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Inactivation of the Human *Fragile Histidine Triad* Gene at 3p14.2 in Monochromosomal Human/Mouse Microcell Hybrid-derived Severe Combined Immunodeficient Mouse Tumors[1]

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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 (*LIMD1, CCR1, CCR2, CCR3, CCR5*), 5 genes located within regions that were homozygously deleted in a variety of carcinomas (*ITGA4L, LUCA1, PTPRG, FHIT, DUTT1*), and 4 other genes in chr3p (*VHL, MLH1, TGM4, UBE1L*). We found that *VHL, MLH1, ITGA4L, LIMD1, UBE1L, LUCA1, PTPRG,* and *DUTT1* were expressed in the MCH lines *in vitro* and also in the derived SCID tumors. No transcripts that originated from the four *CCR* 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[3] 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, *MLH1*, HDs were detected in two lung tumor biopsies (5) and in five NSCLC lines, including ACC-LC5 (6, 7). The *ITGA4L* gene was cloned from the HD region in ACC-LC5 (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 line (17). A HD at 3p14 that encompasses the *FHIT* and *PTPRG* 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 3p21.1–3p14.2, between *D3S1235* and *D3S1067*. ER2 includes markers, *D3S2, ALAS1, 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; *LIMD1*, containing LIM domains 1 gene; *CCR1, CCR2, CCR3, CCR5*, chemokine (C-C motif) receptor (1, 2, 3, 5) genes; *ITGA4L*, integrin α_4-like gene; *LUCA1*, homo sapiens putative tumor suppressor gene; *PTPRG*, protein tyrosine phosphatase, receptor type, γ polypeptide gene; *FHIT*, fragile histidine triad gene; *DUTT1*, deleted in U-twenty twenty gene; *VHL*, homo sapiens Von Hippel-Lindau tumor suppressor gene; *MLH1*, DNA mismatch repair protein homolog gene; *TGM4*, prostate-specific transglutaminase gene; *UBE1L*, ubiquitin-activating enzyme E1-like gene; FISH, fluorescence *in situ* hybridization; RT-PCR, reverse transcription-PCR.

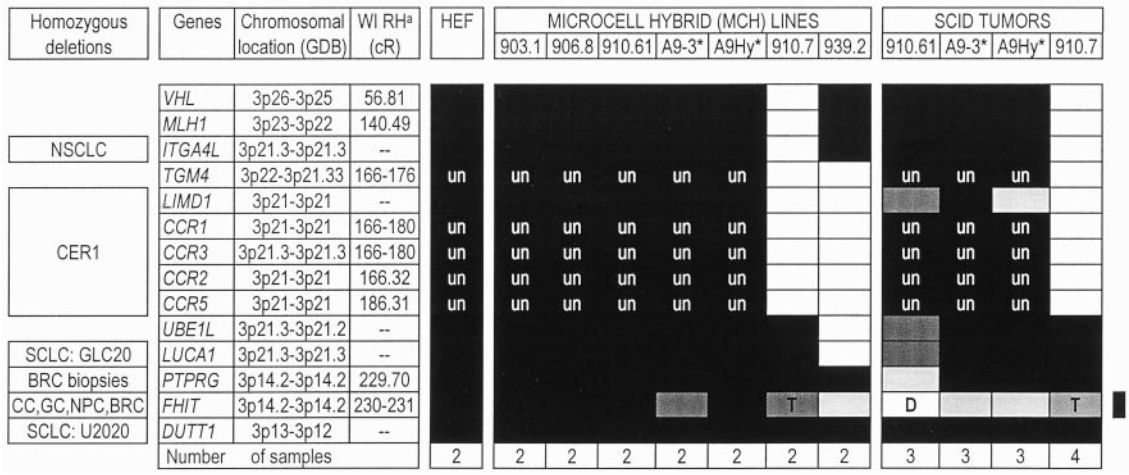

Fig. 1. Comparative RT-PCR analysis of human chr3/mouse MCH lines and derived SCID tumors. □, absence of genomic sequence; ■, transcript detected by one-step RT-PCR; ▨, loss of mRNA expression after tumor growth/already in the line *in vitro*; [D], loss of genomic sequence after tumor growth; ▩, reduced mRNA expression with concomitant coexpression of a truncated mRNA; ▩, reduced mRNA expression compared with the MCH line *in vitro*; [un], mRNA expression undetectable by one-step RT-PCR both in the MCH line *in vitro* and in derived tumors. *HEF*, cultured human embryonic lung fibroblasts; *WI RH*[a], distance in centirays (cR) according to the Radiation Hybrid Map of Chromosome 3 established at the Whitehead Institute/MIT Center for Genome Research; *A9–3**, MCH A9–3Neo; *A9Hy**, MCH A9Hytk3. *NSCLC*, NSCLC lines; *SCLC*, SCLC lines; *BRC*, BRC lines; *CC*, CC lines; *GC*, GC lines; *NPC*, NPC lines. MCH903.1, MCH906.8, MCH910.6, MCH910.61, MCH A9–3Neo, and MCH A9Hytk3 contained cytogenetically normal human chr3. MCH910.7 and MCH939.2 carried del(3)(pter–p21.3) and del(3)(p21.3–p14), respectively. The bar at the right site indicates the regularly affected *FHIT* gene.

(20; see 24 for review). The HDs found in two SCLC lines (1, 3) overlap with ER2.

In the present work, we examine the question of whether known chr3p genes that reside within HDs found in SCLC, NSCLC, BRC, CC, GC, and cervical cancer lines or within CER1 (Fig. 1; Table 1), change their expression upon SCID passage. We have chosen five genes within the HD regions, *ITGA4L* at 3p22–3p21.3, *LUCA1* at 3p21.3, *PTPRG* at 3p14.2, *FHIT* at 3p14.2, and *DUTT1* at 3p13–3p12. We also examined 5 genes within CER1 at 3p21.3, *LIMD1*, *CCR1*, *CCR2*, *CCR3*, and *CCR5*. Four chr3p genes outside these regions, *VHL* at 3p26–3p25, *MLH1* at 3p23–3p22, *TGM4* at 3p22–3p21.33, and *UBE1L* at 3p21.3–3p21.2, were chosen as controls (Fig. 1 and Table 1).

MATERIALS AND METHODS

Cell Lines. Human monochromosome/mouse MCHs were generated by microcell-mediated chromosome transfer as described previously (25). The A9 mouse fibrosarcoma cell line served as recipient. Four MCHs that carried chr3 were used for this study (Figs. 1 and 2). In the case of MCH910.61, MCH910.7 (19), and A9–3Neo, the chromosome donors were neomycin-resistant clones of normal human diploid fibroblast lines that were randomly tagged with the pSV2neo marker. The MCH line A9Hytk3 contained intact chr3 tagged with the bacterial hygromycin phosphotransferase (*Hy*) and herpes simplex virus thymidine kinase (*tk*) genes (26). The MCHs were maintained in growth medium containing geneticin (G418; Sigma, St. Louis, MO) at a final concentration of 400 μg/ml or hygromycin B (Calbiochem Corp., San Diego, CA) at a final concentration of 400 units/ml. Cells were injected s.c. into SCID mice (10^5 cells/mouse). All of the tumors were explanted and cultured *in vitro* for 2–4 passages to obtain the necessary cell number for cytogenetic and molecular analyses.

FISH. Chromosome painting with chr3-specific probe was performed as described previously (19). FITC antidigoxigenin or rhodamine (Rh) antiavidin were used for immunochemical detection. A 4,6-diamidino-2-phenylindole (DAPI) or propidium iodide (PI) counterstaining was applied to visualize G- or R- bands. FISH and image analyses were performed using a fluorescent microscope (LEITZ-DMRB; Leica, Heidelberg, Germany) equipped with a Hamamatsu 4800 cooled CCD camera (Hamamatsu, Herrsching, Germany) and processing software Image-Pro Plus (Media Cybernetics) and Adobe Photoshop. A minimum of 20 metaphase cells was examined for each of the samples hybridized. More than 80% of the examined cells showed an identical pattern for each of the analyzed samples.

Genomic PCR Analysis. High-molecular-weight DNA was prepared by proteinase K digestion and phenol/chloroform extraction according to standard procedures (27). The PCR markers used for the genomic analysis are shown in Table 2. Genomic PCR was carried out using 200 ng of DNA as a template. Thirty cycles of 30 s at 94°C, 60 s at 50–55°C, and 2 min at 72°C, and a final extension of 5 min at 72°C followed initial denaturation at 95°C for 5 min. The PCR products were evaluated by electrophoresis through 2–3% agarose gels, 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 DNaseI-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–p21.1); (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

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

The sequences of the FHIT-exon 6 (FHIT-ex6) primers were published previously (14); the sequences of the pBE/pBI primers (*UBE1L*) were kindly provided by Dr. C. H. C. M. Buys (University of Groningen, the Netherlands), the sequences of the CKR1 and CKR3 primers were published previously (37), the LIMD1-L3/LIMD1-R3 primers were designed by H. Kiss (Karolinska Institute, Stockholm, Sweden). The primers FHIT-CDS have the positions on the *FHIT* sequence at 350- and 1018-nucleotide, respectively, and result in PCR products that contain all translated exons of *FHIT*.

Target gene	Genbank ID no.[a]	Name of primers	GDB accession ID	Sequence (5′–3′)	Size (bp)	
					cDNA	DNA
VHL	U68176	VHL-F21	GDB:375190	F-TACTCTGAAAGAGCGATGCC	171	171
		VHL-R20		R-ATCTCCCATCCGTTGATGTG		
MLH1	U07343	WI-7345	GDB:679141	F-TGCACTGTGGGATGTGTTCT	151	151
				R-AATCAATCCACTGTGTATAAAGGAA		
ITGA4L	D25303	D3S3880	GDB:674546	F-TCACATGACCTGATCACTAGCC	260	260
				R-AGGGTGACCATGCGTGTT		
TGM4	L34840	WI-7947	GDB:677753	F-AAGAGCCAGCAGGTCAAAAA	331	331
				R-GAGAGCACTCCAGTTGACAGG		
LIMD1	AJ132408	LIMD1-L3		F-ACACTTGGGGGCCAGCAC	408	
		LIMD1-R3		R-TGCTCACCCGAAGGCTGAT		
CCR1	L10918	CKR1	D	F-TGAACCTCTTTGGGCTGG	573	573
				R-CGGACTCCATGCTGTGCC		
CCR3	U28694	CKR3	D	F-AGTTTGTGCCCCCGCTGT	645	645
				R-TGAAAAACACCGCCATGA		
CCR2	D29984	WI-9314	GDB:678232	F-GGAAATCACACGTCTGGCTT	113	113
				R-ACTGTTTTCCAACCCAGCTG		
CCR5	U54994	CCR5	D	F-GGAAGGAGGGAGGTATTCGTG	782	782
				R-ATCTGGAATAAGTACCTAAGGCGC		
UBE1L	L13852	PBE		F-TGGTGCTGACTGCTGCAAAG	408	1036
				R-CTCTTGACTTCAGTGATAGCC		
LUCA1	U03056	SHGC-11855	GDB:733936	F-CAGACTGGAATAGTGGCATAAGG	210	210
				R-GGAATGAATAAGTCCACATAAAACG		
PTPRG	U46116	WI-11100	GDB:1220992	F-CTAATAAGGGATGTTCACATGAAGC	177	177
				R-CTGTATTTTAATGGAGTGGATAGCA		
FHIT	U46922	FHIT-ex6		F-ATGTTCTTGTGTGCCCGCTG	145	145
				R-TGCATGGAAAAGGTGAGAGAGG		
	U46922	FHIT-CDS	D	F-CAACTGTGAGGACATGTCGTTCA	668	
				R-TCTGCCTGTCTGAGCCGTTTAG		
DUTT1	Z95705	DUTT1-3	D	F-TGCAATCAGAGTGTACAAATTGTCG	499	499
				R-TTTACAATGTTTCTACCCCATTCGAA		

[a] ID, identification; F, forward; R, reverse; D, designed by us.

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, or maintained parts of it with chimeric translocations in 12 of 19 MCH903.1- and MCH906.8-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. 3*B*). 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. 3*B*). No *FHIT* transcripts were found in any of the MCH A9–3Neo- and MCH A9Hytk3-derived tumors (Figs. 1 and 3*A*). 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 3*E*). 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. 3*A*), 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).

Table 2 *The list of markers*[a,b] used for the PCR analysis of MCHs and SCID mouse tumors

No.	Name of primer	Description	Index	WI RHM[c] (cR)	Cytogenetic position (GDB)
1	D3S1297	$(CA)_n$	N, R, W	13.50	3pter–3p25
2	D3S1304	$(CA)_n$	N, R, W	37.10	3p25–3p24.2
3	VHL-F21/	*VHL*	N, R	56.80	3p26–3p25
4	D3S1263	$(CA)_n$	G, R, W	58.60	3p25–3p24.2
5	D3S1293	$(CA)_n$	G, N, W	(94–96)	3pter–3p24.2
6	SGC32684	*TGFBR2*	R	116.90	3p22–3p22
7	D3S1283	$(CA)_n$	G, N, W	(117–110)	3p24.2–3p22
8	D3S647	$(CA)_n$	A, N, W	(121–116)	3p23–3p23
9	GLB1	*GLB1*	K, N, R	125.80	3p22–3p21.33
10	D3S1611	$(CA)_n$	G, N, R, W	140.50	3p23–3p22
11	WI-7345	*MLH*	W	(140–147)	3p23–3p22
12	D3S1298	$(CA)_n$	G, N, V, W	(140–147)	3p24.2–3p22
13	D3S1260	$(CA)_n$	G, N, V, W	(147–144)	3p24.2–3p22
14	D3S3880	*ITGA4L*	H, K	—[d]	chr3
15	WI-5224	STS	W	(147–144)	chr3
16	WI-6058	*ACAA*	R, W	143.51	3p24.2–3p22
17	D3S3605	$(CA)_n$	G, W	(144)	chr3
18	D3S2343	$(CA)_n$	G, N, W	(144)	3p22–3p21.3
19	D3S3527	$(CA)_n$	G, W	(144)	chr3
20	SGC30812	*MYD88*	R	146.03	chr3
21	D3S3521	$(CA)_n$	G, N, W	(144)	chr3
22	D3S3658	$(CA)_n$	G, W	(173–171)	chr3
23	D3S3559	$(CA)_n$	G, W	(171–166)	chr3
24	WI-4193	STS	R, W	166.43	chr3
25	WI-9364	EST	R, W	166.63	chr3
26	WI-9324	EST	R, W	171.15	chr3
27	NKTR-3	*NKTR*	K, N	—	3p23–3p21
28	BCATE/	*CTNNB1*	K, N	—	3p21.3–3p21.3
29	D3S966	$(CA)_n$	A, K, N, V	—	3p21.32–3p21.31
30	D3S1029	$(CA)_n$	A, C, N, V	—	3p21.31–3p21.2
31	WI-7947	*TGM4*	B1, W	(176–166)	3p22–3p21.33
32	D3S2354	$(CA)_n$	C, G, W	(176–166)	3p22–3p21.3
33	CKR1	*CCR1*	C, W	—	3p21–3p21
34	CKR3	*CCR3*	C, W	—	3p21.3–3p21.3
35	WI-3958	STS	C, R, W	166.32	chr3
36	WI-9314	*CCR2*	C, W	—	3p21–3p21
37	CCR5	*CCR5*	C, W	—	3p21–3p21
38	LTF1/2	*LTF*	B3, C, N	—	3p21.3–3p21.2
39	D3S32	*RFLP*	C, N, V	—	3p21.3–3p21.2
40	WI-2832	STS	C, W	(180–188)	chr3
41	D3S643	$(CA)_n$	A, C, K, N, V	—	3p21.33–3p21.32
42	D3S2420	STS	C, W	(188)	3p21.3–3p21.2
43	WI-10865	STS	R, W	187.94	chr3
44	UNPH-3	*UNPH*	B3, K, R	191.41	3p21.3–3p21.3
45	GPX1	*GPX1*	K, R	195.18	3p21.3–3p21.3
46	NIB1593	*TCTA*	B1, K, N, R	195.20	3p21–3p21
47	WI-16108	*APEH*	B2, K, R	198.33	chr3
48	D3S3888	*MYL3*	B2, R	(182.00)	chr3
49	H3H2-5/	*MST1*	B2, K, N, V	—	3p21.3–3p21.3
50	UBE1L	*UBE1L*	K, N, V	—	3p21.3–3p21.2
51	SEMA4-3	*SEMA4*	L, R	206.40	3p21.3–3p21.3
52	GNAI2E2/	*Gi2*	B4, L, R, V	204.95	3p21.3–3p21.2
53	SGC11855	*LUCA1*	K, L	—	3p21.3–3p21.2
54	D3S1235	$(CA)_n$	L, N, V, W	(203–208)	3p21.3–3p21.2
55	D3S2	RFLP	K, N	—	3p21.3–3p21.3
56	D3S1573	$(CA)_n$	G, L, N, W	(208–210)	3p21.2–3p21.2
57	ALAS1	*ALAS1*	B9, N, R	214.00	3p21.1–3p21.1
58	D3S1578	$(CA)_n$	D, G, R, W	219.15	3p21.2–3p21.1
59	D3S1289	$(CA)_n$	D, G, N, W	(219–217)	3p21.2–3p21.1
60	D3S1076	$(CA)_n$	N	—	3p21.2–3p21.1
61	D3S1067	$(CA)_n$	D, K, N	—	3p21.1–3p14.3
62	D3S1313	$(CA)_n$	D, G, R, W	231.43	3p21.1–3p14.2
63	D3S1547	$(CA)_n$	G, W	(231–224)	3p21.1–3p14.2
64	WI-11100	*PTPRG*	R	229.70	3p14.2–3p14.2
65	D3S1234	$(CA)_n$	D, N, W	(230)	3p21.1–3p14.2
66	D3S1300	$(CA)_n$	D, G, N, W	(230)	3p21.1–3p14.2
67	D3S1312	$(CA)_n$	D, G, R, W	229.70	3p14.2–3p14.1
68	D3S1285	$(CA)_n$	D, G, W, R	233.22	3p14.2–3p14.1
69	D3S1210	$(CA)_n$	D, N, W	(260–256)	3p14.1–3p12
70	DUTT1-3′	*DUTT1*	S	—	3p12–3p12
71	D3S3	RFLP	N, S	—	3p13–3p13
72	PROSA1/centromere	PROS1	K, N, W	—	3p11–3q11
73	D3S1291	$(CA)_n$	G, N, W	(421–481)	3q13–3q13
74	D3S1278	$(CA)_n$	G, R, W	524.25	3q13.2–3q13.3
75	D3S1269	$(CA)_n$	G, N, W	(558–543)	3q21–3q21
76	ACPP	*ACPP*	K, N	—	3q21–3q23
77	D3S1238	$(CA)_n$	N, W	(610–616)	3q21.3–3q25.2
78	D3S1237	$(CA)_n$	N	—	3q25.1–3q25.2
79	D3S1282	$(CA)_n$	G, W	(761–766)	3q25.2–3q26.2
80	GLUT2	*GLUT2*	N	—	3q26.2–3q27
81	D3S1262	$(CA)_n$	G, R, W	853.07	3q27–3q27
82	D3S1314	$(CA)_n$	G, R, W	872.70	3q27–3q27
83	D3S1682	STS	K	—	3q27–3qter

[a] The sequences and the full information for the markers and loci are available at the GDB World Wide Web site (http://gdbwww.gdb.org./).

[b] 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 (38); 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 (htpp://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); G, DNA segment containing (CA) repeat from the genetically mapped polymorphic STS placed on CEPH/Geneton Chromosome 3 Linkage Map (http://www.cephb.fr/ceph-genethonmap.html); H, the gene is located inside the 800-kb region at 3p22–p21.3, which was homozygously deleted in a lung cancer cell line (8); K, unordered at the YAC contig maps probes were placed by MCHs analysis (20); L, markers are mapped at the 600-kb cosmid clone contig surrounding the lung cancer tumor suppressor gene (*TSG*) locus on human chromosome 3p21.3 (3); N, chromosome 3-specific sequences were regionally localized into 23 intervals by a somatic cell hybrid mapping panel (40); R, 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/); S, the gene is cloned from the region at 3p12 that was homozygously deleted in the U2020 cell line (11); W, markers are ordered according to the STS-based Map of Human Chromosome 3 established at the WI/MIT CGR; V, the order of markers was determined upon the results of multiple cross-overs in the family DNA collection of the CEPH, FISH analysis, and deletion-hybrid mapping (41).

[c] Distance in centiRays (cR) from the top of chr3 according to the WI RHM.

[d] —, not known.

$(CA)_n$ markers containing (CA) repeats.

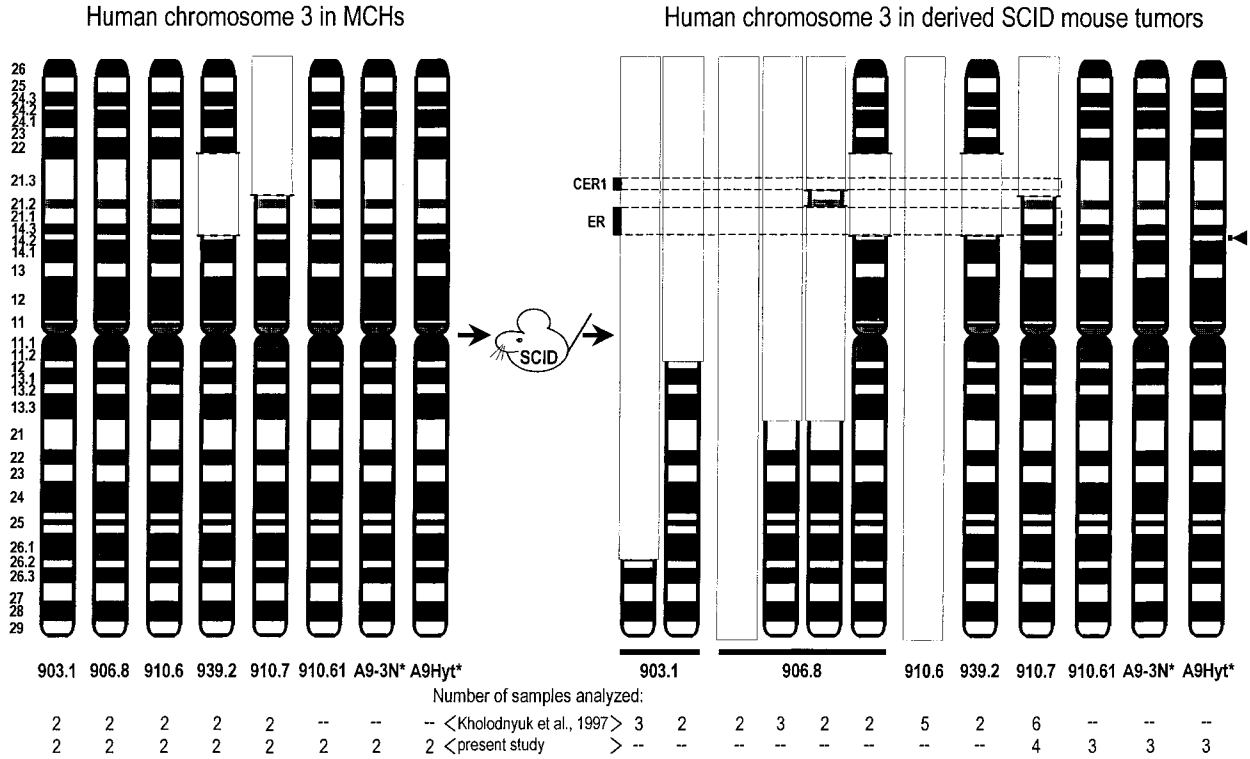

Fig. 2. Experimental design. The ideograms represent the intact and deleted chr3 that have been introduced into mouse A9 cells via MMCT. MCH lines *in vitro* and derived SCID tumors are shown. *Arrowhead on the right*, the *FHIT* region. *Horizontal bars*, CER1 and ER; - - -, not analyzed.

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*, *ITGA4L*, *UBE1L*, *LUCA1*, and *DUTT1* were expressed in the parental MCHs and in derived PCR+ tumors as well (Figs. 1 and 3*A*). MCH910.61-derived tumors showed a significantly reduced mRNA expression of *UBE1L* and *LUCA1*, localized in the 3p21.3–p21.2 region (Fig. 1). The recently identified *LIMD1* 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 *LIMD1* transcript was present in the MCH A9–3Neo-derived tumors at the same level as in the parental MCH line (Fig. 1). *TGM4*, *CCR1*, *CCR2*, *CCR3*, and *CCR5* mRNA was not detected in any of the MCHs or derived tumors by one-step RT-PCR (Figs. 1 and 3*D*).

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

Single chr3 had been introduced into the mouse A9 cells via microcell-mediated chromosome transfer. The transferred cytogenetically normal chr3 were derived from different donors. MCH910.7 and MCH910.61 carried chr3 of the same origin. The recipient A9 mouse fibrosarcoma line and all of the MCHs were tumorigenic in SCID mice, with a take incidence over 80%.

Name of MCH	chr3	Frequency in MCH (%)	Frequency in SCID tumors (%)	
			T1	T3
910.61	Intact	65	6	2
	Translocated/deleted	30	42	45
	No chr3	5	52	53
A9-3Neo	Intact	98	48	16
	Translocated/deleted	0	0	0
	No chr3	2	52	84
A9Hytk3	Intact	96	58	75
	Translocated/deleted	0	24	12
	No chr3	4	18	13
910.7	del(3)(pter–p21.1), intact	98	37	35
	Translocated/deleted	2	nd[a]	nd
	No chr3	0	nd	nd

[a] nd, 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 M_r 16,800 Fhit protein hydrolyzes diadenosine triphosphate (AppA) 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* transcripts have 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–p21.3) (derivatives of MCH910.7), for the expression of human chr3p-genes. All of the five MCH lines that contained an intact chr3p, 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

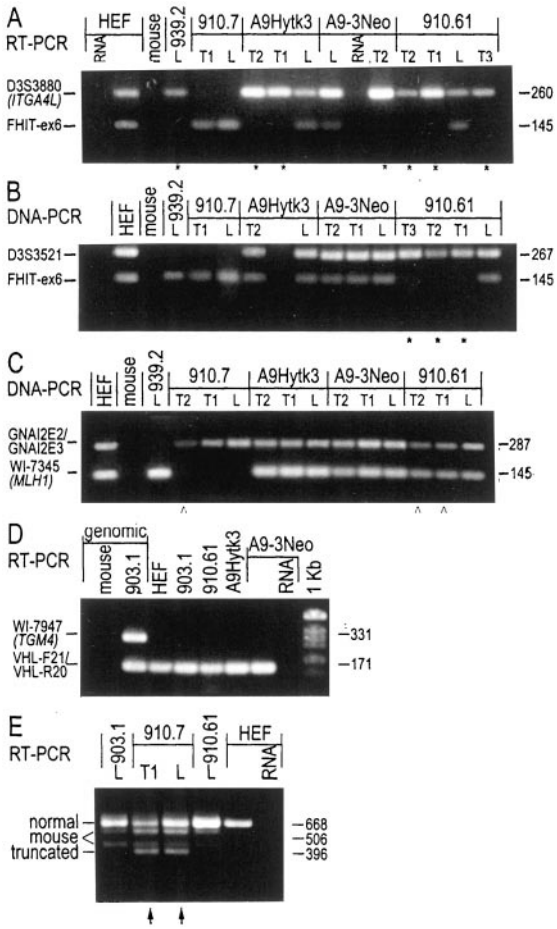

Fig. 3. PCR analysis of human chr3/mouse MCH lines and derived SCID tumors. *A* and *D*, Duplex RT-PCR. *B* and C, Duplex PCR of genomic DNA. Duplex PCR were performed using two pairs of primers simultaneously. ∗, the samples that have lost the genomic sequences of the marker, or fail to express it. *E*, *arrows*, 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*, *UBE1L*, *LUCA1*, *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 Siprashvili *et al.* (35).

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