Inactivation of the Human Fragile Histidine Triad Gene at 3p14.2 in Monochromosomal Human/Mouse Microcell Hybrid-derived Severe Combined Immunodeficient Mouse Tumors

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ABSTRACT

We have previously shown that inoculation of human chromosome 3 (chr3)/A9 mouse fibrosarcoma microcell hybrids (MCHs) into severe combined immunodeficient (SCID) mice was followed by the regular elimination of some 3p regions whereas a 3q region was retained even after prolonged mouse passage. Using this approach, referred to as the elimination test (Et), we have defined a common eliminated region (CER) of ~7 cM at 3p21.3 that was absent in all of the 27 tumors generated from five MCHs. Later, CER was reduced to a 1-Mb region, designated as CER1. Another eliminated region (ER2) at 3p21.1–p14.2 was absent in 21 of the 27 tumors. ER2 borders at but does not include the fragile histidine triad (FHIT) gene, considered as a putative tumor suppressor gene.

In the present work, two new and two previously studied MCHs, and 13 derived SCID mouse tumors were analyzed by fluorescence in situ hybridization (FISH) chromosome painting and by PCR, using 72 chr3p-specific and 11 chr3q-specific markers. Nine tumors generated from three MCHs that carried cytogenetically normal chr3, remained PCR-positive for all of the chr3 markers tested. Designated as “PCR+” tumors, they were examined by reverse transcription (RT)-PCR, together with four of six previously studied tumors derived from MCH910.7, which carried a del(3)(pter–p21.1), for the expression of 14 human genes: 5 genes within CER1 (LIMDI, CRCR, CRCR2, CRCR3, CRCR5), 5 genes located within regions that were homozygously deleted in a variety of carcinomas (ITGA4L, LUCA1, PTRPRG, FHIT, DUTT1), and 4 other genes in chr3p (VHL, MLHI, TGM4,UBE1L). We found that VHL, MLHI, ITGA4L, LIMDI, UBE1L, LUCA1, PTRPRG, and DUTT1 were expressed in the MCH lines in vitro and also in the derived SCID tumors. No transcripts that originated from the four CRC genes or from TGM4 could be detected in any of the MCH lines.

Alone among the 14 genes examined, FHIT showed a tumor growth-associated change. It was expressed in vitro in five of seven MCH lines. Nine of 13 derived tumors had no FHIT transcript. The remaining 4 expressed a truncated mRNA and a reduced amount of the full-length mRNA. We have previously found that FHIT was deleted at the DNA level in 17 of 21 tumors derived from four MCHs. The remaining 4 of 21 had no FHIT transcript. Our compiled data show that FHIT was either physically or functionally impaired in all 34 of the 34 analyzed tumors. Variants with deleted or down-regulated FHIT have a selective growth advantage.

INTRODUCTION

Overlapping HDs have been found within the 3p21.3 region in three SCLC lines (1–3), in a BRC line, and a primary BRC (4). The minimum common deleted region was approximately 120 kb in length. (4) It included the LUCA1 gene. In the 3p22–3p21.3 region adjacent to the DNA mismatch repair protein homologue gene, MLHI, HDs were detected in two lung tumor biopsies (5) and in five NSCLC lines, including ACC-LCS (6, 7). The ITGA4L gene was cloned from the HD region in ACC-LCS (8). HDs within 3p21.3 were also found in three lung tumors in vivo by FISH (9). A large 8-Mb deletion at the 3p13–3p12 was identified in one SCLC cell line, U2020 (10). A gene called Deleted in U-twenty twenty (DUTT1) was characterized as well (11).

The candidate tumor suppressor gene FHIT spans the FRA3B fragile site at 3p14.2 (12). In four CC, two GC, two nasopharyngeal, and one BRC carcinoma lines, the HDs at D3S1300 (3p14.2) were shown to include parts of the FHIT gene (13). Loss of heterozygosity at 3p14.2 was very frequent in 32 lung cancer lines (100% of SCLC and 88% of NSCLC) and 108 (45%) primary NSCLC (14). The same study identified HDs within the FHIT/FRA3B region in 6 (4.4%) of 135 lung cancer lines, whereas the Northern blot showed low or no FHIT expression in most of the lines (14). Hemi- or homozygous deletions, affecting exon 5 of FHIT preferentially, have been found in 86% of the early lesions classified as Barrett’s metaplasia and in 93% of the associated adenocarcinomas (15). Boldog et al. (16) found HDs within FHIT in nearly 90% of cervical and 50% of colorectal carcinoma cell lines. Recently, a HD within FHIT was found in a cervical carcinoma cell line (17). A HD at 3p14 that encompasses the FHIT and PTRPRG genes, was also found in three samples of benign proliferative breast disease associated with familial BRC (18). Each of the deleted regions was considered as the possible site of one or several tumor suppressor genes.

Our previous work has shown that certain human 3p segments were regularly lost from chr3/A9 mouse fibrosarcoma MCHs in the course of tumor growth in SCID mice (19, 20). The same segments were frequently deleted in a variety of cancer-derived cell lines (20). We have proposed that the elimination of chromosome regions from monochromosomal hybrids during tumor growth can indicate the location of tumor antagonizing genes (19, 21). We have identified a CER at 3p22–3p21.3, between D3S1029 and D3S643/D3F15S2, that was lost from all of the 27 SCID tumors derived from 5 different MCHs (20). The region was gradually reduced to 1 Mb and designated CER1 (22, 23). No HDs were reported within CER1 in human tumors. A second eliminated region (ER2) was found at 3p21.1–p14.2, between D3S1235 and D3S1067. ER2 includes markers, D3S2, ALASI, D3S1578, D3S1289 and D3S1076. It was lost in 21 tumors that grew from 4 MCHs, but not in 6 tumors derived from the del(3)(pter–p21.1) carrying MCH910.7 (Fig. 2). ER2 borders on the region of frequent HDs within 3p14.2 that includes the FHIT gene.

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3 The abbreviations used are: HD, homozygous deletion; chr3, human chromosome 3; MCH, microcell hybrid; SCID, severe combined immunodeficient/immunodeficiency; CER, common eliminated region; ER, eliminated region; Mb, megabase; SCLC, small cell lung carcinoma; BRC, breast cancer; NSCLC, non-SCLC; CC, colon carcinoma; GC, gastric carcinoma; NPC, nasopharyngeal carcinoma; LIMDI, containing LIM domains 1 gene; CRCR1, CRCR2, CRCR3, CRCR5, chemokine (C-C motif) receptor 1, 2, 3, 5 genes; DUTT1, integral a12-like gene; LUCA1, homo sapiens putative tumor suppressor gene; PTRPRG, protein tyrosine phosphatase, receptor type, y, polypeptide gene; FHIT, fragile histidine triad gene; DUTT1, deleted in U-twenty twenty gene; VHL, homo sapiens Von Hippel-Lindau tumor suppressor gene; MLHI, DNA mismatch repair protein homologue gene; TGM4, prostatic-specific transglutaminase gene; UBE1L, ubiquitin-activating enzyme E1-like gene; FISH, fluorescence in situ hybridization; RT-PCR, reverse transcription-PCR.
mRNA expression after tumor growth; already in the line in vitro; mRNA expression undetectable by one-step RT-PCR both in the MCH line in vitro and in derived tumors. The MCH line carried del(3)(pter–p21.3) and del(3)(p21.3–p14), respectively. The bar at the right site indicates the regularly affected samples hybridized. More than 80% of the examined cells showed an identical pattern.

Photoshop. A minimum of 20 metaphase cells was examined for each of the cell lines. DNA was stained with ethidium bromide, and photographed. Abnormal results were confirmed by duplex RT-PCR using two pairs of primers simultaneously.

RNA Extraction and cDNA Synthesis. RNA was extracted from the MCH and tumor cell lines using TRizol (Life Technologies, Inc., Grand Island, NY) according to the manufacturer’s protocol. Random-primed, first-strand cDNA were synthesized from 6 μg of DNAse-treated total RNA in a 50-μl volume using Superscript II (Life Technologies, Inc., Grand Island, NY) according to the instructions. Each cDNA synthesis reaction was paired with a control reaction without the addition of reverse transcriptase. The synthesized cDNA and the controls were aliquoted (5 μl/tube) and stored at −80°C.

RT-PCR. Five μl of each synthesized cDNA and the controls (RNA) were subjected to PCR in a volume of 25 μl with the same PCR conditions and cycling as described for the genomic PCR. Primers used for RT-PCR analysis are shown in Table 1. Low-stringency PCR (at 45°C of annealing) was carried out to detect the full-length FHIT transcript and a truncated FHIT transcript. To exclude RNA degradation as an explanation for the absence of FHIT expression, duplex RT-PCR using two pairs of primers simultaneously, was performed.

RESULTS

Two new MCHs, A9–3Neo and A9Hytk3, and a subline of the previously tested MCH910.6, designated MCH910.61, carried cytogenetically normal chr3, derived from three different donors. A fourth previously studied line, MCH910.7 carried del(3)(pter–p21.3); (Fig. 2). The recipient A9 mouse fibrosarcoma line and all MCHs were tumorigenic in SCID mice, with take incidences over 80%. Thirteen SCID passaged tumors were collected, three from each MCH that carried a cytogenetically normal chr3, and four from MCH910.7.

Chromosome Painting (FISH-P). The MCH lines and two derived SCID tumors from each were analyzed by FISH-P (Table 3). We have previously shown that the introduced chromosomes were maintained in the MCHs without significant change during at least 60 days of in vitro cultivation in the absence of G418 (19). We have also found that SCID tumors derived from MCH910.7 and MCH939.2 that
carried del(3)(pter–p21.3) and del(3)(p21.3–p14), respectively, retained the introduced chromosome in its original form (Fig. 2). In contrast, MCHs that carried a normal chr3, had lost the entire introduced chromosome in 7 of 19 MCH906.8- and MCH910.6-derived tumors (Fig. 2).

In the present study, we found that the MCH910.61 line carries a cytogenetically normal chr3 in 65% of the cells whereas 30% of the cells contain chimeric translocations. Derived tumors contained translocations in 42–45% of the cells and a cytogenetically intact chr3 in 2–6% of the cells (Table 3). The MCH A9–3Neo line carries a cytogenetically normal chr3 in 98% of cells, with no translocations. Chimeric translocations were not found in the derived tumors either. A cytogenetically intact chr3 was maintained in T3 and T1 tumors in 16 and 48% of the cells, respectively (Table 3). In contrast, the MCH A9Hytk3 line that carried a cytogenetically normal chr3 in 96% of the cells, has produced after SCID passage numerous chimeric translocations (found in T1 and T3 tumors in 24 and 12% of the cells, respectively). The cytogenetically intact chr3 was still present in 58% of the tumor T1 and 75% of the tumor T3 cells (Table 3). The del(3)(pter–p21.3) chromosome was present in 98% of the cells in the MCH910.7 in vitro line and was maintained in 35–37% of the cells in the derived SCID tumors.

**PCR Analysis.** Thirteen SCID tumors were analyzed by PCR, using 72 chr3p-specific and 11 3q-specific markers (Table 2). All of the tumors that were derived from MCH910.61, MCH A9–3Neo, and MCH A9Hytk3, were PCR-positive for all of the markers tested. They will be referred to as “PCR+” tumors. In the MCH910.61-derived tumors, the markers D3S1547, D3S1234, and D3S1300, located at 3p14.2, amplified a reduced amount of the product (data not shown). The FHIT-ex6 primers (from the exon 6 of FHIT) did not yield any PCR product in any of the MCH910.61-derived tumors (three were examined), in contrast to the normal amplification in all of the three MCH A9–3Neo-derived tumors, three MCH A9Hytk3 tumors, and four MCH910.7-derived tumors (Fig. 3B). The MCH910.7 line that carried del(3)(pter–p21.3), and all derived tumors were negative for all of the PCR-markers from D3S3888 to the telomere. The duplex-PCR is exemplified in Fig. 3, B and C. Taken together with our previously published data, 24 of the 34 MCH-derived tumors were negative for at least one FHIT PCR marker. Ten of the 34 tumors retained all of the FHIT markers tested (Fig. 2).

**Comparative RT-PCR Analysis.** Fig. 1 summarizes the expression of the selected chr3p genes in the MCH lines and derived PCR+ tumors. Only one of the 14 genes studied, FHIT, showed a consistent down-regulation after SCID passage. The three MCH910.61-derived tumors have lost the genomic FHIT sequence (Fig. 3B). No FHIT transcripts were found in any of the MCH A9–3Neo- and MCH A9Hytk3-derived tumors (Figs. 1 and 3A). A truncated FHIT mRNA was found together with a reduced level of the full-length FHIT transcript in four tumors derived from MCH910.7 that carried del(3)(pter–p21.3). The truncated FHIT transcript was already present in the in vitro propagated MCH910.7 line (Figs. 1 and 3E). In our previous experiments (19, 20) we found that the del(3)(pter–p21.3) in MCH910.7 and the del(3)(p21.3–p14) in MCH939.2 were retained in their original intact form after SCID passage. The FHIT transcript was not found in the MCH939.2 line in vitro (Fig. 3A), in derived tumors, and in two MCH906.8-derived tumors that have maintained the FHIT region at 3p14.2 (Figs. 1 and 2). Small lesions (inversions, insertions, and deletions) within the gene might hamper FHIT expression, as reported for a variety of human tumors (see Ref. 24 for review). (The mRNA expression of other chr3p genes was not analyzed in these tumors, because of the lack of the material).

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**Table 1** PCR primers used for the RT-PCR analysis of SCID mouse tumors generated from MCHs

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Genbank ID no.</th>
<th>Name of primers</th>
<th>GDB accession ID</th>
<th>Sequence (5’–3’)</th>
<th>Size (bp)</th>
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</thead>
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<tr>
<td>VHL</td>
<td>U68176</td>
<td>VHL-F21</td>
<td>GDB:375190</td>
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<td>171</td>
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<tr>
<td>MLH1</td>
<td>U07343</td>
<td>VH-LR20</td>
<td>GDB:679141</td>
<td>R-ATCTCCATCGCTGATGTG</td>
<td>151</td>
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<tr>
<td>IGF1</td>
<td>D25303</td>
<td>WI-7134</td>
<td>GDB:674546</td>
<td>F-GGAAATCACACGTCTGGCCT</td>
<td>260</td>
</tr>
<tr>
<td>TGM4</td>
<td>L34840</td>
<td>WI-7947</td>
<td>GDB:677753</td>
<td>R-AGGGTGAGGATGCTGT</td>
<td>331</td>
</tr>
<tr>
<td>LIMD1</td>
<td>AJ132408</td>
<td>CR1</td>
<td>GDB:677753</td>
<td>F-TCACATGACCTGACATGGCC</td>
<td>331</td>
</tr>
<tr>
<td>CCR1</td>
<td>L10918</td>
<td>CCR3</td>
<td>GDB:678232</td>
<td>R-ACCTTGGGCGCCCATGC</td>
<td>573</td>
</tr>
<tr>
<td>CCR2</td>
<td>U28694</td>
<td>CCR5</td>
<td>GDB:678232</td>
<td>F-AGTTTGGCGCCCATGTT</td>
<td>645</td>
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<tr>
<td>CCR5</td>
<td>U54994</td>
<td>PBE</td>
<td>GDB:733936</td>
<td>R-AGGAAGAGGAGGATTCTG</td>
<td>782</td>
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<tr>
<td>UBE1L</td>
<td>L13852</td>
<td>PBE</td>
<td>GDB:733936</td>
<td>F-GGAGGCGAGGATTCTG</td>
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<tr>
<td>LUCA1</td>
<td>U03056</td>
<td>PBE</td>
<td>GDB:733936</td>
<td>R-AGTCTGGCATGCTGCAAG</td>
<td>408</td>
</tr>
<tr>
<td>PTPRG</td>
<td>U46116</td>
<td>PBE</td>
<td>GDB:733936</td>
<td>F-GGAGGCGAGGATTCTG</td>
<td>813</td>
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<tr>
<td>FHIT</td>
<td>U46922</td>
<td>FHIT-ex6</td>
<td>GDB:1220992</td>
<td>R-AGTCTGGCATGCTGCAAG</td>
<td>177</td>
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<tr>
<td>DUTT1</td>
<td>Z95705</td>
<td>DUTT1-3</td>
<td>GDB:1220992</td>
<td>F-AGTCTGGCATGCTGCAAG</td>
<td>177</td>
</tr>
</tbody>
</table>

*ID, identification; F, forward; R, reverse; D, designed by us.*
Markers are listed from the telomere down to the centromere as follows: A, the orientation of the cosmid markers within the 3p23–21.3 region was determined by two-color FISH on human prophase chromosomes and stretched DNA; B, the markers are placed on the PAC-clone-contig maps of the 3p21.3 region reported by Human Genome Sequencing Center at Baylor College of Medicine (http://www.hgsc.bcm.tmc.edu/); C, the order of the markers was identified by the PAC-clone contig covering 1-Mb CER1 at the 3p21.3 (23); D, the order of PCR markers is taken from the YAC map covering 31 cM in 3p21–p14 (39); E, DNA segment containing (CA) repeat from the genetically mapped polymorphic STS markers were placed by MCHs analysis (20); F, the gene is cloned from the region at 3p12 that was homozygously deleted in a lung cancer cell line (8); G, W (761–766) 3q25.2–3q26.2; H, the gene is located inside the 800-kb region at 3p22–p21.3, which was determined upon the results of multiple crossovers in the family DNA collection of the CEPH, FISH analysis, and deletion-hybrid mapping (41); I, U2020 cell line (11); J, WI-7345 MLH1 (140–147); K, unordered at the YAC contig maps probes were placed by MCHs analysis (20); L, markers are mapped at the 600-kb cosmid interval by a somatic cell hybrid mapping panel (40); M, PCR markers are ordered at the Radiation-Hybrids Based Map of Human Chromosome 3 (WIRHM) established at the Whitehead Institute for Biomedical Research/MIT Center for Genome Research (WI/MIT CGR; http://www-genome.wi.mit.edu/); N, the gene is placed on CEPH/Genethon Chromosome 3 Linkage Map (http://www.cephb.fr/ceph-genethonmap.html); O, the gene is located inside the 3p13–3q13 region that was homozygously deleted in the U2020 cell line (11); P, markers are ordered according to the STS-based Map of Human Chromosome 3 established at the WI/MIT CGR; Q, the order of markers was determined upon the results of multiple crossovers in the family DNA collection of the CEPH, FISH analysis, and deletion-hybrid mapping (41).
PTPRG was expressed in the MCH910.61 line but not in three derived tumors. Ten other tumors derived from the three other MCHs maintained their PTPRG expression (Fig. 1). VHL, MLH1, IFGA5L, UBEIL, LUCA1, and DUTT1 were expressed in the parental MCHs and in derived PCR+ tumors as well (Figs. 1 and 3A). MCH910.61-derived tumors showed a significantly reduced mRNA expression of UBEIL and LUCA1, localized in the 3p21.3–p21.2 region (Fig. 1). The recently identified LMD1 gene, located within CER1 (28), showed a reduced RNA expression in the MCH910.61-derived tumors and a total loss of expression in the MCH A9Hytk3-derived tumors. However, the LMD1 transcript was present in the MCH A9-3Neo-derived tumors at the same level as in the parental MCH line (Fig. 1).

Table 3 FISH-painting analysis of microcell hybrids and derived SCID tumors

<table>
<thead>
<tr>
<th>Name of MCH</th>
<th>chr3</th>
<th>Frequency in MCH (%)</th>
<th>Frequency in SCID tumors (%)</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>T1</td>
<td>T3</td>
</tr>
<tr>
<td>910.61</td>
<td>Intact</td>
<td>65</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Translocated/deleted</td>
<td>30</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>No chr3</td>
<td>5</td>
<td>52</td>
</tr>
<tr>
<td>A9-3Neo</td>
<td>Intact</td>
<td>98</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Translocated/deleted</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No chr3</td>
<td>2</td>
<td>52</td>
</tr>
<tr>
<td>A9Hytk3</td>
<td>Intact</td>
<td>96</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Translocated/deleted</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>No chr3</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>910.7</td>
<td>del(3)(pter-p21.1), intact</td>
<td>98</td>
<td>37</td>
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<tr>
<td></td>
<td>Translocated/deleted</td>
<td>2</td>
<td>nd</td>
</tr>
<tr>
<td></td>
<td>No chr3</td>
<td>0</td>
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d, not done.

DISCUSSION

The FHIT gene, cloned in 1996, includes FRA3B, the most common fragile site at 3p14.2 and the hereditary renal cancer t(3;8) translocation breakpoint (12). The gene covers approximately 1 Mb and encodes a 1.1-kb transcript with 10 small exons. Exon 5 is the first protein-coding exon. It is flanked by FRA3B in intron 4 and intron 5. The M16,800 Fhit protein hydrolyzes diadenosine triphosphate (APppA) to ADP and AMP in vitro. Mutation of a central histidine abolishes hydrolytic activity (see Ref. 29 for review). Using RT-PCR and cDNA sequence analysis, Ohta et al. (12) have detected aberrant FHIT transcripts in 13 of 27 uncultured esophagus, stomach, and colon tumors. Normal-sized transcripts were also observed in 8 of the 13 tumors with aberrant transcripts. Aberrant FHIT RNA expression has also been found in 30% of breast carcinomas (30), 80% of primary SCLCs, 40% of NSCLCs (31), and 55% of squamous cell carcinomas of the head and neck (32). Six of 7 cervical carcinoma lines showed no or reduced FHIT expression (33). Aberrant FHIT RNA expression correlated with the lack of the detectable Fhit protein in 10 cancer-derived cell lines of the kidney, colon, stomach, cervix, head and neck, and nasopharynx (34). Expression of exogenous Fhit protein in four FHIT-negative cancer cell lines that originated from different tumors, was found to abrogate their tumorigenicity in nude mice (35).

In the present study, we have tested human chr3/A9 mouse fibrosarcoma MCH-derived SCID tumors that have maintained the entire chr3 (derivatives of MCH910.61, MCH A9–3Neo, and MCH A9Hytk3) or the del(3)(pter–p21.1) (derivatives of MCH910.7), for the expression of human chr3p-genes. All of the five MCH lines that contained an intact chr3, expressed FHIT in vitro. In all of the analyzed tumors, FHIT was functionally impaired. Nine PCR+ tumors derived from three MCHs (three from each), MCH910.61, MCH
in vitro. Eight genes, CCR2, CCR1, truncated mRNA, in parallel with the normal-sized transcript, detected generated from MCH910.61 revealed a deletion including exon 6 of A9 –3Neo, and MCH A9Hytk3, failed to express FHIT in vitro performed using two pairs of primers simultaneously.


dHEF mouse A9 fibrosarcoma cells; on the right Controls

Fig. 3. PCR analysis of human chr3/mouse MCH lines and derived SCID tumors. A and D, Duplex RT-PCR. B and C, Duplex PCR of genomic DNA. Duplex PCR were performed using two pairs of primers simultaneously. *, the samples that have lost the genomic sequences of the marker, or fail to express it. E, arrow, truncated FHIT transcript in the MCH910.7 line in vitro and the derived T1 tumor. On the left, markers; on the right, the sizes of the relevant PCR-products (in bp). Controls: mouse, recipient mouse A9 fibrosarcoma cells; HEF, cultured human embryonic lung fibroblasts.

A9–3Neo, and MCH A9Hytk3, failed to express FHIT. Three tumors generated from MCH910.61 revealed a deletion including exon 6 of FHIT. Four tumors derived from the MCH910.7 line expressed a truncated mRNA, in parallel with the normal-sized transcript, detected at a reduced level. Five of the 14 examined chr3p-genes, TGM4, CCR1, CCR2, CCR3, and CCR5, were not expressed in the MCH lines in vitro. Eight genes, VHL, MLH1, ITGA4L, LIMD1, UBEIL, LUCAI, PTPRG, and DUTT1, were expressed in the MCHs in vitro and in all derived tumors. The down-regulation of FHIT expression after SCID mouse passage contrasts, moreover, with the maintained expression of eight other genes, localized in or near regions that are targets of tumor-associated HDs.

The validity of the A9-based MCH elimination system for the functional detection of tumor suppressor genes is also supported by the finding of Li et al. at our Center (Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden; Ref. 36) showing that the human RB gene, transfected into A9 cells, is either eliminated or down-regulated, after SCID mouse passage. The elimination test provides a possibility to detect malignancy suppressors in frequently deleted, but otherwise unknown, areas of the genome. Meanwhile, FHIT qualifies for the role of a malignancy suppressor, as also indicated by the suppression study of Siprasvili et al. (35).

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FHIT IN MCH TUMORS


Inactivation of the Human *Fragile Histidine Triad* Gene at 3p14.2 in Monochromosomal Human/Mouse Microcell Hybrid-derived Severe Combined Immunodeficient Mouse Tumors

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