The Expression of a Truncated HMGI-C Gene Induces Gigantism Associated with Lipomatosis

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Abstract

Rearrangements of the HMGI-C gene have frequently been detected in human benign tumors of mesenchymal origin, including lipomas. The HMGI-C protein has three AT-hook domains and an acidic COOH-terminal tail. The HMGI-C modifications consist in the loss of the C-tail and the fusion with ectopic sequences. Recent results show that the loss of the COOH-terminal region, rather than the acquisition of new sequences, is sufficient to confer to HMGI-C the ability to transform NIH3T3 cells. Therefore, transgenic mice carrying a HMGI-C construct (HMGI-C/T), containing only the three AT-hook domains, were generated. The HMGI-C/T mice showed a giant phenotype, together with a predominantly abdominal/pelvic lipomatosis, suggesting a pivotal role of the HMGI-C truncation in the generation of human lipomas.

Introduction

HMGI-C belongs to the HMGI protein family (1), also including the HMGI-I and HMGI-Y proteins. HMGI proteins bind to the minor groove of AT-rich DNA sequences, thereby inducing a bend within the DNA (2, 3). They are not able to stimulate initiation of transcription, but they can enhance promoter binding of transcription factors (3–5).

The HMGI-C protein has three separate DNA binding domains referred to as “AT-hook” motifs. The COOH-terminal region contains a highly acidic tail (1). Rearrangements of the HMGI-C gene have been frequently detected in human benign tumors of mesenchymal origin, including lipomas, lung hamartomas, uterine leiomyomas, endometrial polyps, fibroadenomas, and adenolipomas of the breast (6–9). Chromosomal translocations involving the region 12q13–15, where the HMGI-C gene is located, account for these rearrangements. In most of the human benign tumors, breaks occur within the third intron of the gene, resulting in chimeric transcripts containing exons 1–3 of HMGI-C (encoding the AT-hook domains) and ectopic sequences from other genes (6, 7). In some cases, only a few amino acids are fused to the HMGI-C DNA binding domains (8, 10). We have recently demonstrated that a truncated HMGI-C, deprived of the acidic tail, is able to neoplastically transform the murine fibroblasts NIH3T3 and that the acquisition of ectopic sequences does not increase the transforming ability of the truncated form of HMGI-C (11), indicating that the truncation of HMGI-C, rather than its fusion with other genes, is responsible for cell transformation.

Materials and Methods

Generation of Transgenic Mice. The construct carrying the cDNA encoding the truncated form of murine HMGI-C gene (pRC/CMV-HMGI-C/T) has been described previously (11). pRC/CMV-HMGI-C/T construct was electro-roporated into ES AB2.2 cells (13), and G418-resistant clones were selected and analyzed by Southern blot hybridization with a CMV promoter probe (data not shown). Ten positive clones were expanded, and the expression of HMGI-C/T was evaluated by a semiquantitative RT-PCR-based assay, using a construct-specific primer set, described in the following paragraph. The highest two HMGI-C/T expressing ES cell clones were microinjected into C57BL6/J mouse blastocysts and then transferred to pseudopregnant foster mothers (Laboratory Animal Facility, Thomas Jefferson University, Philadelphia, PA). Chimeric mice were crossed to wild-type C57BL6/J mice (Taccon Farm), and germ-line transmission of the transgene was checked by Southern blot analysis of tail DNA from agouti coat-colored F1 offspring.

RT-PCR Analyses. Tissues from transgenic animals were rapidly dissected, frozen on dry ice, and stored at −80°C. Total RNA was extracted using TRI-reagent solution (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s protocol and treated with DNase I (GenHunter Corporation, Nashville, TN). One μg of RNA was reverse transcribed using random exonucleotides as primers (100 nm) and MuLV reverse transcriptase (Perkin-Elmer). Five μl of cDNA was amplified in a 25-μl reaction mixture containing 1 unit of Taq DNA polymerase (Roche Molecular Biochemicals), 0.4 μM dNTPs, 2.0 μM MgCl2, 0.2 μM of each primer. The PCR amplification was performed for 25 cycles (94°C for 30′, 55°C for 30′, and 72°C for 30′), using the Protocol thermal cycler (AMS Biotechnology). Primers designed to specifically amplify the transcripts of the transfected constructs (forward primer, 5’-ATATAAGCTTGTACGGGTAGAGGCAGTGG-3’; reverse primer, 5’-AGTCGAGGCTGATCAGCGAG-3’) overlapped the 5′ end of the cloned gene and the vector pRC/CMV downstream from the cloned gene but upstream from the poly(A) signal site. For detection of the endogenous HMGI-C gene expression, primers specific for exon 1 and 5 (forward, 5’-ATATAAGCTTGTACGGGTAGAGGCAGTGG-3’; reverse, 5’-ATATAAAGCTTACCGTGAGGCGAGTGG-3’) overlapped the 5′ end of the cloned gene and the vector pRC/CMV downstream from the cloned gene but upstream from the poly(A) signal site. For detection of the endogenous HMGI-C gene expression, primers specific for exon 1 and 5 (forward, 5’-ATATAAAGCTTGTACGGGTAGAGGCAGTGG-3’; reverse, 5’-ATATAAACCGTGACGAGTGG-3’) overlapped the 5′ end of the cloned gene and the vector pRC/CMV downstream from the cloned gene but upstream from the poly(A) signal site. For detection of the endogenous HMGI-C gene expression, primers specific for exon 1 and 5 (forward, 5’-ATATAAAGCTTGTACGGGTAGAGGCAGTGG-3’; reverse, 5’-ATATAAACCGTGACGAGTGG-3’) overlapped the 5′ end of the cloned gene and the vector pRC/CMV downstream from the cloned gene but upstream from the poly(A) signal site. For detection of the endogenous HMGI-C gene expression, primers specific for exon 1 and 5 (forward, 5’-ATATAAAGCTTGTACGGGTAGAGGCAGTGG-3’; reverse, 5’-ATATAAACCGTGACGAGTGG-3’) overlapped the 5′ end of the cloned gene and the vector pRC/CMV downstream from the cloned gene but upstream from the poly(A) signal site. For detection of the endogenous HMGI-C gene expression, primers specific for exon 1 and 5 (forward, 5’-ATATAAAGCTTGTACGGGTAGAGGCAGTGG-3’; reverse, 5’-ATATAAACCGTGACGAGTGG-3’) overlapped the 5′ end of the cloned gene and the vector pRC/CMV downstream from the cloned gene but upstream from the poly(A) signal site. For detection of the endogenous HMGI-C gene expression, primers specific for exon 1 and 5 (forward, 5’-ATATAAAGCTTGTACGGGTAGAGGCAGTGG-3’; reverse, 5’-ATATAAACCGTGACGAGTGG-3’) overlapped the 5′ end of the cloned gene and the vector pRC/CMV downstream from the cloned gene but upstream from the poly(A) signal site.

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2 CMV, cytomegalovirus; ES, embryonic stem; RT, reverse transcription; MRI, magnetic resonance imaging.
specific probes labeled with [$\alpha$-32P]dATP using an oligolabeling method (Megaprime, Amersham Pharmacia Biotech) to specific activity equal to or higher than $7 \times 10^8$ cpm/µg. Hybridization and detection were performed as described previously (14).

Fig. 1. Generation of HMGI-C/T transgenic mice through an ES cell-mediated strategy.

1. Electroporation of the HMGI-C/T construct into ES cells
2. Selection of the G418-resistant clones
3. Check for HMGI-C/T expression
4. Injection into blastocyst of HMGI-C/T expressing clones

foster female

HMGI-C/T chimaeric mice

+ wild-type mouse + HMGI-C/T transgenic mouse

Fig. 2. Detection of HMGI-C and HMGI-C/T expression in transfected ES cells and transgenic mouse tissues by RT-PCR. a, expression of the HMGI-C/T gene in transfected ES cells. Construct-specific primer sets, overlapping the 5' of the cloned gene and a pRc/CMV sequence, downstream from the cloned gene were used. RNA from NIH3T3 and NIH3T3 transfected with the HMGI-C/T construct (11) was used as negative and positive control, respectively. WT, wild type; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. b, transgene expression in adult transgenic mouse tissues. c, expression of the endogenous HMGI-C gene. As expected, adult tissues did not express detectable levels of HMGI-C. As positive controls, RNAs from a cell line derived from a spindle cell cutaneous carcinoma (A5) and from NIH3T3 fibroblasts were used. Primers specific for exons 1a and 5o of the HMGI-C gene were used. d, HMGI-C/T expression in “classical” and “ES-mediated” transgenic mice. The expression level of the transgene was lower in classical HMGI-C/T transgenic mice than in transgenic mice obtained by the ES-mediated strategy. Construct-specific primers (see “Materials and Methods”) were used.

Fig. 3. Increased weight of transgenic mice. Mean and 95% confidence intervals of weights of 10 wild-type (□) and transgenic (HMGI-C/T; ◆) male (a) and female (b) mice as a function of age.
Histological Analysis. For light microscopy, tissues were fixed by immersion in 10% formalin and embedded in paraffin by standard procedures. Five \( \mu \)m sections were stained with H&E or hematoxylin and periodic acid/Schiff reagent. Frozen sections, 4–8 \( \mu \)m thick, of wild-type and transgenic tissues were cut in a frozen microtome and allowed to dry for 1 h at room temperature before being fixed in acetone for 10 min. The slides were air dried for 2 h at room temperature and then placed in PBS for 5 min before the immunoperoxidase staining procedure.

MRI. Imaging was performed on a 1.5-T magnet system (GE medical Systems) using local receiver coils and an 8-cm field of view. Coronal slices 3 mm thick were obtained with T1-weighting (TR/TE = 400/11 ms) with and without fat saturation to unequivocally identify lipids in fat.

Results

Generation of Transgenic Mice Carrying a Truncated HMGI-C Construct. An ES cell-mediated strategy has been used to generate transgenic mice. It is summarized in Fig. 1. A truncated HMGI-C cDNA (HMGI-C/T), deprived of the COOH-terminal tail, under the transcriptional control of the CMV promoter, was transfected into the ES cells AB2.2. G418-resistant clones were selected and analyzed by Southern blot hybridization with a CMV promoter probe (data not shown). Ten positive clones were expanded, and the expression of HMGI-C/T was evaluated by a semiquantitative RT-PCR-based assay (Fig. 2a). Two transfected cell clones expressing the highest levels of HMGI-C/T mRNA were microinjected into C57BL/6/J mouse blastocysts, which were then transferred to pseudopregnant foster mothers. Several chimeric mice, identified by the mixed black/agouti coat color, were obtained and crossed with wild-type C57BL/6/J mice. Two independent HMGI-C/T strains, identified by Southern blot hybridization, were generated and examined. Both the mouse lines showed the same phenotype. We used as wild-type controls the offspring mice from the chimera that did not inherit the transgene.

The HMGI-C/T expression was detected by RT-PCR in all of the
analyzed tissues of transgenic animals (Fig. 2b), without any significant difference among them. Conversely, no detection of the endogenous gene was observed both in transgenic and wild-type mice (Fig. 2c). Immunohistochemical and Western blot analyses, confirmed the expression of the HMGI-C/T protein in transgenic mice (data not shown).

HMGI-C/T Transgenic Mice Exhibit a Giant Phenotype Associated with Abdominal/Pelvic Lipomatosis. All of the HMGI-C/T transgenic mice (deriving from both the lines) exhibited a giant and obese phenotype (data not shown). At 12 months of age, they showed an average 15% increase in body length (naso-anal), compared to wild-type littersmates. Moreover, male and female transgenic mice revealed, at the same age, a drastic weight gain (~36%), starting around the third month (Fig. 3).

MRI of the transgenic and wild-type mice was performed to evaluate the extent of fat deposition and the presence of other abnormalities. Transgenic and chimeric mice showed a drastic expansion of the retroperitoneal and s.c. white adipose tissue (Fig. 4a). Abundant fat stores were also observed in the perirenal and epididimal areas (Fig. 4b). Moreover, bladder enlargement was observed (Fig. 4a). MRI analysis has also shown that the accumulation of fat pads becomes apparent at 3 months of age (not shown).

Autopsy examination of the HMGI-C/T transgenic mice confirmed the presence of a very abundant abdominal fat mass. Moreover, large adipose pads were found in other body sites, such as around the kidneys, in the mediastinum, and at the base of the heart (data not shown).

Histological analysis of the s.c. tissues revealed a prominent s.c. adipose tissue, which was not different from the normal adipose tissue (Fig. 5a). In addition to the increase in fat tissue, chimeric and transgenic mice showed varying degrees of urogenital abnormalities, including hydrosalpinx (Fig. 5, b and c), clitoral gland hyperplasia (Fig. 5e) and epidermoid cysts (Fig. 5d), dilated bladder, mild hydrenephrosis (Fig. 5g), cystitis, urinary infections, chronic balanitis (Fig. 5f), and testis hypotrophy with hypozoospermia (Fig. 5h). Compression of the urinary tract by the adipose tissue may account for some of the genitourinary pathology. Because HMGI-C rearrangements have been found in several benign mesenchymal tumors, a detailed histological analysis of all of the tissues was performed to find lipomas or other related tumors. One transgenic female out of 10 animals analyzed showed a well circumscribed lipoma (Fig. 5b). However, the overall hypertrophy of the adipose tissue in certain sectors of the anatomy of these mice is consistent with a diagnosis of abdominal/pelvic lipomatosis.

Analysis of the lipomatous tissue for the expression of several adipocytic terminal differentiation markers, such as adipocyte aP2 gene (aP2), peroxisome-proliferator-activated receptor γ (PPARγ), lipoprotein lipase (LPL), leptin, and phosphoenolpyruvate carboxykinase (PEPCK), by an RT-PCR assay (data not shown) demonstrated
that the differentiation state of the adipose tissue was not affected by the expression of the HMGI-C/T gene.

Transgenic mice have been also generated by the classical approach microinjecting the same HMGI-C/T construct directly into fertilized mouse eggs. The expression levels of the transgene were much lower than those detected in tissues of the mice generated through the ES-mediated strategy (Fig. 2d). Accordingly, only the mice carrying multiple copies of the transgene showed the giant phenotype associated with lipomatosis (data not shown), indicating that the transgene exerts its effects in a dose-dependent manner.

Discussion

Here, we report the generation of transgenic mice carrying a truncated HMGI-C construct deprived of its COOH-terminal tail by using a new ES cell-mediated strategy that allows us to generate mice showing high transgene expression levels. These transgenic mice showed a giant phenotype, together with a drastic expansion of the retroperitoneal and s.c. white adipose tissue. The great expansion of the adipose tissue observed in HMGI-C/T mice suggests a pivotal role of the HMGI-C rearrangements in generation of human lipomas. Moreover, the giant phenotype shown by the HMGI-C/T mice is the mirror image of that of the HMGI-C null mice. In fact, these mice are characterized by a pigmy phenotype with a reduction of the adult body weight, mainly affecting the fat tissue (15). Based on the combined data, we suggest that the truncation of the HMGI-C gene leads to an increased activity of the HMGI-C protein, which in turn stimulates adipocyte cell growth.

Recently, we have shown that the block of the synthesis of the related HMGI(Y) protein, by the expression of an antisense construct, induced a drastic increase in the growth rate of the 3T3-L1 cells. Moreover, single- and double-HMGI(Y) knockout chimeric mice have an enormous increase in fat tissue. These results would indicate that HMGI(Y), unlike HMGI-C, exerts a negative role on adipocytic cell growth and that the regulation of adipocytic cell proliferation may result from the balance of HMGI(Y) and HMGI-C protein functions: a gain in HMGI-C protein activity induces adipocyte cell hyperproliferation, whereas the dominance of HMGI(Y) has an opposite effect.

The phenotype shown by the HMGI-C/T transgenic mice closely resembles a rare benign human disease, known as pelvic lipomatosis, characterized by the proliferation of normal fatty tissue limited to the perirectal and perivisceral spaces of the pelvis; it usually affects men in the fourth decade of life or older (16, 17). Major complications of this syndrome are infection and obstruction of the urinary tract and various degrees of bladder deformity. Moreover, several other cases of familial lipomatosis have also been described (18). Finally, the Proteus syndrome, mainly characterized by partial gigantism, lipomatosis, and vascular tumors (19), shares several features of the HMGI-C/T mouse phenotype.

Therefore, the HMGI-C/T mice may represent an experimental model for these syndromes, and germ-line HMGI-C rearrangements/mutations may be responsible for some of these lipomatosis diseases.

References

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