Expression of Reciprocal Hybrid Transcripts of HMGIC and FHIT in a Pleomorphic Adenoma of the Parotid Gland

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Abstract

The developmentally regulated HMGIC gene, which encodes an architectural transcription factor, has recently been linked to the pathogenesis of benign solid tumors with chromosome aberrations involving 12q13-15. Among these tumors are pleomorphic adenomas of the salivary glands, lipoma, uterine leiomyoma, hamartomas of the breast and lung, fibroadenoma of the breast, angiomyxoma, and endometrial polyps. For most tumor types, however, the translocation partners are variable. At present, no translocation partner genes of HMGIC are known for pleomorphic adenomas. Here, we report that in a primary pleomorphic adenoma of the parotid gland, the FHIT gene, which spans the chromosome 3p14.2 fragile site FRA3B, and is frequently disrupted in tumors, acts as a fusion partner of HMGIC. In addition to normal HMGIC and FHIT transcripts, an HMGIC/FHIT hybrid transcript as well as its reciprocal counterpart, FHIT/HMGIC, were found to be expressed by reverse transcription-PCR. The results establish the concurrent disruption of two tumor-associated genes in a benign tumor.

Introduction

Pleomorphic adenoma is the most frequent benign tumor of the major and minor salivary glands (1) and about 50–80% of these tumors carry chromosome aberrations (2, 3, and references therein). In addition to a subgroup of tumors with apparently normal karyotypes, there are two major subgroups with abnormal karyotypes, i.e., tumors with rearrangements of 8q12 or 12q13-15. Whereas a t(3;8)(p21;q12) is the most frequently observed translocation, many different chromosome segments have been found as translocation partners of both 8q12 and 12q13-15. In the context of a comprehensive search for genes involved in tumor-specific chromosome translocations, we recently identified the HMGIC gene as the target for the 12q13-15 translocations observed in pleomorphic adenomas of the salivary glands and a variety of other benign tumors, including ordinary lipomas, uterine leiomyomas, hamartomas of the breast and lung, fibroadenomas of the breast, angiomyxomas, and endometrial polyps (4, 5). Most breakpoints within the HMGIC gene were found to cluster within the large third intron, which separates the first three exons, encoding the putative DNA-binding domains of HMGIC, from exons 4 and 5, which encode the acidic carboxyl-terminal part of the protein. The FHIT gene is a member of the HMG1 protein gene family (6, 7). The HMGIC protein possesses three AT hooks through which the protein is assumed to bind in the minor groove to appropriately spaced AT tracts in double-stranded DNA. Functionally, the HMGIC protein is thought to act as an architectural factor in the nuclear scaffold, critical for the correct assembly of stereospecific transcriptional complexes (7, 8). Interestingly, gene-targeting studies have indicated that inactivation of the mouse Hmgic gene results in the pygmy phenotype, suggesting an important role of this gene in mammalian growth and development (9).

Thus far, only three fusion partner genes of HMGIC have been identified. The first one is the LPP gene (4, 10), which is located at chromosome 3q27-q28 and encodes a LIM-only protein. LIM proteins are found widespread in nature, from plants to mammals, and have a function in cell signaling and developmental regulation. The second one is a postulated gene which has not yet been characterized in detail but which is predicted to encode for a protein with a serine/threonine-rich domain (5). The third one is the mitochondrial aldehyde dehydrogenase ALDH2 gene, which was found as a translocation partner gene of HMGIC in a uterine leiomyoma (11). In pleomorphic adenoma of the salivary glands, no fusion partner genes have been identified yet. The pathogenetic relevance of the fusion partners remains to be elucidated. The high diversity in chromosomal segments that have been found to participate in translocations could indicate that the critical sequences are abundantly present in the human genome. It has not yet been possible to identify a common structural or functional denominator from the available data of these translocation partner genes.

Here, we report the identification and characterization of the first example of a translocation partner gene of HMGIC in a pleomorphic adenoma of the salivary glands. Using 3' RACE experiments, ectopic sequences fused to HMGIC were isolated. Subsequently, the chromosomal location of these sequences was determined via chromosome assignment using somatic cell hybrids and FISH analysis. Molar characterization of these ectopic sequences led to the identification of the tumor-associated FHIT gene (12-16) as fusion partner of HMGIC.

Materials and Methods

Tumor Material and Cytogetic Analysis. A 36-year-old female sought medical attention because of a swelling over the left parotid gland. The tumor (designated CG992), which measured 1.0 X 1.5 X 1.0 cm, was radically removed by superficial parotidectomy. It had a completely benign histological appearance, typical of pleomorphic adenoma. There were no signs of atypia or malignant transformation in any of the sections examined. Primary cultures were established from fresh, unfixed tumor tissue as described previously (17). Chromosome preparations were made from exponentially growing primary cultures and these were subsequently G-banded and analyzed according to the 1995 guidelines of the International System for Human Cytogenetic Nomenclature. 

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YAC Clones and FISH Analysis. YAC clones were isolated from the CEPH human genomic YAC libraries mark 1 and 3 using a combination of PCR-based screening and colony hybridization analysis as described previously (4). YAC clones are indicated by their unique microtiter plate addresses. FISH analysis was performed as described previously (18). The following FISH probes were used: whole chromosome painting and a satellite probes for chromosomes 3, 10, and 12; ML12q1315 (microdissection library specific for 12q13-15), YAC clones 145F7, 405C8, and 850A6 (containing different parts of the FHIT gene); and LL12N01-derived cosmids 142H1 and 27E12 (containing the 5' and 3' parts of HMIGC, respectively; Ref. 4).

Nucleotide Sequence Analysis. Nucleotide sequences were determined according to the dideoxy-mediated chain termination method using the T7 polymerase sequencing kit from Pharmacia/LKB or the dsDNA Cycle Sequencing System (Life Technologies, Inc.). Sequencing results were analyzed using an A.L.F. DNA sequencer (Pharmacia Biotech) on standard 30 cm, 6% Hydrolink, Long Range gels (AT Biochem). Sequence data were evaluated using Lasergene Biocomputing Software for Windows (DNASTAR, Inc., Madison, WI, United Kingdom).

RACE and RT-PCR Analysis. 3' RACE was performed using the 3' exon trapping protocol of Life Technologies, Inc. with slight modifications. For first-strand cDNA synthesis, adapter primer (AP2; 94M2363)5'-AAG GAT CCG TCG ACA TCA(T)3'-3' was used. For both initial and secondary rounds of PCR, the universal amplification primer (UAP2; 95L736)5'-CUA CUA CUA CUA AAG GAT CCG TCG ACA TCA(T)3'-3' was used as reverse primer. In the first PCR round, the following HMIGC-specific forward primers were used: 94N1501, 5'-CTT CAG CCC AGG GAC AAC-3' (exon 1); 94N701, 5'-CAA GAG GCA GAC CTA GGA-3' (exon 3); or 94M1892, 5'-AAC AAT GAT CAG AAG AAG CCF GCT-3' (exon 4). In the second round, the following HMIGC-specific forward primers (nested primers as compared to those used in the first round) were used: 94N1502, 5'-CAU CAU CAU CAU CGC TCC AGA AGA GAG GAC-3' (exon 1); 94N700, 5'-CAU CAU CAU CAU GGT CAG AAG AAG CCT GCT-3' (exon 4); or 94M1908, 5'-CAU CAU CAU CAU TGG ATC TGA TAA GCA AGA GTG GG-3' (3'-UTR). RT-PCR was carried out as described by Krizman and Berget (19). Briefly, cDNA was synthesized from 5 µg of total RNA. RT was performed according to the first-strand cDNA synthesis protocol from Life Technologies, Inc. using FHIT-specific cDNA synthesis primer (96C2322) 5'-TGC CTG TCT GAG CCG TTT AGG T-3' and HMIGC-specific cDNA synthesis primer (95C3362) 5'-TAC AGT TTG TTA CTA CTA-3'. Specific primers used in the various experiments are listed in the legend to Fig. 2. The PCR products were subcloned in a TA-cloning kit vector and subsequently used for sequencing.

RNA Isolation and Northern Blot Analysis. For 3' RACE and RT-PCR analysis, total RNA was isolated using the guanidinium thiocyanate extraction method described by Chomczynski and Sacchi (20). For Northern blot analysis, commercially obtained Multiple Tissue Northern Blots (Clontech) containing poly(A)-selected RNA from adult human tissues were hybridized according to standard procedures.

Results and Discussion

To study the possible role of translocation partner genes of HMIGC in benign solid tumors, we have analyzed a case of pleomorphic adenoma of the parotid gland. Cytogenetic analysis of short-term cultured tumor cells revealed a pseudodiploid karyotype with three complex marker chromosomes, i.e., a 3p−, a 10p + q−, and a 12p−q− marker (Fig. 1A). Detailed comparison of the G-banding pattern in combination with FISH analysis enabled us to determine the origin and nature of these three markers. FISH analysis using the ML12q1315 microdissection library as a probe revealed that sequences from the 12q13-15 segment were deleted from one chromosome 12 homologue and juxtaposed to the short arms of the der(3) and der(10) (Fig. 1B). The 12q sequences were inserted close to pter of the der(3). FISH analysis using cosmids 142H1 and 27E12, which correspond to the 5' and 3' parts of the HMIGC gene, respectively, indicated that the complete HMIGC gene was inserted into the der(3). Hybridization signals were only observed on the normal chromosome 12 and the der(3) (Fig. 1C). There was no translocation of sequences from chromosomes 3 or 10 to any other chromosome as determined.
3pter ← 3p14.2 → cen(3)
Sal Sfi
single YAC coverage
405C8
145F7
275A12
405C8
145F7
275A12
850A6
850A6
20 kb

Fig. 3. A, long-range physical map of a 740-kb genomic DNA region located at human chromosome segment 3p14.2. Data have been deduced from a YAC contig consisting of four overlapping CEPH YAC clones. The long-range physical map of the composite genomic DNA covered by the YAC inserts is represented by a solid line with the relative positions of the various restriction sites of rare-cutting enzymes indicated. The DNA region in which additional SfiI sites are present is indicated by arrows. Below the long-range physical map, the sizes and relative positions of the overlapping YAC clones are represented by solid lines. The DNA marker D3S1234 is shown in a box, and its relative position within the restriction map as well as that of the translocation breakpoint in intron 8 of FHIT (asterisk) are indicated. Unique CEPH microsatellite marker addresses of the YAC clones are listed. STS CH223, which is located in the 3' UTR of FHIT, corresponds to part of the ectopic sequences found in the HMGIC fusion transcript obtained from pleomorphic adenoma C0592. Restriction sites: B, BssHII; K, KspI; M, SalI; SfiI; Sfi. spliced mRNA isoforms or correspond to two different genes with closely related regions remains to be established.

To establish the chromosomal origin of the ectopic sequences, chromosome assignment using somatic cell hybrid analysis was performed using the National Institute of General Medical Sciences human/rodent somatic cell hybrid mapping panel 2 (Coriell Cell Repositories). These studies revealed that they were derived from chromosome 3. To more precisely map these sequences, the CEPH map and 3 YAC libraries were screened with PCR probe pCH223, which corresponds to the ectopic sequences. This resulted in the isolation of three CEPH-A YAC clones (145A7, 275A12, and 405C8) and 13 mega-YAC clones (750F1, 752F4, 752F5, 768A7, 768B7, 768D2, 775B3, 775B6, 805F12, 850A6, 894H3, 918A4, and 944D4). A YAC query at http://www-genome.wi.mit.edu/cgi-bin/contig/phys_map showed that these mega-YACs all mapped within M.I.T. YAC contig WC-3.10 located at 3p14.2-21. A long range physical map was constructed with the overlapping YAC clones 145F7, 275A12, 405C8, and 850A6 and, subsequently, the relative position of the ectopic sequences (pCH223) within this map was established (Fig. 3A). FISH analysis of normal metaphase chromosomes using YAC clone 850A6 as a molecular probe confirmed the localization of this YAC to 3p14. Using FISH and cosmids corresponding to the 5' and 3' parts of HMGIC, we could demonstrate that both parts of the gene were inserted within the 3p14 region. The rearrangement of HMGIC resulting in the expression of a fusion transcript cannot easily be explained by a simple insertion. Additional intrachromosomal rearrangements including e.g., inversions and/or deletions, must have occurred as well.

While this study was in progress, a routine BLAST search by E-mail at blast@ncbi.nlm.gov revealed that the ectopic sequences fused to HMGIC sequences were identical to those of the human FHIT gene (fragile histidine triad gene). The FHIT gene was recently shown to span the chromosome 3p14.2 fragile site and the renal carcinoma-associated t(3;8) translocation breakpoint (12) and encodes a dinucleotide 5'-5''-P1-P3-triphosphate hydrolase. Comparative evaluation of the HMGIC fusion transcript and the published genomic organization and nucleotide sequence data of the FHIT gene (12) showed that the first three exons of the HMGIC gene, encoding the three AT hook domains, were fused to exons 9 and 10 of the FHIT gene, which encode the last 31 carboxyl-terminal amino acids of this gene (Fig. 2B). FISH analysis using YACs 145F7, 405C8, and 850A6 (containing different parts of the FHIT gene; Fig. 3) revealed signals only on the normal chromosome 3 and the der(3) (Fig. 1D). This observation along with the HMGIC FISH data indicate that both fusion genes are located on the der(3). Expression of the HMGIC/FHIT fusion transcript was also confirmed by RT-PCR. Using the appropriate HMGIC- and FHIT-specific primers, RT-PCR resulted in a product of 481 bp (Fig. 2A, Lane 3). The reciprocal FHIT/HMGIC transcript was also expressed as indicated by the presence of a 686-bp RT-PCR product (Fig. 2A, Lane 4). These results clearly show that the HMGIC and FHIT genes are reciprocal fusion partners in this pleomorphic adenoma.

The detection of HMGIC/FHIT and FHIT/HMGIC transcripts in the tumor raised the question as to whether normal transcripts of these genes were also expressed. Results of RT-PCR analysis using the appropriate primer sets (Fig. 2) suggested that indeed this was the case. RT-PCR products of 518 and 602 bp were detected which correspond to normal HMGIC (Fig. 2A, Lane 2) and FHIT (Fig. 2A, Lane 5) transcripts, respectively. Nucleotide sequence analysis revealed that the coding regions of both RT-PCR products had the wild-type configuration (data not shown).

Our finding of reciprocal fusion transcripts between HMGIC and FHIT in a primary pleomorphic adenoma of the parotid gland represents the second example in which FHIT is disrupted by a tumor-specific chromosome translocation. The first one being the recently described hereditary renal cell carcinoma associated t(3;8)(p14.2;q24) translocation (12). FHIT is also the first known translocation partner gene of HMGIC in pleomorphic adenomas. Interestingly, chromosome 3p is frequently affected in pleomorphic adenomas as a result of a recurrent t(3;8)(p21;q12) which is the most common chromosome abnormality in this tumor type (2, 3). To test whether FHIT may be the target of these translocations, we hybridized two FHIT-containing YACs (850A6 and 750F1) to pleomorphic adenomas with t(3;8). Both YACs were clearly proximal to the 3p breakpoint (data not shown), demonstrating that FHIT is not the preferential translocation partner gene of 8q12 in pleomorphic adenomas. The breakpoint in the FHIT gene in the present case was found in intron 8, downstream of the sequences encoding the highly conserved functionally critical histidine-triad domain, resulting in the exchange of 31 carboxyl-terminal amino acids of the FHIT protein by 26 amino acids of the acidic tail of HMGIC. To what extent the physiological function of wild-type FHIT is affected remains to be established. Furthermore, it should also be noted that, due to the reciprocal exchange, the putative mRNA destabilizing AUUUA motifs, which are present in the 3' UTR of HMGIC, become linked to the hybrid FHIT/HMGIC transcript. This could result in a decrease in the physiological levels of FHIT, a scenario also observed in a number of breast carcinomas (15). Finally, no common functional domain in the presently well-known translocation partners of HMGIC, including the case presented here, is exchanged for particular 3' HMGIC sequences (4, 5, 10, 11). A common denominator, however, seems to be that the rearrangements...
of HMGIC invariably lead to separation of its DNA-binding domains from its acidic carboxyl-terminal tail, which is replaced by alternative sequences resulting in fusion proteins. These data suggest that such an alteration of the wild-type HMGIC protein is a crucial oncogenic event. On the other hand, we recently molecularly characterized a case of pleomorphic adenoma in which the HMGIC rearrangement occurred in the 3′ noncoding region of the gene, leaving the complete coding region of HMGIC intact but resulting in the removal of eight AUUUA motifs, which are potential mRNA-destabilizing motifs (21). The other HMGIC allele had the wild-type configuration. Furthermore, we recently obtained suggestive evidence that similar rearrangements in the 3′ noncoding region of HMGIC may also occur in lipoma and uterine leiomyoma. The latter observations suggest that alterations in the 3′ noncoding region of HMGIC might also be pathogenetically relevant. The possibility that other critical genes are affected in these cases can, however, not be excluded.

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