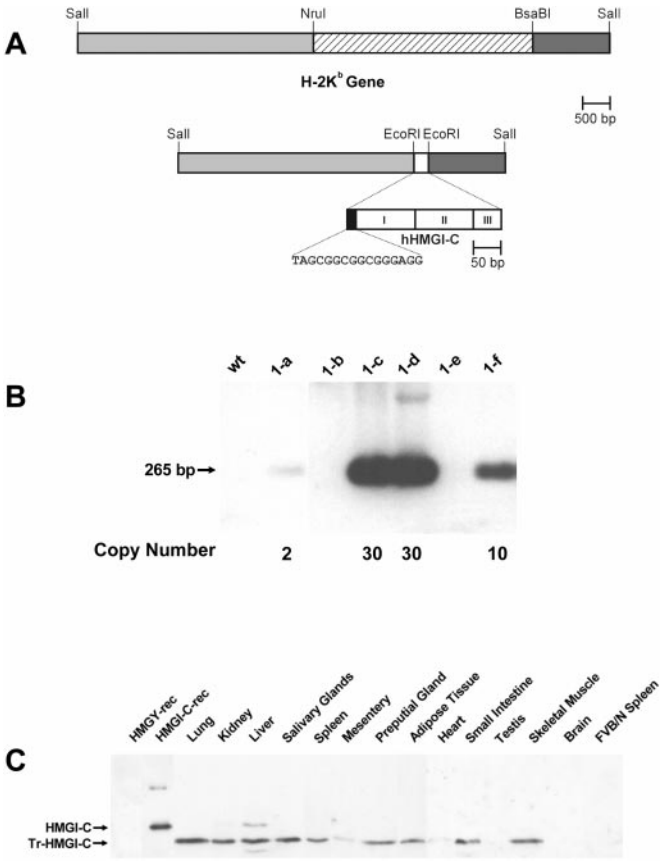

FIG. 1. **Schematic of the H-2K^b-HMG I-C (1–3) transgene and analysis of its expression pattern in the transgenic mice.** *A*, the class I promoter/enhancer fragment *Sal*I-NruI is shown in *light gray*. The *Nru*I-*Bsa*BI fragment from the class I gene containing all coding exons is shown with *hatching*. The class I polyadenylation and splice sequence is *shaded dark gray*. The *Nru*I-*Bsa*BI portion of the gene was removed, and a generated *Eco*RI cloning site used to insert the truncated HMG I-C cDNA fragment generated by polymerase chain reaction. The exact nucleotide sequence of the upstream sequence of the HMG I-C insert and a schematic showing the entire first three exons that encode the three A-T hooks are shown in the bottom construct. A stop codon was also inserted at the end of the HMG I-C coding region. The figure is to scale as indicated by the 500-base pair and 50-base pair rulers. *B*, Southern blot analysis of transgenic mice from the F_0 generation. A band at 265 base pairs corresponding to the HMG I-C portion of the transgene is shown. Four founder mice (designated as 1-a, 1-c, 1-d, and 1-f) are identified. The estimated number of copies of the transgene in each founder mouse is indicated beneath each lane. *C*, Western blot analysis. PCA protein extracts from different tissues of a representative transgenic mouse (from founder line 1-f) and from the spleen of a nontransgenic mouse are analyzed by Western blot. Bands corresponding to the full-length and truncated HMG I-C polypeptides are indicated as HMG I-C and Tr-HMG I-C, respectively. HMG I-C rec and HMG Y rec correspond to recombinant full-length, high pressure liquid chromatography purified HMG I-C and HMG Y, respectively.

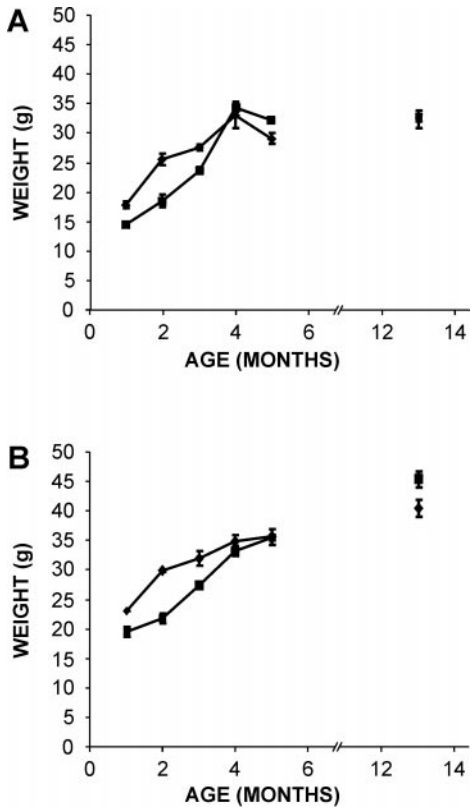

FIG. 2. **Analysis of body weights of transgenic and control nontransgenic mice.** Transgenic mice exhibit enhanced body weight in the first three months of life. Age- and sex-matched nontransgenic (*squares*) and transgenic (*diamonds*) mice are compared. *A*, female mice. *B*, male mice. A minimum of five mice were weighed for each category of age. A Student's *t* test was used to assess the statistical significance of the differences observed in body weights between transgenic and wild type animals. The comparison of wild type and transgenic female mice produced *p* values of 0.001066, 0.001791, and 0.001078 at 1, 2, and 3 months, respectively. Similarly, the comparison of transgenic and wild type male mice produced statistically significant differences in their body weights up to 3 months of age, with *p* values of 0.010202, 2.4316^{-7}, and 0.015792 at 1, 2, and 3 months, respectively. *p* values >0.05 resulted from the comparison of mice at 4, 5, and 13 months of age, thus no significant differences were observed at these ages. Animals from both founder lines 1-a and 1-f were weighed. No data points were obtained between 5 and 13 months of age.

weight of the transgenic mice is a consequence of enhanced adipogenesis. No significant increase in muscle mass or skeletal development was observed in the transgenic mice.

Although an increase in body weight was observed only in young animals (up to 3 months of age), at least 24% of the older animals (10–12 months of age) still showed overabundance of fat at many anatomic sites. The most dramatic examples were found at the hilus of the kidney and at the base of the heart. Fig. 3*A* shows a typical cross-section of a nontransgenic heart. A corresponding section from an age- and sex-matched transgenic mouse is shown in Fig. 3*B*. Note the substantial increase in adipose tissue at the base of the transgenic heart. Fig. 3*C* shows a cross-section view of a nontransgenic kidney. The portion of the kidney surrounding the hilus is shown. Note the lack of significant adipose tissue in this normal animal. In marked contrast, Fig. 3*D* shows substantially larger amounts of adipose tissue in the same location of an HMG I-C transgenic animal. A careful histologic analysis of over 33 transgenic mice indicated that most of the mice exhibited an abundance of fat at some location in the body. 24% percent of the mice had a large fat pad either at the hilus of the kidney or in the mediastinum surrounding the base of the heart (Table I).

Development of Lipomas—Although the abundance of fat observed in the transgenic mice is consistent with a role for the HMG I-C protein in adipogenesis, the general morphology of the fat tissue in these situations was normal. However, approximately 21% of the transgenic mice exhibited histologic abnormalities of the adipose tissue, suggesting that the transgene also predisposes adipose tissue for lipomagenesis. Seven of the transgenic mice developed large lipomas (Fig. 4), whereas no lipomas were observed in any of the nontransgenic controls. In our extensive work with the FVB/N strain of mouse, we have never observed lipomas in more than 1,000 mice analyzed, and to our knowledge, no evidence of lipoma development in aging FVB/N wild type mice has been reported in the literature (44). Although lipomas are very common tumors in humans, they very rarely occur as spontaneous lesions in mice, thus further

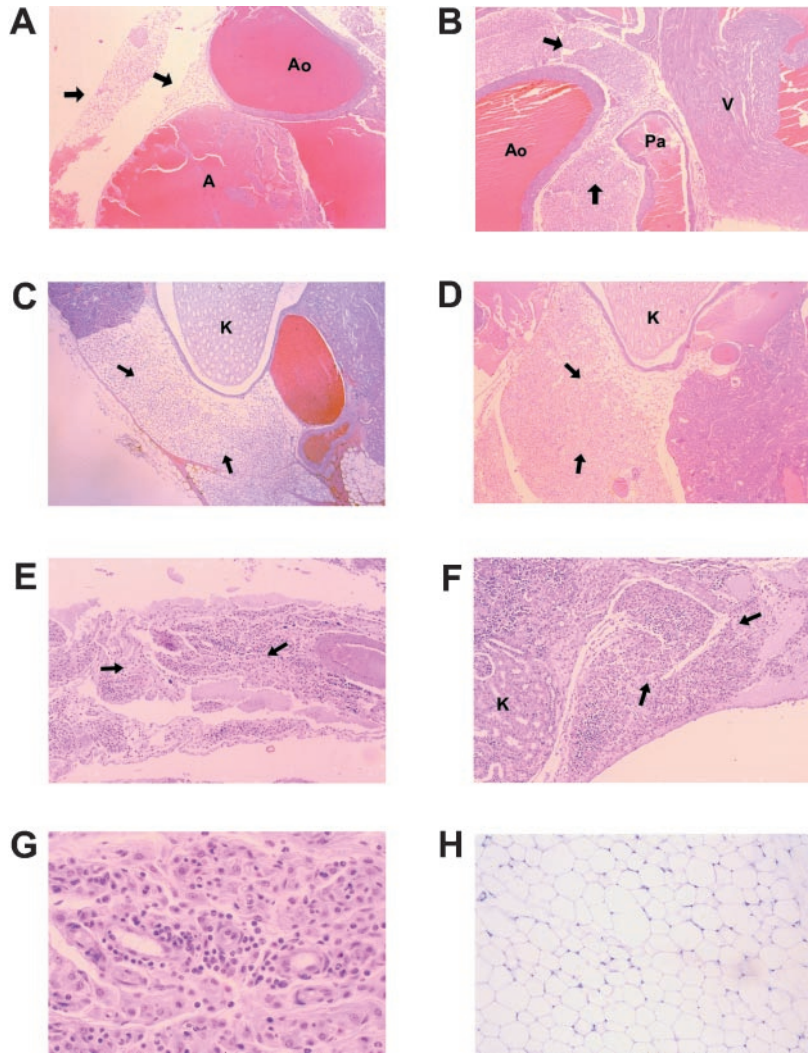

FIG. 3. **Transgenic mice exhibit an abundance of fat and adipose tissue inflammation.** *A*, a representative section of cardiac tissue from a nontransgenic animal. Nominal amounts of brown adipose tissue are observed in the mediastinum at the base of the heart and are indicated by *arrows*. *B*, a typical view of a section obtained from the heart of a 10-month-old HMG I-C transgenic mouse (founder line 1-a). A substantially larger mass of adipose tissue, brown fat, is visible at the base of the heart and is indicated by *arrows*. *A*, atrium; *Ao*, aorta; *Pa*, pulmonary artery; *V*, ventricle. *C*, a typical section of kidney obtained from a nontransgenic mouse. *Arrows* are pointing at the brown fat tissue; normal amounts of adipose tissue are present. *D*, a section form a 10-month-old HMG I-C transgenic mouse (founder line 1-a) demonstrating abundant adipose tissue (brown fat) located adjacent to the hilus. *K* indicates kidney tissue in the region of the hilus. *E*, a section of adipose tissue at abdominal location from a 12-month-old transgenic mouse. *Arrows* are pointing at a large markedly inflamed area. *F*, a view of inflamed adipose tissue from the same mouse at perirenal location. *K*, kidney. *G*, low magnification of the section shown in *E*. *H*, wild type adipose tissue from an age- and sex-matched FVB/N control animal. Notice the lack of foci of inflammation in this sample.

TABLE I

Summary of the phenotypes observed in the Tr-HMG I-C transgenic mice

Wild type control mice (data not shown) and two lines of transgenic mice expressing a different transgene driven by the same H-2K^b promoter are included in this study as negative controls.

	H-2K^b-Tr-HMG I-C					H-2K^b/SV40-SV40 T-antigen (different founders; 1–50 copies)		H-2K^b-TIMP-1 (different founders; 1–50 copies)	
	1-a (2 copies)		1-d (30 copies)	1-f (10 copies)					
	Male	Female	Female	Male	Female	Male	Female	Male	Female
Number of mice analyzed	7	4	1	12	9	48	30	88	63
Lipomas	2	1	0	1	3	0	0	0	0
Steatitis	4	1	1	3	4	0	0	0	0
Excess amount of fat[a]	3	0	0	4	1	0	0	0	0

[a] This category includes the transgenic mice that developed enlarged fat pads either at the hilus of the kidney or at the base of the heart.

supporting a central role for HMG I-C in the genesis of these tumors in our transgenics (45). Fig. 4*A* shows a photo of a typical excised abdominal lipoma from a 10-month-old transgenic mouse. Fig. 4 (*B* and *C*) shows a cross-section of this lipoma. Lipomas presented as encapsulated nodules of fat cells and therefore appeared on gross examination to be circumscribed tumors that could be distinguished from enlarged, otherwise normal, fat pads. In certain cases, where the lipomas were unusually large and pedunculated, necrotic fat tissue was observed (Fig. 4C), probably resulting from a cutoff in the blood supply to the tumor. Similar necrotic fat tissue was also observed in different body locations (data not shown). Moreover, two transgenic mice developed bronchiolar/alveolar adenomas of the lung, and two showed mesothelial proliferation on the pleura at the lung (data not shown). Bronchiolar-alveolar neoplasms appear to be common neoplastic lesions in FVB/N mice at 14 months of age (44) and therefore might not be transgene-related.

As additional controls, we also compared our transgenic mice to mice containing the same H-2K^b promoter/enhancer combination and polyadenylation signal, but driving the expression of other transgenes. In particular, a chimeric construct that is exactly analogous to the HMG I-C construct, but containing the TIMP-1 (tissue inhibitor of metalloproteinase-1) coding sequence in lieu of the HMG I-C open reading frame, was used to generate multiple transgenic mouse lines. This transgenic line was developed in the same genetic background (FVB/N) as the HMG I-C transgenics. Although the TIMP-1 transgenic mice

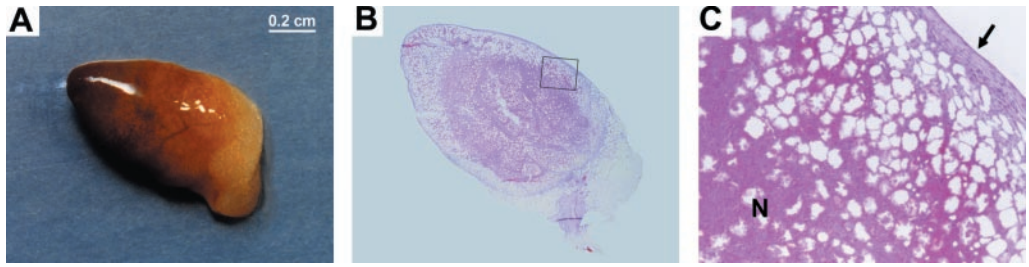

FIG. 4. **HMG I-C transgenic mice develop lipomas.** *A*, low magnification photograph of a typical lipoma isolated from a discrete abdominal mass (10-month-old transgenic mouse from founder line 1-f). *B*, low magnification photomicrograph of the mass as shown in *A*. *C*, detail of the photomicrograph shown in *A* in the region highlighted by the *square*. The *arrow* indicates the fibrous capsule at the edges of the lipoma. *N* indicates an area of necrotic fat tissue at the center of the tumor.

showed appropriate tissue-specific expression of the transgene, a survey of over 150 transgenic progeny mice, observed for periods up to 15–18 months, was unable to detect any phenotype. In sharp contrast to our observations with the HMG I-C transgenic mice, we could not see any evidence of either obesity or lipoma formation in any of the TIMP-1 mice studied (data not shown). All of the TIMP-1 mice included in the study were autopsied, and 50 were subjected to exhaustive histologic analysis of every tissue.

Separately, the same H-2K^b enhancer but in combination with the SV40 promoter and driving expression of the SV40 T-antigen coding sequence was used to produce transgenic mice in the CD-1 genetic background. These transgenic mice developed choroid plexus tumors and multiple endocrine neoplasia involving the pancreas, pituitary gland, thyroid gland, adrenal gland, and testis. They also developed sporadic tumors in the heart, kidney, and muscles, but never involving the adipose tissue. These observations strongly suggest that the expression vector, in the absence of the HMG I-C sequence, could not by itself have induced the observed phenotypic changes in our transgenic mice. A comparison among three transgenic lines for the phenotypes described above is shown in Table I. Information about the correlation between the number of copies of the transgene harbored by each mouse and the phenotypes observed are also included in the same table. We have also performed Western blot analysis to compare the levels of HMG I-C protein expressed in the founder lines 1-a, 1-d, and 1-f (data not shown). We found that equivalent levels of HMG I-C were produced in 1-d and 1-f mice and that 1-a mice expressed approximately 70% of that expressed in the other founder lines. Because the three founder mice expressed very similar levels of HMG I- C protein, it is not surprising that lipomas developed at similar frequency in mice from both the 1-a and 1-f lineages.

Our observation of lipoma development provides direct evidence that the expression of a truncated HMG I-C protein can be a transforming event. This view is supported by the fact that NIH 3T3 cells stably transfected with constructs expressing a truncated HMG I-C polypeptide analogous to the one used in this study are able to form colonies on soft agar and to induce tumors in nude mice (33). Because this truncated polypeptide was expressed in practically all cells of the transgenic mice, it is significant that tumors arose in a very restricted fashion. We note the lack of tumors in other tissues such as salivary glands or fibrous tissues. This is in marked contrast to the expression of other types of oncogenes, which function in a more pleiotropic fashion in transgenic animals.

Development of Adipose Tissue Inflammation (Steatitis)—Remarkably, over 40% of the transgenic mice also developed adipose tissue inflammation (steatitis) at different locations. This condition is virtually absent in nontransgenic mice in the age range studied, and we found no evidence for a similar phenotype in mice expressing a transgene other than HMG I-C (Table I) or in FVB/N wild type mice. Fig. 3 (*E*—*G*) shows representative cross-sections of adipose tissue from a 12-month-old transgenic mouse at abdominal (*panel E*) and perirenal (*panel F*) locations, respectively; in both cases the tissue looks markedly inflamed, with substantial macrophage and eosinophil infiltration (*panel G*). Fig. 3*H* shows a cross-section of adipose tissue from an age- and sex-matched wild type mouse for comparison. As expected there is a lack of inflammatory cells infiltrating this sample. The lack of inflammatory events in tissues other than the adipose tissue and in wild type control mice supports the view of a tissue-specific role of the truncated HMG I-C protein in the development of this phenotype.

Gene Expression Profile in Transgenic Adipocytes and Lipomas—The high frequency of development of lipomas and increased adiposity in HMG I-C transgenic mice prompted us to use this animal model to probe HMG I-C-related molecular pathways involved in the appearance of these phenotypes. Considering the role of HMG I-C as an architectural factor involved in gene transcription regulation, we have begun a search for candidate HMG I-C target genes in transgenic adipose tissue and transgenic lipomas (as compared with wild type adipose tissue).

Differential display analysis was performed as described under "Experimental Procedures" using total RNA from the adipose tissue and a lipoma of a 10-month-old transgenic mouse from founder line 1-a and from the adipose tissue of a wild type age- and sex-matched FVB/N mouse. A portion of the gel after autoradiography is pictured in Fig. 5 to show that the three tissues do indeed exhibit different gene expression profiles and that this model can be useful for the identification of HMG I-C target genes. Twenty arbitary primers were used in this study to scan the entire mouse genome for differentially expressed mRNAs (several were observed). 14 of the differentially expressed DNA bands were eluted from the gel and sequenced using the M13 reverse primer to obtain sequence from the 5′ end. All the sequences were compared with the GenBank^{TM} data base using the BLAST service provided by National Center for Biotecnology Information.

Four of the differentially expressed genes had no homology to known or unknown sequences in the data base, whereas 10 of the sequenced fragments were homologous to known messengers. Among them, we found particularly interesting that genes for Cbl-associated protein (CAP) and fatty acids synthase were differentially expressed because they are already known to participate in fat metabolism.

CAP is a signaling protein that interacts with both c-Cbl and the insulin receptor and is strongly suspected to be involved in the specific tyrosine phosphorylation of c-Cbl upon insulin stimulation of its receptor in 3T3-L1 adipocytes and other tissues metabolically sensitive to insulin (46, 47). Therefore, CAP is very likely to mediate some of the metabolic effects of insulin in adipocytes. CAP messenger is up-regulated in transgenic, histologically normal adipose tissue, when compared

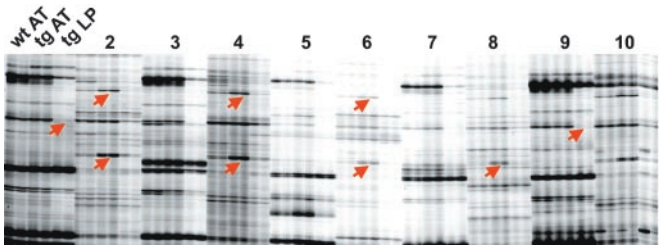

FIG. 5. **Differential display analysis on total RNA samples from wild type adipose tissue (*wt AT*), transgenic adipose tissue (*tg AT*), and transgenic lipoma (*tg LP*).** A typical close-up picture of a portion of an autoradiograph is shown, and *arrows* indicate representative differentially expressed bands. Several differentially expressed mRNAs were detected throughout the gel using 20 ARPs covering the entire mouse genome (some indicated with *red arrows*). The first six lanes show products generated using primer pair 1. The following sets of six lanes each (designated *2–10*) refer to the nine additional ARPs used and shown in this portion of the original gel. The lane orders are the same as shown in primer pair 1.

with wild type adipose tissue and transgenic lipoma. This observation suggests that some of the phenotypic abnormalities developed by the HMG I-C transgenic mice may be related to CAP-mediated enhanced insulin responsiveness of transgenic adipocytes *versus* wild type counterparts.

Interestingly, we also observed a down-regulation of the fatty acids synthase gene (48) expression in transgenic fat and lipoma *versus* wild type fat. We suspect that some still unknown compensatory "feedback"-like mechanisms may be involved in the down-regulation of this gene in our transgenic mice and are actively investigating this possibility.

DISCUSSION

Our experiments suggest that the C-terminally truncated HMG I-C protein that is expressed as a component of various fusion proteins in many human lipomas has an important and specific role in lipoma formation. Transgenic expression of the C-terminally truncated HMG I-C protein in most mouse tissues resulted in an increase in neonatal mouse adipose tissue, occurrence of adipose tissue inflammation, and high incidence of lipomas. Abnormal growth of other tissues did not occur, consistent with the hypothesis that the N-terminal HMG I-C protein specifically facilitates growth of adipocytes. An aspect of this result is reinforced by the observation that the phenotype of the homologous knock-out of HMG I-C included a drastic reduction in body fat content (23). However, the specificity of the effect of the transgenic C-terminally truncated HMG I-C is somewhat surprising in light of the more global effect of the knock-out on the growth of many tissues with a resultant pygmy phenotype, the association of the fusion transcript with uterine leimyomas and salivary gland adenomas as well as lipomas in humans, and previous experiments in which expression of the C-terminally truncated protein lowered the serum dependence and lessened the contact inhibition of NIH 3T3 cells (33–38).

The restricted effect of the C-terminally truncated HMG I-C on early adipogenesis and the incidence of lipoma *versus* the more general effect of the fusion protein that are found in human mesenchymal tumors could be due to a dependence of the effects on the interaction of the truncated HMG I-C protein component with adipocyte-specific transcription factors on the interaction of proteins induced by the truncated HMG I-C with adipocyte-specific proteins, on the importance of the fusion protein partner in broadening the effects, or on a species-specific effect that results in relative suppression of the C-terminally truncated HMG effects in muscle or glandular tissues.

An important aspect of our data and this transgenic model is that a lipoma developed in 7 mature mice of 33 that expressed the C-terminally truncated HMGI-C protein. These data indicate that lipomas are a rare outcome of expression of the C-terminally truncated HMGI-C in all cells in most tissues. Most likely, additional genetic change(s) must occur in adipose tissue before the effect of the HMG I-C can be evident. The increase in adiposity among young transgenic animals is most consistent with the possibility that the transgenic construct increases the growth or survival of adipocytes and that secondary genetic change results in enhanced growth and survival of a single cell that develops into an adenoma. The transgenic construct has the DNA binding activity of HMG I-C[2] and is expected to bind to DNA and affect transcription (10). An expectation of this hypothesis is that the C-terminally truncated HMG I-C effect changes in the transcription of adipocyte genes that enhance adipocyte growth or survival. The transcriptional effect may be specific for the adipocyte or may be more general and play out in enhanced growth or survival in the adipocyte. Thus, there may be general effects of C-terminally truncated HMGI-C protein transgenic expression or more specific effects in adipocytes. For these reasons, transcriptional profiling of adipocytes from normal and transgenic animals is expected to reveal differences and provide an indication of the genetic basis for lipoma development. Further differences should be evident in the comparison between normal adipose tissue and lipoma tissue in the transgenic animals. These latter differences would reflect the effect of postulated genetic changes that are specific to the lipoma cell and may be important in the identification of those specific changes. Comparison of gene expression patterns in transgenic and wild type adipose tissue and transgenic lipoma by differential display analysis shows that the three tissues do differ in their expression profiles, thus further supporting the central utility of this new transgenic model as a tool to understand the molecular pathways of lipoma development.

While this manuscript was in preparation, Battista and coworkers (49) reported the development of embryonic stem cell-derived transgenic mice expressing high levels of a truncated HMGI-C polypeptide similar to the one used in the present study. There are both similarities and important differences between their model and the one we describe in this paper. Consistent with our work, their transgenic mice showed increased adiposity (although throughout development). In sharp contrast, it is not clear whether their mice also develop lipomas at a high frequency. In their report a single case of lipomatosis (in 10 mice analyzed; from a single founder) was described in the manuscript. In our comprehensive analysis of over 33 transgenic mice, seven cases of encapsulated lipomas (from two independent founder lines) were observed. Because no conclusions can be made on the lipomagenic role of truncated HMG I-C in their study, our mice remain the only established model of lipoma development. A second important distinction between the models is that our transgenic mice also develop an abnormally high incidence of inflammation affecting the adipose tissue, further extending the adipose tissue-specific function of the truncated HMG I-C protein *in vivo.* We do not yet understand the molecular basis of steatitis observed in the transgenic mice. However, the mice also represent the first animal model of this disease.

Our identification of gene products that are differentially expressed in adipocytes expressing the truncated HMG I-C protein *versus* their wild type counterparts illustrates the utility of this unique transgenic model to dissect molecular events participating in the development of lipomas and steatitis. Further experiments are in progress in our laboratory to extend

[2] P. Arlotta and S. J. Ono, unpublished data.

these studies and establish/understand the functional significance of these differences for adipocyte survival, tumorigenesis, and inflammation. Thus, the central utility of this transgenic model for lipoma development resides not only in understanding the role of HMG I-C in adipocyte growth and survival (early molecular events) but also in providing lipomas that can be analyzed for genetic changes that are later events in the genesis of the lipomas.

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Transgenic Mice Expressing a Truncated Form of the High Mobility Group I-C Protein Develop Adiposity and an Abnormally High Prevalence of Lipomas

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