Germ Line Mutations of the *HMGA2* Gene are Rare Among the General Population

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Abstract. *Proteins encoded by the HMGA family are architectural transcription factors, which induce conformational changes in the DNA and thus influence gene expression. Despite the obvious association of the expression of high mobility group protein genes with human cancer, very little is known about the variation of the HMGA proteins within human populations. Therefore, the coding regions of HMGA2 from 87 normal healthy donors were sequenced with the aim of detecting single nucleotide polymorphisms. There was only one sequence divergence leading to an amino acid change in coding regions of HMGA2. Thus, HMGA2 is not only well conserved between species but there is also a high intra-individual conservation of HMGA2, further supporting the important role of HMGA proteins in cellular processes. This analysis clearly demonstrates that as a rule, germ line mutations of HMGA2 are not the cause for benign tumors, e.g. uterine leiomyomas, or human malignant solid tumors.*

During the past ten years, the causal role of proteins of the high mobility group family HMGA in a number of diseases has emerged. HMGA proteins were known to be expressed in malignant tumors (1-5) and during embryonic development, while they were expressed at very low levels or even absent in adult normal tissues (6, 7). Nevertheless, the first clear evidence indicating their causal involvement in the development of tumors comes from the positional cloning of the breakpoints of recurrent translocations in benign tumors. By positional cloning of the 12q14-15 breakpoint region affected by structural chromosomal aberrations in a variety of benign tumors, *e.g.,* lipomas, pulmonary hamartomas, pleomorphic adenomas of the salivary glands and uterine leiomyomas, *HMGA2* was found to be the target gene (8, 9). Later, the other gene encoding HMGA proteins, *i.e.*, *HMGA1*, was shown to be affected by chromosomal aberrations in other subgroups of most of the latter tumors (10, 11). The prominent role of the HMGA proteins in proliferation of mesenchymal cells was clearly underlined later by two groups of experiments. Knock-out mice for the *HMGA2* gene (12) and double-knock-out mice for the leptin receptor and the *HMGA2* gene (13) indicated the role of *HMGA2* in fat cell proliferation. In addition to aberrant growth of mesenchymal cells, malignant epithelial growth can be decreased apparently by the suppression of *HMGA* gene expression. Scala *et al.* (14) were able to show that an adenovirus carrying the *HMGA1* gene in an antisense orientation induced programmed cell death of different tumor cell lines. Furthermore, a high HMGA protein level is characteristic for tumor phenotype of spontaneous and experimental malignant neoplasms. As a rule, high HMGA protein expression correlates with the presence of a highly malignant phenotype and worse prognosis (4, 15, 16).

Despite a number of interesting findings, very little is known about variation of the HMGA proteins within populations. The coding regions of *HMGA2* of a number of healthy volunteers were sequenced with the goal of detecting single nucleotide polymorphisms that may predispose to benign tumors and cancer.

Materials and Methods

Blood samples taken from 87 healthy volunteers were included in the present study. Genomic DNA was isolated from 5 ml peripheral blood by the salt-extraction procedure described by Lahiri and Nurnberger (17).

Specific oligonucleotide primers were designed according to the genomic HMGA2 DNA sequence (8) (GenBank accession number AH003120). Primer sets were designed for PCR to amplify each of the 5 exons of *HMGA2* (see Table I). PCR was performed in a reaction volume of 50 μl containing 500 ng of genomic DNA template, 10 mM Tris/HCl pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 100 μM dATP, 100 μM dTTP, 100 μM dGTP, 100 μM dCTP, 200 nM forward primer, 200 nM reverse primer, 1 unit/100 μl DNA Taq polymerase (Sigma, Deisenhofen, Germany). For the fragment containing exon 5, a nested PCR was performed by addition of 0.5 μl

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Table I. *Primer sequences and conditions for PCR amplification of HMGA2.*

Exon	Primer sequences[a]	Annealing temperature (˚C)	Size of product (bp)
1	(F) ACCTCATCTCCCGAAAGGTGCTG	66	290
	(R) ACTGGGCTCCCGCACTCCG		
2	(F) CCACCTTTTCAATATGGAAGATTGTTG	57	816
	(R) TTCCTCACTTCTACCCGTTTTAAGGTT		
3	(F) TCTTTTAGGGCGAGGGGTTGCATAGAT	57	553
	(R) GAAGAGTGGAGCCTCTTTTGATGTTGA		
4	(F) AGCTGGTCTACAACTCCTGGGCTCAAG	60	420
	(R) GGCTACAACACATTTTCAGAATACTGC		
5	(F1) TGCCCTGACATTCTCTGGACAGC	60	532
	(R1) TCCCTGTGTTACAGCAGTTTT		
	(F2) CCTCAATTACGGTTTAAGAAG	60	426
	(R2) AGGGATTACAAAGAAGGTGATTA		

[a]F=forward; R=reverse.

of the primary PCR product to a second PCR mix. PCR experiments were performed for 35 cycles (1 min denaturation at 94˚C, 1 min annealing at 57-66˚C for a single primer pair, see Table I and 1 min extension at 72˚C). The cycles were preceded by an initial denaturation step of 4 min at 94˚C and were followed by a final extension step of 10 min. The amplification products were separated on 1.5% agarose gels.

Each PCR fragment was sequenced on both strands using the primers shown in Table II on an automated ABI 377 sequencer according to the manufacturer's protocol (PE-Applied Biosystem, Connecticut, USA). The resulting sequences were aligned for the presence of mutations (nonsense, insertion, or deletion mutations) with the DNASTAR's SeqMan II program.

Table II. *Primer sequences for sequencing of HMGA2.*

Exon	Primer sequences[a]
1	(F) ACCTCATCTCCCGAAAGGT
	(R) ACTGGGCTCCCGCACTC
2	(F) TGAACGTGTTCCAACAGCTC
	(R) GAAATGAGTCTTGTGTTACCCAG
3	(F) CGATACGTCATCTGCAAAGC
	(R) CTGGATGGAGCCTAGTGGTA
4	(F) CTCAAGCCATCCGTCCGC
	(R) TTCAGAATACTGCAGGACGA
5	(F) GCTGTGGAAACAGGTTACCT
	(R) CCATGGCAATACAGAATAAGTGG

[a]F=forward; R=reverse.

Results

All five exons of *HMGA2* containing the complete ORF (open reading frame) were amplified by PCR. Using the DNA of 87 individuals, all PCR experiments resulted in fragments of the expected sizes (Table I). Direct sequencing revealed only one G→A point mutation in exon 1 of *HMGA2*, which leads to a substitution of glycine with glutamic acid at position 9 of the protein.

Discussion

Proteins encoded by the *HMGA* family are architectural transcription factors whose interaction with the minor groove of AT-rich DNA sequences leads to conformational changes in the DNA. Due to this chromatin remodelling, HMGA proteins can affect the assembly of transcriptional complexes, thus influencing gene expression. This function of HMGA proteins explains their involvement in a number of diseases including various malignancies and several benign tumors. Although somatic mutations in *HMGA*-genes have been associated with several diseases, relatively few examples exist of germ line mutations in these genes. Ligon *et al.* (18) described an 8-year-old boy with a *de novo* pericentric inversion of chromosome 12 that was found to truncate *HMGA2*, with breakpoints at p11.22 and q14.3 and a phenotype including extreme somatic overgrowth, advanced endochondral bone and dental ages, a cerebellar tumor and multiple lipomas.

As to the regulation of *HMGA2*, a functional polymorphic dinucleotide repeat (TCTCT$(TC)_n$) 500 bp upstream of the ATG translational start codon was found to regulate the human *HMGA2* promoter with an activation pattern that correlates to its TC-repeat length (19). Based on the concept that frequent single nucleotide polymorphisms of that gene exist that are associated with genetic predisposition for diseases characterized by abnormal allele proliferation, we herein analyzed 174 alleles of the *HMGA2* gene. In the complete ORF of *HMGA2*, there was only one sequence divergence leading to an amino acid change. Most likely, the high intra-individual conservation of the protein is due to the fact that most base pair substitutions leading to an amino acid exchange lead to

proteins which do not behave like the wilde-type protein. This hypothesis is also supported by comparative genomics. The coding region of the murine *HMGA2* shows an identity of 92% in comparison to its human counterpart, allowing for the expression of a protein which differs in only 5 out of 110 amino acids (96% identity) (20, 21). However, this is the first report of the sequence analysis of the *HMGA2* gene in a larger number of individuals. From the results, it can be clearly deduced that sequence variations of *HMGA2* are not only well conserved between species but there is also a high intra-individual conservation of *HMGA2,* further supporting the important role of conserved HMGA proteins in cellular processes.

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