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Intron 3 of *HMGIC* is the Most Frequent Target of Chromosomal Aberrations in Human Tumors and Has Been Conserved Basically for at Least 30 Million Years

A particular region of chromosome 12, i.e. 12q14-15 is well known to be affected by chromosomal rearrangements in a large variety of human benign tumors, e.g., lipomas, pleomorphic adenomas of the salivary glands, uterine leiomyomas, and pulmonary chondroid hamartomas [1]. Recently, we were able to show that the breakpoints of these chromosomal abnormalities are often clustered within the third intron of the *HMGIC* gene coding for a member of the high mobility group family of proteins [2]. The third intron has a size of about 140 kb and separates the part of the gene encoding for the DNA binding domains from the 3′ part encoding for the acidic tail. By the structural rearrangements, the 5′ part of the gene becomes fused to ectopic DNA sequences derived from the different translocation partners of chromosome 12 [2, 3].

Some of the tumor entities with *HMGIC* rearrangements are most frequent, most likely making intron 3 of *HMGIC* the most frequent target of structural chromosomal abnormalities in such tumors. The molecular structure of the third intron of *HMGIC* and its evolutionary conservation are thus interesting questions as for comparative mapping. In this study we have used cosmid clones assigned to that third intron for mapping studies on the Old World monkey *Macaca mulatta*, two great apes, i.e., *Pongo pygmaeus* and *Gorilla gorilla*, and the New World monkey *Callithrix jacchus*.

For each of the four species 10 metaphases were cytogenetically analyzed. All four animals showed normal female (*Pongo pygmaeus*, *Macaca mulatta*) or male (*Gorilla gorilla*, *Callithrix jacchus*) karyotypes. For fluorescence in situ hybridization (FISH) on metaphase spreads of *Gorilla gorilla*, *Pongo pygmaeus*, and *Macaca mulatta* we used a set of two human cosmids (185H2 and 142H1) [2] from intron 3, respectively flanking a region of about 100 kb of the human *HMGIC* gene. For FISH analyses on *Callithrix jacchus* the cosmids 142H1 and 27E12 flanking a region of about 140 kb were used, 142H1 belongs to intron 3 and 27E12 contains the complete 3′ end of *HMGIC*.

To map the human cosmids unambiguously, GTG-banding was performed before FISH. Specific signals were assigned to chromosome 9 of *Pongo pygmaeus* and *Callithrix jacchus*, and to chromosome 10 of *Gorilla gorilla*, respectively (Fig. 1). The cosmids mapped to the regions 9q14-15 and 10q14-15 cytogenetically corresponding to the chromosomal region 12q14-15 in humans. In *Macaca mulatta* the signals were mapped to the region q14-15 of chromosome 12 corresponding to the same region in humans (Fig. 1). The intensity of the fluorescent signals was identical in all species.

HMGIC belongs to the high mobility group family of DNA binding proteins and is about 160 kb in length. The *HMGIC* protein contains three DNA binding domains binding to the minor grooves of AT-rich DNA [4]. As for the *HMGIC* gene, its part encoding for these DNA binding domains is separated from that encoding an acidic C-terminal domain by a space of about 140 kb in length. Because of the DNA binding domains it has been suggested that *HMGIC* may play a role in organizing satellite chromatin and may thus act as an architectural transcription factor [5, 6].

In the human genome, *HMGIC* maps to 12q15 [7]. Recently we established a cosmid contig covering *HMGIC* [2] and clones from this contig were used for the present study as well. The signal patterns indicate that the sequence homology of *HMGIC* between Old World monkeys, great apes, marmosets, and humans was at least high enough to result in signals on metaphase chromosomes under stringent hybridization conditions. It seems unlikely that the hybridization was only because of the relatively small coding region of the gene of 330 bp because the largest part of the cosmid inserts consists of noncoding *HMGIC* sequence, thus indicating also a high similarity of the noncoding sequences of the *HMGIC* gene between humans and the monkeys tested so far. As the marmoset and *Homo sapiens* are thought to have diverged from a common ancestor, a primitive anthropoid, approximately 30 million years ago [8], we can conclude that basically the genomic structure and the chromosomal localization of *HMGIC* has been conserved for at least 30 million years.

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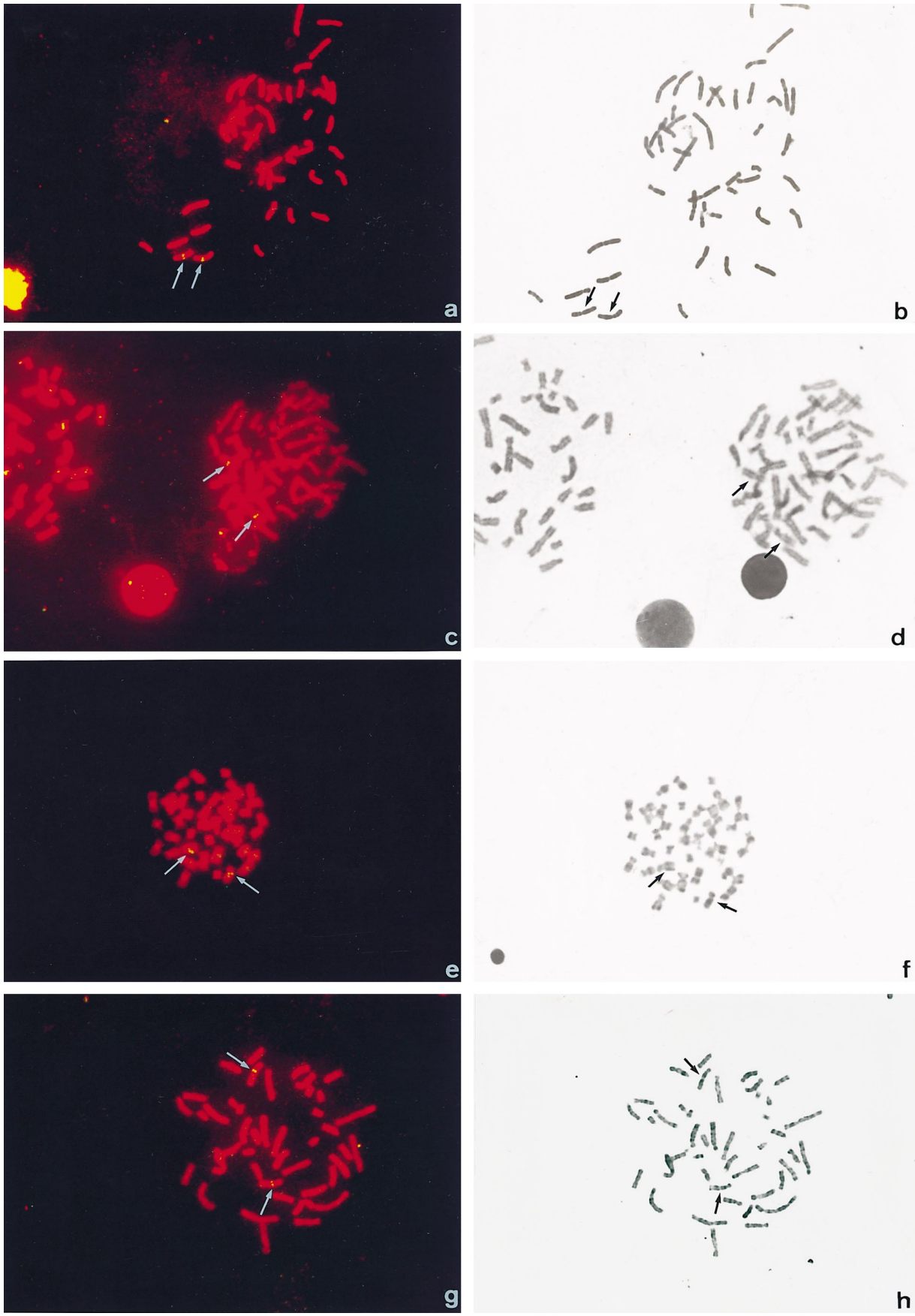
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REFERENCES

1. Mitelman F (1994): Catalog of Chromosome Aberrations in Cancer. 5th Ed. Wiley-Liss, New York.
2. Schoenmakers EFPM, Wanschura S, Mols R, Bullerdiek J, Van den Berghe H, Van de Ven WJM (1995): Recurrent rearrangements in the high mobility group protein gene, HMGI-C, in benign mesenchymal tumours. Nature Genet 10:436–444.
3. Kazmierczak B, Rosigkeit J, Wanschura S, Meyer-Bolte K, Van de Ven W, Kayser K, Krieghoff B, Kastendiek H, Bartnitzke S, Bullerdiek J (1996): *HMGIC* rearrangements as the molecular basis for the majority of pulmonary chondroid hamartomas: a survey of 30 tumors. Oncogene 12:515–521.
4. Reeves R, Nissen MS (1990): The AT-DNA-binding domain of mammalian high mobility group I chromosomal proteins: a novel peptide motif for recognizing DNA structure. J Bio Chem 265:8573–8582.
5. Reeves R, Langan TA, Nissan MS (1991): Phosphorylation of the DNA-binding domain of nonhistone high-mobility group I protein by cdc2 kinase: reduction of binding affinity. Proc Natl Acad Sci USA 88:1671–1675.
6. Thanos D, Maniatis T (1992): The high mobility group protein HMGI(Y) can stimulate or inhibit for NF-κB-dependent virus induction of the human tFN-β gene. Cell 71:777–789.
7. Strickberger MW (1990): Evolution. Jones and Bartlett, Boston.
8. Wanschura S, Kazmierczak B, Schoenmakers EFPM, Meyen E, Bartnitzke S, Van de Ven WJM, Bullerdiek J, Schloot W (1995): Regional fine mapping of the multiple aberration region involved in uterine leiomyomas, lipomas and pleomorphic adenomas of the salivary gland to 12q15. Genes Chromosom Cancer 14:68–70.

Figure 1 (a–h): Chromosomal localization of *HMGIC* on metaphase spreads of *Gorilla gorilla* (a,b), *Pongo pygmaeus* (c,d), *Macaca mulatta* (e,f), and *Callithrix jacchus* (g,h). The chromosomes were first GTG-banded (b,d,f,h) and then in situ hybridization with biotin-labeled cosmid probes on the same metaphase spreads followed (a,c,e,g). For probe detection FITC-labeled antibodies were used and the chromosomes were counterstained by propidium iodine. In *Pongo pygmaeus* (c,d) and *Callithrix jacchus* (g,h) the *HMGIC* gene was mapped to chromosomal region 9q14-15, in *Gorilla gorilla* (a,b) to the region 10q14-15, and in *Macaca mulatta* (e,f) to chromosomal region 12q14-15. Arrows indicate the localization of the signals after FISH and the same chromosomes after GTG-banding. To identify the chromosomes unambiguously, FISH analysis was performed after GTG-banding of the same metaphase spreads with different cosmids (insert length of about 40 kb each) spanning parts of intron 3, exon 3, and the 3′ end of *HMGIC*, as initially described by Schoenmakers et al. [2].