

COMMENTARY

Role of the high mobility group A* proteins in human lipomas

Monica Fedele¹, Sabrina Battista¹,
Guidalberto Manfioletti², Carlo Maria Croce³,
Vincenzo Giancotti² and Alfredo Fusco^{1,4,5}

¹Centro di Endocrinologia ed Oncologia Sperimentale del Consiglio Nazionale delle Ricerche c/o Dipartimento di Biologia e Patologia Cellulare e Molecolare, Facoltà di Medicina e Chirurgia, Università degli Studi di Napoli, Via Pansini, 5, I-80131 Naples, Italy, Istituto Nazionale dei Tumori Fondazione Senatore Pascale, via M. Semmola, I-80131 Naples, Italy,

²Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, University of Trieste, Italy, ³Kimmel Cancer Center, Jefferson Medical College, Philadelphia, PE 19107, USA and ⁴Dipartimento di Medicina Sperimentale e Clinica, Facoltà di Medicina e Chirurgia di Catanzaro, Università degli Studi di Catanzaro, via Tommaso Campanella 5, I-88100 Catanzaro, Italy

⁵To whom correspondence should be addressed
Email: afusco@napoli.com

The HMGA family is comprised of four proteins: HMGA1a, HMGA1b, HMGA1c and HMGA2. The first three proteins are products of the same gene, HMGA1, generated through an alternative splicing mechanism. The HMGA proteins are involved in the regulation of chromatin structure and HMGA DNA-binding sites have been identified in functional regions of many gene promoters. Rearrangements of the HMGA2 gene have been frequently detected in human benign tumors of mesenchymal origin including lipomas. 12q13-15 chromosomal translocations involving the HMGA2 gene locus, account for these rearrangements. The HMGA proteins have three AT-hook domains and an acidic C-terminal tail. The HMGA2 modifications consist in the loss of the C-terminal tail and fusion with ectopic sequences. A pivotal role of the HMGA2 rearrangements in the process of lipomagenesis is suggested by experiments showing that transgenic mice carrying a truncated HMGA2 gene showed a giant phenotype together with abdominal/pelvic lipomatosis. As HMGA2 null mice showed a great reduction in fat tissue, a positive role of the HMGA2 gene in adipocytic cell proliferation is proposed. More recently, similar alterations of the HMGA1 gene have been described. As the block of the HMGA1 protein synthesis induces an increase in growth rate of the pre-adipocytic cell line 3T3-L1, we suggest a negative role of the HMGA1 proteins in adipocytic cell growth and, therefore, we propose that adipocytic cell growth derives from the balance of the HMGA1 and HMGA2 protein functions.

Introduction

*HMGA protein family**

HMGA1/Hmga1 and *HMGA2/Hmga2* are two genes (human/murine, respectively) that encode four proteins named

*The nomenclature of the high mobility group (HMG) proteins has been recently revised; see Chromosomal Proteins Nomenclature Home Page: <http://www.informatics.jax.org/mgihome/nomen/genefamilies/hmgfamily.shtml>. In this review we use the new nomenclature, but we report the old one in parenthesis. HMGA1a, high mobility group A1a (HMGI); HMGA1b, high mobility group A1b (HMGY); HMGA1c, high mobility group A1c (HMG-IR); HMGA2, high mobility group A2 (HMGI-C).

HMGA1a, HMGA1b, HMGA1c and HMGA2, being the first three proteins spliced forms of the *HMGA1/Hmga1* gene.

The HMGA1a, HMGA1b and HMGA2 proteins (previously HMGI, HMGY and HMGI-C, respectively) are composed of 107, 96 and 108 amino acid residues, respectively (1–3). Each protein contains three basic domains, named AT-hooks and an acidic C-terminal region. The HMGA1a protein differs from HMGA1b in that it has an additional insertion of 11 amino acid residues between the first and the second AT-hook domains. The structure of HMGA2 protein is very similar to that of HMGA1a; however, the first 25 amino acid residues are totally different. Moreover, in HMGA2 there is a short peptide of 12 amino acid residues between the third AT-hook and the C-terminal acidic tail. These proteins are very well conserved during evolution, and only a few differences can be detected between the human and the murine HMGA sequences (4–10). A new HMGA1 isoform has been recently isolated and found to be a variant of HMGA1a. It has been designated HMGA1c and has a deletion of 67 nucleotides compared with the HMGA1 sequence. This deletion results in a frameshift so that the two proteins are identical in their first 65 amino acids and differ thereafter. HMGA1c encodes a protein of 172 amino acids and has a molecular weight of 26–27 kDa (11). Little is known about this form that, however, appears to be the only isoform present in normal human and mouse testis (Chieffi *et al.*, manuscript in preparation).

The three AT-hook domains are responsible for binding to AT-rich sequences inside the minor groove of DNA (12,13) and are characterized by the amino acidic sequence BBXRGRPBB where B is a basic amino acid residue (K or R) and X is a glycine or proline residue (14). Structural analysis of the protein–oligonucleotides complex has shown that the conserved sequence RGR is important for this interaction. Although each AT-hook binds with a low specificity to DNA, a high affinity has been described when two or three AT-hook domains bind simultaneously to a single DNA molecule. The C-terminal region (15 amino acid residues) is highly acidic and contains several hydroxylic amino acids that are phosphorylated by casein kinase II. This region may be important in protein–protein interaction and in the negative regulation of cell growth, as it is lost in human benign tumors.

The genomic structure of the HMGA genes

The *Hmga2* gene (see Figure 1) consists of five exons and spans for >200 kb with a large intronic sequence (>100 kb) separating the third from the fourth exon. The *Hmga2* transcripts, among which the major one is of 4.1 kb, originate from multiple transcription initiation sites in the 5' flanking region and have a long 3' untranslated region. The first exon encodes the first 37 amino acid residues which includes the first AT-hook domain. The second and third AT-hook domains are encoded by the second and third exon, respectively. The fourth exon encodes the 12 amino acid residues that separate the third AT-hook domain from the C-terminal acidic domain, which is encoded by the last exon (7,15).

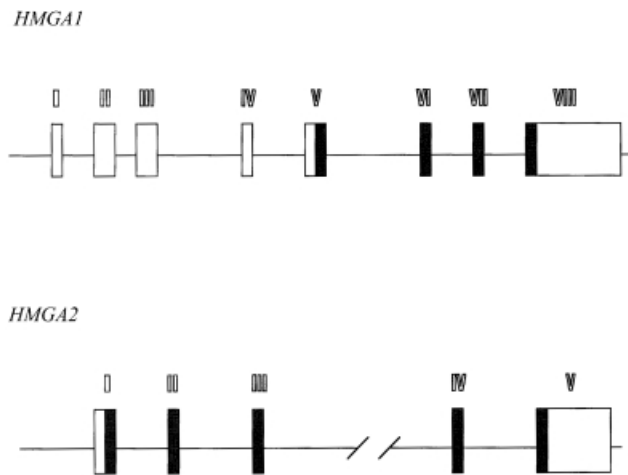


Fig. 1. Schematic representation of the *HMGA* genomic organization. White and shaded boxes indicate non-coding and coding sequences, respectively.

The *HMGA1* gene has the same exon–intron organization except it lacks the region corresponding to the fourth exon of *HMGA2*. The *HMGA1* gene spans 10 kb; it is processed in a mRNA of 1.8 kb that encodes three different isoforms through an alternative splicing. At the 3' end of the *HMGA1*-specific mRNA there is a long untranslated region of 1.3 kb. The *HMGA1* gene has eight exons and a number of different transcription start sites (5,16).

Post-translational modifications of the *HMGA* proteins

Phosphorylation. In rapidly growing mammalian cells, the HMGA1 proteins are among the most extensively phosphorylated chromosomal proteins (17). They are substrates of various protein kinases, including protein kinase C (PKC), cdc2 and casein kinase II. Two major (Ser43 and Ser63) and four minor PKC phosphorylation sites have been identified (18,19). These sites are distinct from those phosphorylated by cdc2 kinase, which phosphorylates Thr52 and Thr77 (20,21), and by casein kinase II, which phosphorylates two or three serine residues at the C-terminus of the protein (22,23). The two cdc2 kinase phosphorylation sites are adjacent to the N-terminus of two of the three AT-hooks. Moreover, one of the major PKC phosphorylation sites, Ser63, is adjacent to the C-terminus of the second AT-hook, whereas Ser43 is located within the region spanning between the first and the second AT-hook. Phosphorylation of HMGA1a by both PKC or cdc-2 kinases resulted in a strong reduction of the DNA-binding affinity. These phosphorylations occur in a cell cycle-dependent manner, at the beginning of S phase and during the G₂/M transition, suggesting that phosphorylation of HMGA1a plays a critical role in cell cycle progression. Very recently, a link between apoptosis induced in leukaemic cells and the degree of phosphorylation of HMGA1a protein has been described. At the early stages of the apoptotic process, the HMGA1a protein is hyper-phosphorylated. Subsequently, when the apoptotic bodies are formed, the HMGA1 protein becomes almost completely de-phosphorylated (23).

Acetylation. In addition to undergoing phosphorylation, HMGA1a and HMGA1b proteins are also regulated by acetylation. The transcriptional coactivators CBP/p300 (CREB-binding protein) and P/CAF (CBP-associated cofactor) acetylate HMGA1a at distinct lysine residues, causing distinct effects on transcription (24). Specifically, CBP preferentially

acetylates Lys65 whereas P/CAF preferentially acetylates Lys71. In the context of the human β -interferon gene expression, acetylation of HMGA1a by both CBP and P/CAF is required for the enhanceosome activation, whereas only CBP acetyltransferase activity is required for enhanceosome destabilization and post-induction turn-off (24).

Other modifications. Some other post-translational modifications have also been reported for HMGA proteins (25,26). In fact, both ADP-ribosylation and methylation have been reported, but a clear relationship between these modifications and biological events, in which they are involved, has not been reported yet.

HMGA1 proteins in the transcriptional complexes and chromatin

HMGA proteins are involved in the regulation of chromatin structure and function (18,25,27,39). HMGA1a DNA-binding sites have been identified in functional regions of many gene promoters (28–30,32,33,35–38,40,41). This protein has been demonstrated to bind to other transcription factors (28–41), such as NF- κ B, Elf-1 and Tst-1/Oct-6, through protein–protein interaction in highly conserved regions, i.e. Ets domain of Elf-1 and POU domain of Tst-1/Oct-6. As the *HMGA1* proteins form complexes on specific promoters that interact with the basal transcription machinery, they are considered able to induce transcription.

One of the best characterized examples of synergistic interactions between transcription factors is provided by the virus-inducible enhancer of the interferon- β (IFN- β) gene (35–40). Transcripts of IFN- β mRNA are not detected in uninfected cells, but after virus infection the gene is activated to very high levels and then undergoes a rapid post-induction turn-off. Detailed analysis of the IFN- β gene upstream sequence has revealed a highly compact and complex organization of *cis*-acting regulatory elements (PRDI through PRDIV). PRDII, PRDIV and PRDIII are recognized by NF- κ B, ATF-2/c-Jun heterodimer, and several members of the IRF family, respectively. HMGA1a plays a key role in the activation of this gene by functioning as the essential architectural component for the assembly and stability of the IFN- β gene enhanceosome (36–38). Binding of HMGA1a to the enhancer alters the structure of the DNA, allowing cooperative recruitment of the IFN- β gene activators that, together with HMGA1a, assemble into a remarkably stable higher order nucleoprotein complex termed the IFN- β enhanceosome (37,39,40). Thus, the assembly and function of the IFN- β enhanceosome requires a complex network of protein–DNA and protein–protein interactions orchestrated by the HMGA1a protein.

Involvement of HMGA1a protein has been reported not only at the promoter region of specific genes, but also at more global nuclear structures related to higher order chromatin bound to the nuclear matrix and forming distinct nucleoprotein loops (42–45). Such structures have been called MARs (Matrix Attachment Regions) or SARs (Scaffold Attached Regions) and it has been shown that they contain, together with specialized AT-rich DNA sequences with high unwinding aptitude called BURs (Base-unpairing Regions), a variety of proteins including histone H1, topoisomerase II, SAF-A and SAF-B (Scaffold Attachment Factor A and B), lamin B1, p53, SATB1, nucleolin, p114 and HMGA1a/b proteins (44–49). HMGA1a/b proteins specifically bind to BURs (48), although binding should regard DNA regions different from those to which histone H1 is bound. In fact, it has been reported that

HMGA1a displaces histone H1 from chromatin and nuclease sensitive chromatin releases HMGA1a, HMGA1b and HMGA2 proteins but not histone H1 (49). At the same time, in an immunocytochemical study it has been demonstrated that topoisomerase II and HMGA1a/b proteins co-localize in the interphase nucleus of HeLa cells (50).

The mutually exclusive localization of histone H1 and HMGA1a protein could account for a different involvement of these two factors in the processes of chromatin condensation–de-condensation which are in turn related to the phosphorylation of these proteins, both well known substrates for cyclin-dependent kinases p34-Cdc2 and Cdk2 (20,21,51).

HMGA1a protein could occupy several distinct subnuclear positions roughly grouped into two categories: specific micro-regions, in which HMGA1a acts as ‘architectural transcription factor and is involved in both positive and negative gene regulation, and macro-regions, in which HMGA1a is a structural special component of the chromatin of cancer cells as suggested (48), being possible that micro-regions are included in macro-regions. The understanding, by ultrastructural techniques, of the identity of the nuclear domains in which HMGA1a protein localizes and the identification by immunoprecipitation of its proteic partners could constitute an advancement of the simple use of the expression of HMGA1a protein as immunohistochemical marker of neoplastic transformation.

Expression of the HMGA proteins in normal and neoplastic tissues

The *HMGA2* gene is not expressed in any of the several adult mouse (3) and human tissues tested (52). A very low expression has been also observed in CD34 positive hematopoietic stem cells (53), and recently in mouse pre-adipocytic proliferating cells (54). The *HMGA1* gene is expressed at low levels in adult murine and human tissues: a higher expression was observed in testis, skeletal muscle and thymus. Conversely, both the genes are widely expressed during embryogenesis (55,56).

Hmga1 and Hmga2 over-expression was first described in rat thyroid transformed cells and in experimental thyroid tumors (57–59). Over-expression of the HMGA proteins was then found to be a common feature of experimental and human malignant neoplasias, including thyroid (60–62), prostate (63), uterus (64), breast (Chiappetta *et al.*, manuscript in preparation), colorectum (65–67), ovary (Masciullo, V., personal communication) and pancreas (68) carcinomas. Moreover, the expression level of the HMGA proteins is significantly correlated with parameters of a poor prognosis in patients with colorectal cancer (67).

Over-expression of the HMGA proteins is a necessary event in *in vivo* cell transformation. This was demonstrated by experiments in which *Hmga2* expression was blocked by transfecting rat thyroid cells with an antisense *Hmga2* cDNA construct. When these cells were infected by the myeloproliferative sarcoma virus (MPSV) and the Kirsten murine sarcoma virus (KiMSV) carrying the *v-mos* and *v-ras-Ki* oncogenes, respectively, they did not acquire the typical markers of neoplastic transformation (ability to grow in soft agar and induce tumors after injection into athymic mice). Conversely, these markers were shown by the untransfected rat thyroid cells infected with the same murine retroviruses. Interestingly, the cells carrying the *Hmga2* antisense construct did not express HMGA1a/b proteins suggesting the possibility that the block of cell transformation may be dependent on the inhibition of the *Hmga1* expression (69). Further investigations

showed that over-expression of HMGA1 proteins is also essential in the development of cancer in humans so that it is possible to suggest a cause and effect relationship. In fact, an adenovirus carrying the *HMGA1* gene in an antisense orientation induces programmed cell death in carcinoma cell lines derived from human thyroid, lung, colon and breast cancers (70). Moreover, it has been reported that the over-expression of HMGA1a or HMGA2 leads to neoplastic transformation of both Rat-1a fibroblasts and CB33 cells, whereas the decrease of HMGA1a/b expression abrogates transformation in Burkitt’s lymphoma cells (71,72).

The lack of induction of AP-1 transcriptional activity by suppression of HMGA1 and HMGA2 synthesis may account for the inhibition of the neoplastic cell transformation in absence of the HMGA proteins. In fact, thyroid neoplastic transformation is associated with a drastic increase of AP-1 activity, which is prevented by the suppression of the HMGA protein synthesis. The absence of AP-1 transcriptional activity induction, directly or indirectly regulated by the HMGA proteins, would inhibit the expression of AP-1-dependent genes, such as *VEGF*, collagenase I and stromelysin, which are required for cell neoplastic transformation (73).

HMGA genes in human lipomas

Rearrangements of HMGA2 and HMGA1 genes in human benign tumors

Several cytogenetic studies have demonstrated a non-random association between rearrangements of bands 12q13-15 or 6p21 and a variety of benign tumors mainly of mesenchymal origin: uterine leiomyomas, lipomas, pulmonary chondroid hamartomas, aggressive angiomyxomas, pleomorphic adenomas of the salivary glands, and fibroadenomas and adenolipomas of the breast (74–91). The translocation occurred with different chromosome partners. In lipomas, for example, translocations have been identified between 12q13-15 and chromosomes 1 through 7, 10, 11, 13, 15, 17, 21 and X, even though translocations involving 12q13-15 with chromosomal region 3q27-28 and the long arm of chromosome 13 are known as the most frequent.

These data suggested that on the chromosomal region 12q13-15 should be located a gene playing a critical role in these tumors. This gene has been identified as the *HMGA2* gene, which is affected by translocation breakpoints mapping to the chromosome region 12q13-14 (92,93).

In one study, the *HMGA2* gene was identified by positional cloning, that led to the identification of a 175-kb region in which breakpoints were identified in eight benign tumors with 12q13-15 aberrations (92). Then, by using 3’ terminal exon trapping, sequences identical to *HMGA2* gene have been identified. Another study (94) started from earlier work that suggested an important role of the *HMGA2* gene in adipocytic cell growth and development. In fact, it has been shown that mice carrying a disrupted *HMGA2* presented a *pygmy* phenotype with a drastic reduction of the adult body weight, mainly affecting fat tissue (55). Moreover, this group located the *HMGA2* gene in a region close to the translocation breakpoint. Then fluorescent *in situ* hybridization (FISH) analysis, using the cloned human *HMGA2* gene, demonstrated that it was disrupted in all three lipomas analyzed.

The chromosome breakpoints occurring at 12q13-15 are preferentially clustered in the large (>160 kb) third intron of *HMGA2*, which separates the third DNA-binding domains from the acidic domain. It is noteworthy that intron 3 of

HMGA2 has been basically conserved for at least 30 million years (95). As a consequence of translocation, the *HMGA2* gene is disrupted and the AT-hooks fused to ectopic sequences. However, in some of these mesenchymal tumors, only a few amino acids are fused to the *HMGA2* DNA-binding domains (96,97).

HMGA2 gene modifications in human lipomas

The lipoma preferred partner (*LPP*) gene, located on chromosome 3q27-28, is the most likely fusion partner gene of *HMGA2* (98–99). The *LPP* gene product is a proline-rich protein that shares 41% of sequence identity with the focal adhesion protein zyxin (100). *LPP* protein localizes in focal adhesions as well as in cell-to-cell contacts; it binds VASP, a protein implicated in the control of actin organization. In addition, *LPP* protein accumulates in the nucleus of cells upon treatment with leptomycin B, an inhibitor of the export factor CRM1. The nuclear export of *LPP* depends on an N-terminally located leucine-rich sequence that shares sequence homology with well-defined nuclear export signals. The *LPP* protein contains a leucine-zipper motif in its N-terminal region and three LIM domains in its C-terminal region (100,101). The fusion with the *HMGA2* gene generally involves the three LIM domains of the *LPP* protein (98), and results in *HMGA2/LPP* and *LPP/HMGA2* fusion transcripts in lipoma cells carrying a t(3;12)(q27;q14-q15) translocation (98,99). The LIM domains are cysteine-rich, zinc-binding sequences that are present in a variety of proteins involved in cell fate regulation and differentiation (100–102). They dimerize with other nuclear proteins, many of which are transcription factors (103,104). Therefore, the LIM domains of the *LPP* protein may serve as a scaffold upon which distinct protein complexes are assembled in both cytoplasm and nucleus.

Although the *HMGA2*–*LPP* fusion has been frequently found in human lipomas, it does not seem exclusive of this kind of tumor, as the FISH analysis of five pulmonary chondroid hamartomas all showing a t(3;12)(q27;q14-q15) revealed that both *HMGA2* and *LPP* are disrupted by this translocation, which indicates that this fusion is not specific for lipomas (105).

In one case of lipoma, the fusion occurs with DNA sequences located on chromosome 15. These sequences encode an acidic peptide rich in serine and threonine residues. These features have been observed in a number of transcriptional activation domains, including the C-terminal domains of homeobox proteins and NF- κ B. So the acquisition of a *trans*-activation domain by the DNA-binding domains of *HMGA2*, which normally possesses a transcriptional inactive acidic domain, can easily be reconciled with aberrant regulation of the *HMGA2* target genes (96).

Recently, a novel human gene, *LHFP* (lipoma *HMGA2* fusion partner), that acts as a translocation partner of *HMGA2* in a lipoma with t(12;13), has been isolated (106). The *LHFP* gene was mapped to the long arm of chromosome 13, a region recurrently targeted by chromosomal aberrations in lipomas. By northern blot analysis, a transcript of 2.4 kb was detected in a variety of human tissues. Nucleotide sequence analysis of the composite *LHFP* cDNA revealed an open reading frame encoding a protein of 200 amino acids. BLAST searches showed that the *LHFP* protein belongs to a new protein family consisting of at least four or five members. In the lipoma studied, the expressed *HMGA2*–*LHFP* fusion transcript encodes the three DNA-binding domains of *HMGA2* followed by 69 amino acids encoded by frameshifted *LHFP* sequences.

Some studies suggest that, besides intragenic *HMGA2* rearrangements, transcriptional activation of the gene can also initiate tumor growth. In fact, three pulmonary chondroid hamartomas (PCH) showing a rare variant type of the translocation t(12;14)(q14-15;q24) with presence of two normal chromosomes 12 and a der(14), but missing the der(12), showed that the breakpoint is located 5' to *HMGA2* (107). However, so far no extragenic rearrangements have been described in lipomas. It cannot be excluded that this kind of alteration could occur also in lipomas. Lipomatosis induced by *HMGA2* wild-type over-expression in transgenic mice (Fedele *et al.*, submitted for publication) and the ability of *HMGA2* to transform rat fibroblasts in culture (72) seem to confirm a potential role of *HMGA2* over-expression in the process of lipomagenesis.

HMGA2 amplification in atypical lipomatous tumors

Atypical lipomatous tumors (ALTs) are a distinctive subset of mesenchymal neoplasms, characterized by a mature adipocytic differentiation and a tendency to arise in the somatic soft tissue of the limbs and in the retroperitoneum. These tumors often tend to recidive. The cytogenetic hallmark of these lesions is the presence of ring and/or long marker chromosomes derived from the chromosomal region 12q13-15. Eighty-three percent of ALTs showed *HMGA1a* immunopositivity associated with *HMGA2* amplification. It is likely that higher expression of *HMGA2* in ALTs compared with benign lipomas may be responsible for the greater aggressiveness of ALTs (108–110).

What is the role of the HMGA2 rearrangement in the generation of human lipomas?

The findings summarized above raised several important questions.

- (i) Do rearrangements of the *HMGA2* gene have a causal role in the development of human lipomas or are they only casually associated with them?
- (ii) Does the translocation partner have a specific role in tumorigenesis or does it merely deregulate expression of the DNA-binding region of *HMGA2*?
- (iii) Is the lack of the C-terminal tail sufficient to confer cell growth advantage? Indeed, even though some published data seem to suggest that the fusion partner has a specific role in tumor formation, many more data indicate that the simple over-expression of the first three exons of *HMGA2* is sufficient to cause transformation (52). In fact, in some of these mesenchymal tumors, only few amino acids are fused to the *HMGA2* DNA-binding domains, suggesting that the truncation of the *HMGA2* gene, rather than its fusion with other genes, is the event responsible for cell transformation.
- (iv) Does the loss of the C-terminal tail in *HMGA2* result in a gain-of-function or in a loss-of-function? The answer to this question determines whether the rearranged *HMGA2* should be viewed as the generation of a new oncogene or as the disruption of a gene able to inhibit cell growth and consequently considered as a suppressor gene.

A rearranged HMGA2 gene is able to transform NIH 3T3 cells

In order to answer the above-mentioned questions, the murine fibroblasts NIH 3T3 were transfected with cDNAs coding for (i) a truncated form of the *HMGA2* protein constituted by only the three DNA-binding domains (*HMGA2/T*); (ii) a fusion protein constituted by the three DNA-binding domains of *HMGA2* and the LIM domains of the *LPP* protein (*HMGA2/C*);

(iii) the wild-type HMGA2 protein. *HMGA2/T* and *HMGA2/C* caused malignant transformation of NIH3T3 cells. Conversely, the wild-type *HMGA2* cDNA did not exert transforming activity. Moreover, the acquisition of ectopic sequences did not increase the transforming ability of the *HMGA2* truncated form. The number of foci was significantly lower (~30-fold) than that obtained transfecting *Ret/MEN2A* or activated *ras* genes. Moreover, these foci appeared with a longer latency period (3–4 versus 1–2 weeks for the appearance of *Ret/MEN2A* foci). Finally, the colony-forming efficiency in soft-agar of the *HMGA2/T* and *HMGA2/C* transfectants was lower (30–35%) compared with that of *Ret/MEN2A* transfectants (80%) (111).

These results led to the following conclusions:

- (i) the rearranged forms of *HMGA2* must be considered oncogenes;
- (ii) the loss of the two last exons of *HMGA2* is sufficient to confer transformation ability;
- (iii) the fusion with ectopic sequences does not increase the transforming ability of the rearranged *HMGA2* gene;
- (iv) the transforming ability of the rearranged forms of the *HMGA2* gene is quite weak. This is consistent with the benign and non-aggressive behaviour of tumors associated with rearranged *HMGA2* forms.

Transgenic mice carrying a truncated HMGA2 gene develop abdominal/pelvic lipomatosis

Subsequent to these findings, two independent groups generated transgenic mice carrying a truncated *HMGA2* construct. In fact, transgenic mice provide a powerful experimental approach to define the role of oncogenes in neoplastic processes *in vivo*.

In one study (112), the transgenic mice were obtained with an innovative ES mediated strategy. The truncated *HMGA2* cDNA (*HMGA2/T*), deprived of the C-terminal tail, under the transcriptional control of the cytomegalovirus promoter (CMV), was transfected into the ES cells AB2.2, and G418-resistant clones were selected. Two transfected cell clones expressing the highest levels of *HMGA2/T* mRNA were microinjected into C57BL6/J mouse blastocysts, which were then transferred to pseudopregnant foster mothers. Several chimeric mice were obtained and crossed with wild-type C57BL6/J mice to generate *HMGA2/T* mouse strains. This technique resulted in a very high expression of the transgene. Indeed, the authors showed that the expression of the transgene in these mice was higher than that detected in mice generated using the classical approach of microinjecting the *HMGA2/T* construct into fertilized mouse eggs (112). The second group generated the transgenic mice microinjecting the *HMGA2/T* construct under the transcriptional control of H-2K^b, a well characterized class I major histocompatibility complex promoter/enhancer, into fertilized mouse eggs (113). In both the studies, the transgene was expressed in all the tissues, and transgenic mice showed an increased body weight with a drastic expansion of the retroperitoneal and subcutaneous white adipose tissue (Figure 2). Moreover transgenic mice had an 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. In both the studies, 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 demonstrated that the differentiation state of the

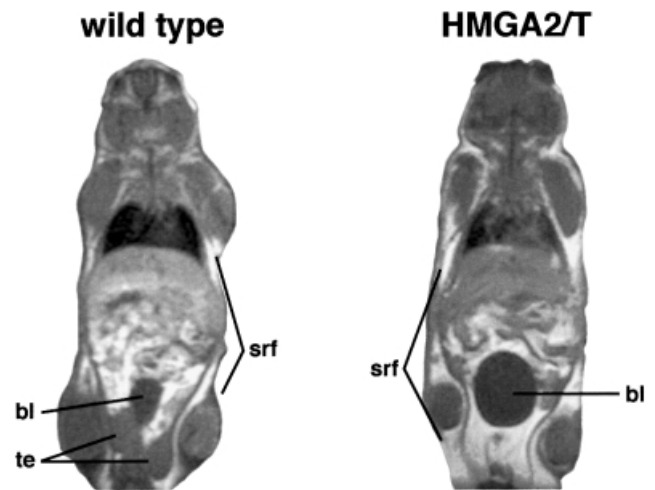


Fig. 2. Magnetic resonance imaging analysis. Frontal sections of 1-year-old wild-type and *HMGA2/T* transgenic mice (112) showing testes (te), bladder (bl) and subcutaneous and retroperitoneal (srf) fat.

adipose tissue was not affected by the expression of the *HMGA2/T* gene. However, in the first study only one classical lipoma was detected in 10 mice; conversely seven lipomas among 33 mice analyzed were found in the second study. In the first study there was an average increase of 15% in body length (naso-anal) in 12-month-old animals, compared with wild-type littermates. Moreover, the animals generated in the first study showed bladder enlargement (Figure 2) consequent to compression of the urinary tract by the adipose tissue. The differences shown by animals generated in the two studies may be explained by the different expression of the transgene.

The dramatic expansion of the adipose tissue observed in *HMGA2/T* mice suggests that *HMGA2* rearrangements play a pivotal role in the generation of human lipomas. Moreover, the giant phenotype shown by the *HMGA2/T* mice is the mirror image of that of the *HMGA2* null mice. In fact, these mice are characterized by a *pigmy* phenotype with a reduction of the adult body weight, mainly as a result of a decrease in fat tissue (55). Based on these data, we suggest that the truncation of the *HMGA2* gene leads to an increased activity of the *HMGA2* protein, which in turn stimulates adipocyte cell growth.

HMGA1 alterations in human lipomas

Recent studies have demonstrated that also the *HMGA1* gene is rearranged in the benign tumors characterized with 6p21 chromosome aberrations (114–117).

So far two cases have been described in human lipomas. In both instances there were deletions in the *HMGA1* gene. In the first case, 10 bp of exon 6, 51 bp of exon 7 and 961 of exon 8, for a total of 1022 nucleotides, were deleted. The predicted protein includes the first two DNA-binding domains fused in-frame to an uninterrupted open reading frame (ORF) encoding 108 amino acids with a high content in proline which is indicative of an unusually huge content of domains potentially regulators of transcription. In the second case, 923 nucleotides of exon 8 were deleted, and this does not affect the *HMGA1* ORF (118).

Role of the HMGA2 and HMGA1 in process of adipogenesis

The first evidence implicating *Hmga2* in adipogenesis came from the observation that *Hmga2*^{-/-} mice express a *pigmy* phenotype together with a drastic reduction (87%) of the

adipose tissue (55). Conversely mice carrying a truncated *Hmga2* gene showed an obese phenotype (112).

More recently it was found that *Hmga2* gene was not expressed in wild-type adipose tissue, whereas it was expressed in fat deposits of both wild-type and genetically obese mice (*Lep^{ob}/Lep^{ob}* and *Lepr^{db}/Lepr^{db}*) after 1 week of high fat diet. Moreover, disruption of *Hmga2* gene prevents both diet- and gene-induced obesity: in fact, *Lep^{ob}/Lep^{ob}*, *Hmga2*^{-/-} double-homozygous mice (54) do not show an obese phenotype. *HMGA2* appears epistatic to *Lep* in the fat tissue. However, as other phenotypic features of *Lep^{ob}/Lep^{ob}* mice are retained in *Hmga2*^{-/-}, *Lep^{ob}/Lep^{ob}* mice, *HMGA2* and *Lep* seem to function via independent genetic pathways. It has been proposed that *HMGA2* regulates the proliferation of undifferentiated pre-adipocytes: the inability of these cells to proliferate in absence of *Hmga2* could explain the reduced adipose tissue mass in *Hmga2*^{-/-} mice. This conclusion is also supported by data showing that the suppression of *Hmga2* expression by antisense technology suppresses the growth of 3T3-L1 pre-adipocytic murine cells (Battista *et al.*, manuscript in preparation). The model-system of mouse 3T3-L1 fibroblasts, which rapidly differentiate to adipocytes upon treatment with several agents (119), has been used to better define *HMGA1* involvement in adipocytic growth and differentiation (120). *HMGA1* gene expression was induced soon after differentiation of the pre-adipocytic 3T3-L1 cell line. Suppression of *HMGA1* expression by antisense technology dramatically increased growth rate and impaired adipocytic differentiation in these cells. Moreover, the authors show that *HMGA1* proteins physically interact C/EBP β and C/EBP α (transcription factors involved in adipocytic cell differentiation) and bind to their specific binding sites on DNA, suggesting a functional cooperation between *HMGA1* proteins and these proteins. Moreover, *HMGA1* proteins enhance the transactivation of a c/EBP β responsive promoter, such as the leptin promoter, an adipocytic specific gene. Taken together, these results indicate that *HMGA1* proteins exert a critical role in adipocytic cell growth and differentiation (120).

From these data, the role of *HMGA1* seems to be multiform, depending on the cellular context. Indeed, *HMGA1* proteins have been often associated with cell proliferation and transformation: in fact *HMGA1a/b* have been found over-expressed in several experimental and human malignant tumors, and *HMGA1a* over-expression causes transformation of Burkitt's lymphoma cells. The hypothesis that the different cellular context may account for the different effects of the *HMGA1* gene over-expression, is supported by recent data showing that *HMGA1* over-expression impairs the growth of normal PC Cl 3 rat thyroid cells by inducing apoptosis. In our opinion, this hypothesis seems to be likely as several genes involved in the control of cell proliferation can induce different biological effects, such as cell growth, differentiation and apoptosis, depending on the cellular context.

Conclusions

The block of the synthesis of the *HMGA1a/b* proteins, by the expression of an antisense construct, induced a drastic increase in the growth rate of the 3T3-L1 cells. Moreover, preliminary data indicate an enormous increase in fat tissue in heterozygous-*Hmga1* knockout mice [the *Hmga1*^{-/-} status is lethal at the embryonic level (Fedele *et al.*, manuscript in preparation)]. These results would indicate that *Hmga1a/b* proteins exert a

negative role on adipocytic cell growth. Conversely *Hmga2* expression seems to be necessary for physiological proliferation of adipocytes. Indeed, *Hmga2* knockout mice display a pygmy phenotype with a remarkable reduction of the adipose tissue (55) and disruption of *Hmga2* gene prevents both diet- and gene-induced obesity (54). Moreover, the suppression of *HMGA2* synthesis blocks the proliferation of the 3T3-L1 pre-adipocytic cells (Bañista *et al.*, in preparation).

These data would suggest that the regulation of adipocytic cell proliferation could result from the balance of *HMGA1a/b* and *HMGA2* protein functions: a gain in *HMGA2* protein activity induces adipocyte cell hyperproliferation, whereas the dominance of *HMGA1a/b* has an opposite effect. Therefore, even though the mechanisms underlying the counter-acting activity of *HMGA1a/b* and *HMGA2* proteins needs to be defined, it is reasonable to think that any modification of this balance, by impairing the *HMGA1a/b* activity or by increasing the *HMGA2* function, may affect adipocytic cell growth resulting in the generation of lipomas.

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