Misexpression of Disrupted HMGI Architectural Factors Activates Alternative Pathways of Tumorigenesis

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ABSTRACT

Cancer arises from aberrations in the genetic mechanisms that control growth and differentiation. HMGI-C and HMGI(Y) are members of the HMGI family of architectural factors expressed in embryonic or undifferentiated cells and highly associated with transformation. Translocations of 12q13–15 in lipomas (fat cell tumors) disrupt HMGI-C and fuse its DNA-binding domains to novel transcriptional regulatory domains. This study shows that in a rare, karyotypically distinct group of human lipomas, rearrangements of 6p21–23 produce internal deletions within HMGI(Y). Activation of the rearranged alleles leads to expression of aberrant HMGI(Y) transcripts in differentiated adipocytes. A molecular analysis of these transcripts demonstrates that fusion of HMGI DNA-binding domains to putative transcriptional regulatory domains was not necessary for lipoma formation. However, such fusions may facilitate tumor development because activation of the wild-type HMGI allele, normally required for tumorigenesis, is bypassed in lipomas which express chimeric HMGI proteins. We hypothesize that HMGI misexpression in a differentiated cell is a pivotal event in benign tumorigenesis, and the molecular pathway of tumor development depends upon the precise nature of HMGI disruption.

INTRODUCTION

Elucidation of the genetic mechanisms that control growth and differentiation (1) have led to a greater understanding of mammalian development and its various abnormalities, including, most prominently, cancer (2, 3). A number of genes that function both in development and transcriptional regulation (4) are known to be mutated in tumor cells. In addition to the well-documented role of c-myc in B-cell ontogeny and leukemias (5), other examples include the c-rel protein, expressed in the hematopoietic organs of the mouse embryo (6), as well as the oncproteins ets-1 and ets-2, which act during development of lymphoid and neuronal tissues (7).

Recently, the embryonically expressed accessory transcription factor HMGI-C was shown to be disrupted in human fat cell tumors, lipomas. HMGI-C is a member of the HMGI family of architectural factors (8), which at present consists of two members, HMGI-C and HMGI(Y). The important structural feature shared by both HMGI proteins is the presence of three DNA-binding domains, termed AT-hooks because of their ability to bind to AT-rich DNA in the minor groove (9). A series of elegant experiments with HMGI(Y) at the human IFN-β promoter have demonstrated its involvement in transcriptional regulation (10). HMGI(Y) was shown to be an essential component of the enhanceosome (11), a higher order transcription enhancer complex that forms when several distinct transcription factors assemble on DNA in a stereospecific manner (12). As an architectural component of the enhanceosome, HMGI(Y) promotes activation of gene expression by subtly modulating DNA conformation (12).

A number of studies, in particular with HMGI-C, have provided insight into the role of the HMGI family in growth and development. Hmgi-c is expressed exclusively during murine embryogenesis (13) and is undetectable in newborn and adult tissues (13, 14). Similarly, Hmgi(y) is predominantly expressed until 16.5 days post coitum in the mouse embryo (13). Inactivation of Hmgi-c produced a dramatic disruption of both pre- and postnatal growth, resulting in the pygmy phenotype (15). Pygmy mice exhibit significant growth retardation, which results in a 60% weight reduction compared to their wild-type littermates (16). Interestingly, Hmgi-c inactivation does not affect the growth hormone/insulin-like growth factor endocrine pathway (13), suggesting that Hmgi-c functions in a previously unknown growth-regulatory mechanism.

During development, the processes of proliferation and differentiation are often interdependent (17), and studies with the HMGI genes have implied that HMGI expression may be also associated with the biological, rather than only the proliferative, state of the cell (18). Expression of Hmgi-c and Hmgi(y) in a thyroid cell line is dramatically increased after transformation, although the rate of proliferation of the transformed cells does not increase (19, 20). Interestingly, several cell lines described in these reports differed markedly in their degree of transformation. A partially transformed cell line that retained its differentiated phenotype revealed low levels of Hmgi expression, whereas Hmgi expression was high in fully transformed cell lines that lost their differentiation markers (20). Finally, preliminary studies from our laboratory have demonstrated that in the mouse embryo, Hmgi-c is localized to less differentiated mesenchymal cells and is no longer present in their terminally differentiated counterparts (Ref. 13 and data not shown). In combination, these results indicate that the function of the HMGI proteins may be to maintain the undifferentiated cellular state (21).

A direct role for HMGI-C in tumorigenesis has been demonstrated by the analysis of several distinct types of solid human tumors (22, 23). Disruptions of HMGI-C were linked to the pathogenesis of a wide variety of benign mesenchymal neoplasms that have consistent rearrangements at chromosomal band 12q13–15 (24). Translocations of HMGI-C generate novel chimeric transcripts that consist of the HMGI-C DNA-binding domains fused to ectopic sequences provided by the translocation partner (22, 23). These novel sequences were predicted to encode for transcriptional regulatory domains, suggesting that aberrant transcription of the HMGI-C target genes contributes to tumor formation (22).

The diverse set of mesenchymal neoplasms in which HMGI-C is frequently disrupted by translocations of 12q13–15 includes lipomas, uterine leiomyomas, pulmonary hamartomas, and pleomorphic adenomas of salivary gland (22, 23). Another cytogenetic subgroup that can be identified in this set of tumors is characterized by rearrangements at 6p21–23 (24, 25). Intriguingly, HMGI(Y) has previously been localized to this chromosomal area (26). Conservation of the HMGI DNA-binding domains (9) and the cognate expression patterns of the HMGI proteins (13, 14) suggested Hmgi(y) as a likely candidate for involvement in mesenchymal neoplasia.
HMGI(Y) REARRANGEMENTS IN LIPOMAS

Identification and Characterization of Aberrant Transcripts in Lipomas

Lipoma specimens were obtained from patients at the time of surgery and processed as described (24). Karyotypes of lipomas were: ST88-08203 [46, XX, t(6;11)(p23;q13), t(11;12)(q13q22)] and ST92-24269 [46, XX, t(4;6)(q27;p21)]. Isolation of RNA from frozen tumors, reverse transcription, 3′RACE and specific RT-PCR were performed essentially as described (22). 3′RACE on RNA isolated from lipomas ST88-08203 and ST92-24269 was performed using an HMGI(Y)-specific exon five primer to amplify HMGI(Y) cDNA (Fig. 3). Sequencing of the aberrant transcript in ST92-24269 cDNA; and 391 bp from the aberrant transcript in ST88-08203 revealed a fully intact HMGI(Y) open reading frame. Further analysis demonstrated that this transcript removed 923 bp of the wild-type sequence from exon eight (Fig. 3). Interestingly, the rearrangement was limited to only the 3′UTR of the gene, leaving the coding sequence intact. Therefore, the aberrant transcripts isolated from the lipomas with rearrangements of 6p21–23 are produced by internal deletions within the HMGI(Y) gene. Although the translocations do not directly disrupt the HMGI(Y) gene, the existence of submicroscopic molecular aberrations in the close proximity to a chromosomal translocation site is often observed (30).

MATERIALS AND METHODS

Isolation and Analysis of Aberrant HMGI Transcripts. Tumors obtained from patients at the time of surgery were processed for culturing and karyotyped as described previously (24). A molecular analysis was performed on lipomas ST92-24269 (t4;6) and ST88-08203 (t6;11) with translocations involving 6p21–23 (24). RNA was isolated directly from frozen tumor samples (27), and 3′RACE (28) was performed with an HMGI(Y)-specific exon five primer to amplify HMGI(Y) transcripts to give an expected wild-type product of approximately 1.5 kb. Surprisingly, aberrant HMGI(Y) products of 592 and 591 bp were observed in tumors ST92-24269 and ST88-08203, respectively (Fig. 1A). At the same time, HMGI-C expression was not detected in these tumors, and both HMGI(Y) and HMGI-C genes were not expressed in normal, adult fat (Fig. 1B). Sequencing of the aberrant HMGI(Y) cDNAs revealed heterologous sequences that followed the 5′ end of HMGI(Y) in both lipomas. The presence of aberrant transcripts in ST92-24269 and ST88-08203 was confirmed by an independent RT-PCR in which a novel sequence- specific reverse primer, rather than oligo(dT), was used for reverse transcription and subsequent PCR (Fig. 2).

The anomalous HMGI(Y) cDNAs were further characterized by a detailed molecular analysis. In lipoma ST92-24269, comparison of the novel sequence to the GenBank database revealed that it was derived from the 3′UTR of wild-type HMGI(Y) (Fig. 3). PCR analysis of the genomic DNA from tumor ST92-24269 determined that the transcript was produced by an internal deletion of both exonic and intronic sequences (data not shown) that removed 922 bp from the wild-type HMGI(Y) cDNA (Fig. 3). Sequencing of the aberrant transcript in lipoma ST88-08203 revealed a fully intact HMGI(Y) open reading frame. Further analysis demonstrated that this transcript removed 923 bp of the wild-type sequence from exon eight (Fig. 3). Interestingly, the rearrangement was limited to only the 3′UTR of the gene, leaving the coding sequence intact. Therefore, the aberrant transcripts isolated from the lipomas with rearrangements of 6p21–23 are produced by internal deletions within the HMGI(Y) gene. Although the translocations do not directly disrupt the HMGI(Y) gene, the existence of submicroscopic molecular aberrations in the close proximity to a chromosomal translocation site is often observed (30).

RESULTS

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Fig. 1. HMGI expression in human lipomas with chromosomal rearrangements of 6p21–23. In A, 3′RACE with HMGI(Y)-specific primers on RNA from lipomas ST92-24269 and ST88-08203 yields aberrant 592- and 591-bp products, respectively; the predicted wild-type 1.5-kb fragment is amplified only in ST88-08203. No detectable expression of HMGI(Y) was observed in normal s.c. fat (Fat), which is mainly composed of differentiated adipocytes (24). OLD-i is a colorectal adenocarcinoma cell line that expresses wild-type HMGI(Y) and HMGI-C (29). Previously, aberrant transcripts involving HMGI(Y) were observed in differentiated adipocytes (24). Expression of HMGI(C) was observed in normal s.c. fat (Fig. 1B). Sequencing of the aberrant HMGI(Y) cDNAs revealed heterologous sequences that followed the 5′ end of HMGI(Y) in both lipomas. The presence of aberrant transcripts in ST92-24269 and ST88-08203 was confirmed by an independent RT-PCR in which a novel sequence-specific reverse primer, rather than oligo(dT), was used for reverse transcription and subsequent PCR (Fig. 2).

Fig. 2. Sequence-specific RT-PCR confirms the presence of HMGI(Y) aberrant products in RNA isolated from lipomas with rearrangements of 6p21–23. RT-PCR products were amplified using primers located on either side of the deletion within HMGI(Y). Expected product sizes are: 1314 bp from wild-type transcript; 392 bp from the aberrant transcript in ST92-24269 cDNA; and 391 bp from the aberrant transcript in ST88-08203 cDNA. In ST92-24269 and ST88-08203, 359- and 358-bp bands, respectively, are produced by the alternative splicing of HMGI(Y) (26).
**Distinct Features of the Proteins Encoded by the Aberrant HMGI Transcripts.** In lipoma ST92-24269, the predicted HMGI(Y) fusion protein consists of the first two DNA-binding domains of HMGI(Y) fused in frame to an uninterrupted ORF encoding for 108 amino acid residues (Fig. 4). A detailed examination of the ORF revealed an unusually high content of proline (17%), which is indicative of a potential transcriptional regulatory domain (31). Therefore, the overall structure of this HMGI(Y) fusion protein is remarkably similar to proteins produced by disruptions at 12q13–15 that juxtaposed the DNA-binding domains of HMGI-C to putative transcriptional regulatory domains (22).

In the tumor ST88-08203, translation of the HMGI(Y) aberrant transcript predicted a normal protein. This is in contrast to the lipomas described previously (22, 23), where chimeric HMGI-C transcripts encoded for novel fusion proteins, the formation of which was proposed to be necessary for lipoma development (22). Furthermore, disruption of HMGI-C by t(12;13) in lipoma ST91-198 (22) resulted in a truncated protein that consists mainly of the HMGI-C AT-hooks (data not shown). We conclude that fusion of transcription regulatory domains to the DNA-binding domains of disrupted HMGI proteins is not required for lipoma formation.

**Lipomas Can Bypass Expression of the Wild-Type HMGI Allele.** Expression of the wild-type HMGI proteins is highly associated with transformation (20, 32) and can be detected in a wide variety of tumors. Moreover, inhibition of HMGI-C synthesis was shown to render several distinct cell types intrinsically to retrorival transformation, suggesting that HMGI expression is required for tumorigenesis (33). In agreement with this hypothesis, appreciable levels of wild-type HMGI(Y) expression were observed in tumor ST88-08203 (Figs. 1 and 2). Surprisingly, the nonrearranged allele was not expressed in lipoma ST92-24269 (Figs. 1 and 2), where an HMGI(Y) fusion protein was identified.

Next, we examined expression of wild-type HMGI-C in lipomas with rearrangements of 12q13–15 that fuse HMGI-C DNA-binding domains to heterologous sequences (22). Again, the nonrearranged allele was expressed in tumor ST91-198 (data not shown), where t(12;13) produced a truncated HMGI-C but not in lipoma ST93-724 (data not shown), in which HMGI-C DNA-binding domains were fused to LIM transcriptional regulatory domains (22, 34).

**DISCUSSION**

**Rearrangements within HMGI Genes Are Required for Lipoma Development.** The studies on HMGI-C and HMGI(Y) now provide insights into the molecular mechanism of tumor formation in lipomas and, by extrapolation, in related solid mesenchymal neoplasms. In all 12 analyzed lipomas (22, 23), chromosomal rearrangements have produced disruptions in translocated HMGI alleles. An aberration common to these tumors is a deletion of, or within, the highly conserved and unusually large 3'UTR that is a distinguishing feature in HMGI genes (35). The best example is lipoma ST88-08203, where the aberrant transcript codes for the wild-type HMGI(Y) protein, and the deletion is restricted to the 3'UTR. Rearrangements of the 3'UTR of HMGI-C have also been observed in leiomyoma and pleomorphic adenoma of salivary gland (23). This suggests that 3'UTRs of the HMGI genes may contain sequences required for the regulation of HMGI expression and that disruptions of the 3'UTRs are necessary for the activation of the disrupted allele. We conclude that the minimal requirement for tumor formation is a disruption within an HMGI gene that involves a deletion within the 3'UTR.

The aberrant transcripts isolated from lipomas with rearrangements of 6p21–23 were generated by internal deletions within the translocated HMGI(Y) allele. This observation suggests that in lipomas and related benign mesenchymal tumors, HMGI genes may contain internal deletions and other submicroscopic rearrangements that would be undetectable by cytogenetic techniques. Therefore, the contribution of the HMGI genes to tumorigenesis is likely to be underestimated and more significant than thought previously.
**Misexpression of HMG1 Genes in a Differentiated Cell Results in Tumorigenesis.** Lipomas are composed of mature adipocytes (24) that, similar to other terminally differentiated cells, normally do not express HMG1 proteins (13, 14). However, transcriptionally active 12q13—15 or 6p21—23 activate an HMG1 allele normally silent in adult cells, and the resulting misexpression of the HMG1 protein in the context of a differentiated mesenchymal cell is a crucial step in tumor development. In this model, tumorigenesis results from the temporally inappropriate expression in an adult cell of a gene that is normally expressed during prenatal development in an embryonic cell of the same lineage.

These observations are somewhat similar to the studies in B-cell leukemias where activation of c-myc expression in a precursor of the B-cell lineage results in neoplasia (5). Unlike the HMG1 family members, however, the endogenous expression of c-myc is not restricted to embryogenesis, and its inappropriate expression takes place at the same time in the life of the organism when it is normally expressed (36). In greater contrast to the misexpression of HMG1 proteins in lipomas is the ectopic expression of HOX11 in a subset of T-cell acute lymphoid leukemias, which result from activation of HOX11 in cells of the inappropriate lineage (37).

**Distinct Molecular Pathways of Tumorigenesis Exist in Lipomas.** The molecular analysis of the lipomas described above yields valuable information about the expression state of the nonrearranged HMG1 alleles. Wild-type HMG1 expression, normally associated with tumorigenesis (20, 32), was readily detectable in lipomas ST88-08203 and ST91-198, where chromosomal rearrangements resulted in normal HMG1(Y) and truncated HMG1-C proteins, respectively. In contrast, the nonrearranged HMG1 allele was not expressed in tumors ST92-24269 and ST93-724, where the aberrant HMG1 transcripts encoded for proteins consisting of the HMG1 DNA-binding domains fused to putative transcriptional regulatory domains.

Lack of expression of the nonrearranged HMG1 allele in lipomas in which chimeric transcripts encode for transcriptional regulatory domains can now be combined with results reported previously (22, 23) into a mechanistically coherent model of lipoma development (Fig. 5). Although speculative at this stage, tumor development appears to be initiated when the chromosomal rearrangement disrupts an HMG1 allele, resulting in HMG1 misexpression in a differentiated mesenchymal cell. A deletion within the 3'UTR is probably the minimal rearrangement necessary for tumor formation. Subsequently, one of the alternative tumorigenic pathways is selected based on the precise nature of the HMG1 disruption. When a chromosomal rearrangement produces an HMG1 protein with no intrinsic transcriptional activity, tumor development is dependent upon subsequent activation of the nonrearranged allele. However, the requirement for HMG1 expression in tumorigenesis (33) is circumvented when, as a result of a translocation, HMG1 DNA-binding domains are fused with a transcriptional regulatory domain (Fig. 5). The reduced number of events involved in tumor formation would readily explain the most frequently observed translocation in lipomas, t(3;12)(q29;q15) (25, 38), because it juxtaposes DNA-binding domains of HMG1-C with LIM domains that are involved in transcriptional regulation (34). The alternative explanations for activation of the nonrearranged allele by an HMG1 autoregulatory mechanism is unlikely because it would postulate that the presence of transcriptional regulatory domains in the fusion proteins found in lipomas ST92-24269 and ST93-724 interferes with a putative autoregulatory function. In conclusion, distinct rearrangements of a single gene can activate alternative molecular pathways of tumor pathogenesis.

**HMG1(Y) and HMG1-C, two distinct members of the embryonically expressed HMG1 family of architectural factors (39), have now been...**
shown to be disrupted in identical tumors. Rearrangements of HMGI-C, first reported in lipomas (22), were later described in other mesenchymally derived neoplasms with translocations of 12q13–15 (23). We predict that, similar to HMGI-C, disruptions of HMGI(Y) will be also responsible for uterine leiomyoma, pulmonary hamartoma, pleomorphic adenomas of salivary gland, and other mesenchymal tumors with recurrent aberrations at 6p21–23 (24–26). Previously, aberrations of 12q13–15 were reported as the most frequent karyotypic abnormality in lipomas, whereas rearrangements of 6p21–23 occur rarely in these neoplasms (24, 25). Notably, HMGI-C encompasses a much larger genomic region (>100 kb; Refs. 22 and 6p21—23occur rarely in these neoplasms (24, 25). Notably, HMGI-C

Benign tumors, unlike their malignant counterparts, are characterized by one or only a few highly specific genetic alterations (40). Therefore, it has been proposed that the molecular analysis of these neoplasms would identify genes of major importance for growth and proliferation (41). The above studies with HMGI(Y) and HMGI-C in lipomas (22, 23) demonstrate the significance of the HMGI family in adipogenesis and tumor progression (42, 43).

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