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Detailed Genetic and Physical Map of the 3p Chromosome Region Surrounding the Familial Renal Cell Carcinoma Chromosome Translocation, t(3;8)(p14.2;q24.1)[1]

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ABSTRACT

Extensive studies of loss of heterozygosity of 3p markers in renal cell carcinomas (RCCs) have established that there are at least three regions critical in kidney tumorigenesis, one most likely coincident with the von Hippel-Lindau gene at 3p25.3, one in 3p21 which may also be critical in small cell lung carcinomas, and one in 3p13-p14.2, a region which includes the 3p chromosome translocation break of familial RCC with the t(3;8)-(p14.2;q24.1) translocation.

A panel of rodent-human hybrids carrying portions of 3p, including a hybrid carrying the derivative 8 (der(8)(8pter→8q24.1::3p14.2→3pter)) from the RCC family, have been characterized using 3p anchor probes and cytogenetic methods. This 3p panel was then used to map a large number of genetically mapped probes into seven physical intervals between 3p12 and 3pter defined by the hybrid panel. Markers have been physically, and some genetically, placed relative to the t(3;8) break, such that positional cloning of the break is feasible.

INTRODUCTION

Cellular proliferation is regulated by both positive and negative pathways. Perturbations in either type of pathway can result in imbalances that lead to tumor formation (for review, see Ref. 1). While perturbations in the positive pathways, protooncogene activation, were initially characterized, there is increasing evidence that perturbations in negative pathways, tumor suppressor inactivations, are critical and ubiquitous in tumor formation (for review, see Ref. 2). Inactivation or loss of function of a tumor suppressor gene can occur by a variety of genetic mechanisms such as point mutation, translocation, or deletion. Since both deletions and translocations are often cytogenetically visible, they have served as landmarks for genes that encode these regulatory signals. Both deletions and translocations involving the short arm of chromosome 3 (3p) have been detected in a number of human malignancies, including RCC[4] (3–5) and LC (6). LOH studies have not only confirmed these cytogenetic observations for RCC (7–13) and LC (14–20) but have expanded upon them by demonstrating nonrandom allelic losses in breast carcinoma (21), nasopharyngeal carcinoma (22), testicular carcinoma (23), and other tumor types (24). Despite numerous LOH studies, pinpointing the location of the critical 3p region(s) harboring the target gene(s) had been hampered by the paucity of well-localized, widely available molecular probes. Recently, efforts to isolate and localize large numbers of 3p molecular probes have been undertaken (25–28). As the probe density on 3p increased, in parallel with recent LOH studies, it became clear that multiple independent loci on 3p were involved (summarized in Refs. 29 and 30).

It has been proposed that multiple loci may be involved within a single tumor type (31), as previously shown for the 11p loci involved in Wilms' tumor (32); also, an individual locus may be involved in more than one tumor type (33). For RCC, it now appears that three separate loci at 3p13–14, 3p21, and 3p25 contribute to tumorigenesis through loss of alleles. The 3p13–14 region encompasses the breakpoint of the constitutional translocation, t(3;8)(p14.2;q24.1), associated with the development of hereditary RCC in a large Italian-American family (34), and may include the homozygous deletion found in the LC-derived cell line U2020 (35). Thus, molecular evidence supports the suggestion that the gene affected by the t(3;8) translocation is important in both sporadic and inherited forms of RCC.

With the increased number of markers now available for 3p, positional cloning is feasible. We are using this approach to identify genes involved in the pathogenesis of RCC by attempting to clone the breakpoint of the t(3;8) translocation. In order to clone this breakpoint, we require a high density of genetically mapped and physically ordered probes in the vicinity of the break and some idea of locus order. We have thus established a rodent-human somatic cell hybrid panel with emphasis on the region that encompasses the breakpoint at 3p14.2; we have characterized the hybrids using Southern blot hybridization and cytogenetic analyses, including chromosome 3 painting and FISH to metaphase chromosomes, in order to define locus position and order. This hybrid panel has been used to define the regions directly surrounding the breakpoint and to saturate these flanking regions with DNA probes so that the marker density within these regions will allow positional cloning of the breakpoint. Furthermore, we have demonstrated that the t(3;8) breakpoint and the U2020 deletion affect independent genes.

MATERIALS AND METHODS

Cell Lines. Human fibroblast cell lines GM1533, GM2808, hamster-human hybrid cell line GM7297, and the mouse cell line, A9, were purchased from the Human Genetic Mutant Cell Repository (Camden, NJ) and maintained as suggested by the supplier. The RCC-derived cell line, Caki 1, was purchased from the ATCC and maintained as suggested by the supplier. The human lymphoblastoid cell lines, TL9944 and JL215 (kindly provided by Linda Cannizzaro and Joan Overhauser, respectively), and the lung carcinoma-derived cell line, U2020 (kindly provided by Kenneth Nilsson), were maintained in RPMI with 10% fetal bovine serum and gentamicin (100 ng/ml). The hamster auxotrophic cell line, Urd-C (kindly provided by David Patterson), was maintained as recommended by the contributor.

Rodent-Human Hybrid Cell Lines. Hybrid clones 297 cl1, cl3, cl5, and cl9 are sister clones derived from the hybrid cell line GM7297. GM7297 was

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[4] The abbreviations used are: RCC, renal cell carcinoma; LC, lung carcinoma; LOH, loss of heterozygosity; FISH, fluorescent *in situ* hybridization; HAT, hypoxanthine, amethopterine and thymidine medium; TK, thymidine kinase; HPRT, hypoxanthine phosphoribosyl transferase; PCR, polymerase chain reaction; DAPI, 4,6-diamidino-2-phenylindole; cM, centimorgan; SCLC, small cell lung carcinoma; ATCC, American Type Culture Collection; GDB, Genome Data Bank.

Table 1 *Parental cell lines and resultant rodent-human hybrids*

Hybrid clone	Human parent	Chromosome 3 abnormality	Rodent parent	Portion(s) of 3 retained	Cytogenetic results
297 cl9	GM3200 lymphoblastoid	None	CHL V79 HPRT$^-$	3pter → 3qter	Chromosome 3 in [a] most cells; non 3 chromosome fragment in all cells
297 cl3	GM3200 lymphoblastoid	*De novo* break in 3p with 3p14–21 → 3pter lost	CHL V79 HPRT$^-$	3p14–21 → 3qter	3p$^-$ in all cells, [a] non 3 frag. in all cells
297 cl1	GM3200 lymphoblastoid	*De novo* break in 3p	CHL V79 HPRT$^-$	3p12 → 3qter	3p$^-$ in all [a] cells, non 3 fragment in all cells
SLF3	GM2808 fibroblast	Constitutional t(3; 17) (p21; p13)	IT22 TK$^-$	3pter → p21	der17, 1–2 copies/cell; several other chromosomes; 1 arm of der17 painted
SLF4	GM1533 fibroblast	Constitutional t(X;3) (q26; p12)	A9 HPRT$^-$	3pter → p12	
5L.8	2954 fibroblast	Constitutional t(3; 8) (p14.2; q24.1)	IT22 TK$^-$	3pter → 3p14.2	der8 present in most cells; several other chromosomes; 3p portion of der8 painted
SLF5	JL215 [b] lymphoblastoid	Constitutional t(3; 11)(p13; p15)	Urd-C	3p13 → qter	der3 present in 1–2 copies/cell; painting confirmed
C36.2	Caki 1 RCC	3pdel(p13-p25) [c]	IT22 TK$^-$	3p14–13 [d]	single small 3 fragment painted in all cells
SLF67	9944 lymphoblastoid	t(3; 8)(p14.2; q24.1)	Urd-C	3p14-cen [d]	two 3 fragments painted

[a] Hybrids 297 cl9, cl3 and cl1 were previously described (38). Chromosome painting has shown that the fragment retained in all three hybrids (and in a sister clone, cl5, which has lost all of 3, not shown) is not a fragment of chromosome 3; preliminary data suggest it is a fragment of chromosome 2.

[b] The JL215 cell line was established by Joan Overhauser by Epstein-Barr virus infection of lymphocytes from a patient carrying this constitutional translocation (J. Overhauser and L. Jackson, personal communication).

[c] The Caki I cell line was karyotyped by Z. Gibas (unpublished data) and found to exhibit the 3p deletion described.

[d] Presumably a *de novo* rearrangement in hybrid resulted in retention of this portion of the chromosome.

originally developed from the fusion of a human lymphoblastoid cell line, GM3200, to the hamster cell line CHL V79 in HAT-selective medium. Because CHL V79 is HPRT deficient, growth in HAT medium allowed for the selective retention of the human X chromosome. Cytogenetic analysis at passage 6 showed that GM7297 retained chromosome X in all cells and chromosome 3 as the only other apparent human component. To generate hybrid cell lines retaining only human chromosome 3, GM7297 was passaged in medium supplemented with 6-thioguanine to select against cells carrying chromosome X, and subclones 297 cl1, 3 and 9 were isolated. The hybrid cell lines SLF3, SLF4, SLF5, SLF67, 5L.8, and c36.2 were clonally derived from fusions of the respective human parents to rodent cell lines in the presence of polyethylene glycol (Table 1), followed by selection in ouabain to eliminate human parental cells and either dialyzed fetal bovine serum or HAT to eliminate hamster or mouse parent cells, respectively. Parental mouse cell lines with deficiencies in TK (IT22) or HPRT (A9) allowed retention of either human chromosome 17 or X or retention of the translocation derivative chromosome (hybrids SLF3 and SLF4; Table 1) carrying the complementary human gene, TK or HPRT (36). The hamster cell line Urd-C is a mutant line with a deficiency in uracil glycosylase and requires exogenous uridine for growth or complementation by the human gene on the long arm of chromosome 3 (37). This facilitated the development of hybrids SLF5 and SLF67, which were selected in uridine-deficient medium. Hybrids 5L.8, and c36.2 selectively retained human chromosome 17 and other chromosomes, in addition to the indicated portion of chromosome 3 (Table 1).

Filter Hybridization. High molecular weight DNA was obtained from cell lysates by standard phenol/chloroform extraction. Southern blotting and hybridization were performed as previously described (38). Molecular probes were radiolabeled by random priming. The hybridized filters were washed at high stringency [final wash, 0.1× standard sodium citrate (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate; pH 7.4), 65°C] and exposed to XAR-2 X-ray film for 16–72 h at −80°C.

Table 2 *Chromosome 3 DNA markers*

Locus	Probe name	Source	Primer name [a]	Ref.
RAF1	p627	U. Rapp		41
GLB1		GDB	GLB1/GLB2	
D3S32	pEFD145.1	ATCC		42
ZNF35	pHF10	L. Lania	HF10A/HF10B	43
LTF		GDB	LTF1/LTF2	
D3S11		GDB	DEM2/DEM3	
PDHB		GDB	PDHB1/PDHB2	
ACY1	ACY1-E	ATCC		44
ARH12		R. Axel		45
D3F15S2	pH3H2	ATCC		46
D3S2	pHF12–32	ATCC	D3S2–1/D3S-2	47
D3S6	pD3S6 [b]	GDB	D3S6–1/D3S6–2	
PTPRG	pA5	(see text)		38
D3S30	pYNZ86.1	ATCC	D3S30–1/D3S30–2	48
D3S3	pMS1–37	GDB	D3S3–1/D3S3–2	49
D3S4	pB67	M. Lerman		50

[a] All primer sequences were obtained from GDB (individual Ref. cited in Ref. 29).

[b] Cloned PCR product.

PCR Amplification. PCR reactions were carried out in a total volume of 50 μl with 100 ng of DNA, 1 μM of each primer, 250 μM of each deoxynucleotide triphosphate, and 3 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT) in 1× reaction buffer supplied by the manufacturer. The reaction cycle was individually optimized for each primer pair. Generally, DNAs were subjected to 30 rounds of denaturation at 95°C for 30 s, annealing between 55 and 65°C for 30 s, and amplification at 72°C for 60 s. Amplification products were subsequently size fractionated on 2% agarose, stained with ethidium bromide, and visualized with UV light.

Chromosome Painting. The portion of chromosome 3 retained in hybrid cell lines was identified by chromosome painting using the Bethesda Research Laboratories whole chromosome-painting system. The whole chromosome-painting probe specific for chromosome 3 was applied to fresh slides containing metaphase chromosomes from hybrids, with results as summarized in Table 1. The chromosome 3-painting probe was directly labeled with spectrum orange fluorophore and combined with unlabeled Cot-1 DNA to increase specificity and reduce binding to repeat sequences on both the target and nontarget DNA.

The application and hybridization of the probe to the chromosomal DNA was carried out according to the procedure recommended by Bethesda Research Laboratories. Briefly, slides were denatured at 70°C for 2–10 min, passed through an ethanol series, and then air dried. The mixture containing the spectrum orange-labeled probe and Cot-1 DNA was denatured and placed on warmed slides (45°C), and hybridization was carried out overnight at 37°C. Slides were washed in a series of formamide rinses at 45°C, with a final wash in 2× standard sodium citrate-0.1% Nonidet P-40. The slides were air dried and counterstained with DAPI. Visualization of spectrum orange-labeled sites was achieved with a Leitz N2 filter (excitation, BP(bandpass)350–56 nm; emission, LP(longpass)580 nm), and visualization of DAPI was with a Leitz A2 filter (excitation, BP270–380 nm; emission, LP410–510 nm).

FISH. The procedure used was a modification of previously described methods (39, 40). Probes were prepared by nick translation using biotin-labeled 11-dUTP (Bionick kit, Bethesda Research Laboratories). Hybridization of biotin-labeled probes was detected with fluorescein isothiocyanate-conjugated avidin. Metaphase chromosomes were identified by Hoechst-33528 staining and UV irradiation (365 nm), followed by DAPI staining to produce the banding pattern. The fluorescence signal was observed with filter block I3 (BP450–490/LP515; Leitz Orthoplan) on the background of red chromosomes stained with propidium iodide. Q-banding was observed with filter block A (BP340–380/LP430). Micrographs were taken with Kodak Ektachrome 400 film, and both color and black and white prints were prepared.

Table 3 *Physical order of loci on 3p*

The rodent-human hybrids carrying defined regions of 3p are listed across the top of the table with probe or gene names which were mapped onto the hybrid panel listed vertically on the left. +, presence of a specific probe in the respective hybrid; –, absence of a probe; blank space, the probe/hybrid combination was not tested. For some probes a band position is indicated next to the probe or gene name. While there may not be complete agreement in the literature concerning band positions of some of these markers, we have attempted to put the consensus position from updated sources (GDB, Reports of the chromosome 3 committees, Ref. 29 and 30). The portions of chromosome 3 retained in the hybrids divides chromosome 3p into eight intervals; interval A1 covers 3p21.3–3pter and is defined by the region of 3p retained in hybrid SLF3; interval A2, defined by the region of 3p present on the der8 (8pter → 8q24.1::3p14.2 → 3pter) and absent in both hybrid 297 cl3 and hybrid SLF3, covers the proximal portion of the large 3p21 band, extending from 3p14.2 through 3p21.2; region B1 is directly centromeric to the 3p14.2 break of the t(3; 8) and extends to the centromeric end of the SLF4 3p break (as shown in Table 1, the SLF4 hybrid was derived from a human cell with a reported constitutional translocation break at 3p12 (our data suggest a probable break in 3p14); intervals B2, B3, and B4 are contiguous regions extending from 3p14 through 3p12 in the order shown. These intervals are defined by the 3p differences between hybrids SLF5 and C36.2. Interval B2 is absent in both hybrids, interval B3 is present only in C36.2, and interval B4 is present in both hybrids. Hybrid cell lines 297 cl9, cl3, cl1, and SLF5 contain the entire long arm of chromosome 3 (3q) (not shown). Hybrid SLF67, retaining markers from D3S6 through PROS 1, has allowed division of the B1 region into B1a (proximal to the t(3; 8) break) and B1b representing the distal portion of B1 adjacent to region B2. Anchor probes are in bold face type.

Probes	cl9	SLF3	5L.8	cl3	cl1	SLF4	SLF5	C36.2	SLF67	
RAF-1 p25	+	+	+	–	–	+	+	–	+	
GLB1 p23–22	+	+	+	–	–	+	–	–		
D3S77	+	+	+	–	–	+				
D3S89	+	+	+	–	–	+				
D3S76	+	+	+	–	–	+				
D3S32 p21	+	+	+	–	–	+				A1
ZNF35 p21	+	+	+	–	–	+	–	–	+	pter-p21.3
D3S34	+	+	+	–	–	+				
LTF p21	+	+	+	–	–	+	–	–	–	
D3S11 p21	+	+	+	–	–	+	–	–	–	
PDHB p21	+	+	+	–	–	+		–		
D3S1029	+	–	+	–	–	+	–	–	–	
D3F15S2 p21.3	+	–	+	–	–	+	–	–		
ARH12 p21.2	+	–	+	–	–	+				
Col7 p21	+	–	+	–	–	+				A2
GNAI	+	–	+	–	–	+	–	–	–	p21.2-p14.3
ACY1 p21.1	+	–	+	–	–	+	–	–		
D3S2 p21.1	+	–	+	–	–	+	–	–	–	
D3S1076	+	–	+	–	–	+	–	–	–	
D3S1067	+	–	+	–	–	+	–	–	–	
D3S1187	+	–	+	–	–	+	–	–	–	
PTPRG p14.2	+	–	–	+	–	+	–	–	–	
Lib 25–38	+	–	–	+	–	+	–	–	–	
Lib 14–88	+	–	–	+	–	+	–	–	–	B1a
Lib 32–47	+	–	–	+	–	+	–	–	–	p14.2
Lib 14–09	+	–	–	+	–	+	–	–	–	
Lib 7–51	+	–	–	+	–	+	–	–	–	
Lib 14–4	+	–	–	+	–	+			–	
D3S1188	+	–	–	+	–	+	–	–	–	
Lib 12–50	+	–	–	+	–	+	–	–	+	
Lib 11–15	+	–	–	+	–	+			+	
Lib 9–99	+	–	–	+	–	+			+	
Lib 18–9	+	–	–	+	–	+			+	B1b
Lib 24–34	+	–	–	+	–	+	–	–	+	p14.2-p14.1
Lib 12–55	+	–	–	+	–	+	–	–	+	
Lib 26–92	+	–	–	+	–	+	–	–	+	
Lib 17–69	+	–	–	+	–	+	–	–	+	
LSP	+	–	–	+	–	+	–	–	+	
D3S6 p21–14	+	–	–	+	–	+	–	–	+	
D3S642	+	–	–	+	–	+	–	–	+	
D3S227	+	–	–	+	–	–	–	–	+	B2
D3S30 p14–13	+	–	–	+	–	–	–	–	+	p14-p13
D3S39	+	–	–	+	–	–	–	+	+	
Lib 12–83	+	–	–	+	–	–	–	+	+	
Lib 17–31	+	–	–	+	–	–	–	+	+	B3
Lib 23–34	+	–	–	+	–	–	–	+	+	p13
D3S3 p14–13	+	–	–	+	–	–	–	+	+	
Lib 16–81	+	–	–	+	–	–	+	+	+	
D3S78	+	–	–	+	–	–	+	+	+	B4
D3S4 p14–13	+	–	–	+	–	–	+	+	+	p13-p12
PROS1 p11-q11	+	–	–	+	+	–	+	–	+	B5
D3S1 q12	+	–	–	+	+	–	+	–	–	q12

Probes. Anchor probes, used in characterization of the hybrid panel and anchoring of the physically ordered intervals to the cytogenetic and genetic maps of chromosome region 3p, are listed in Table 2. Probes were mapped to hybrids by Southern analysis or by PCR amplification using specific primers which were synthesized based on sequences provided in the GDB. A large panel of 3p-specific probes isolated and mapped previously (25, 26, 51) were mapped by Southern analysis into the physical intervals defined by the hybrids.

RESULTS

Characterization of the 3p Hybrid Panel. After clonal expansion of hybrid cell lines in selective media, DNA was isolated and analyzed for the presence of previously mapped 3p markers (Table 2), using both Southern blot hybridization and PCR amplification. For Southern

blot analysis, the presence of specific 3p markers in hybrids was determined by autoradiographic detection of the human-specific fragment after probe hybridization; for PCR analysis, the human-specific amplification product produced, using primer pairs for 3p markers, was observed in an ethidium bromide-stained gel. We included commonly available markers in the characterization of the hybrid panel to facilitate direct comparison and integration of results with available genetic and physical maps.

Hybrid clones were first tested for presence of 3p markers that would allow identification of clones retaining derivative 3 chromosomes in the absence of normal chromosome 3 or reciprocal derivative chromosomes. Markers were selected based on respective chromosome 3 abnormalities present in the human parental cell lines (Table 1). Cytogenetic analysis of hybrid clones, including whole chromosome 3 painting, was performed to confirm the absence of additional human chromosome 3 fragments. Results are summarized in Table 1. Cytogenetic analysis revealed *de novo* rearrangements of 3p in some hybrid clones. The *de novo* derivative 3 chromosomes present in hybrid clones 297 cl1 and cl3 were previously characterized by G-banding and G11 staining, which did not differentiate between the der3 chromosomes of 297 cl1 and 3, while Southern hybridization suggested a larger portion of 3p in 297 cl3 (38). In the present study, whole chromosome 3 painting of 297 cl1 and cl3 confirmed that 297 cl3 contains a larger portion of 3p in the absence of 3p fragments. Hybrid clone C36.2 also exhibited a *de novo* rearranged 3p. Southern hybridization with 3p markers suggested that it contained, as its only human chromosome 3 component, a portion of 3p in the 3p13–14 region. Whole chromosome 3 painting demonstrated a single small human labeled fragment in most cells analyzed. Hybrid SLF67, derived from fusion of lymphoblasts from a t(3,8) carrier (Table 1), also exhibited a fragmented chromosome 3; combined chromosome 3 painting results and chromosome 3 marker retention are consistent and suggest the presence of two fragments of chromosome 3. The larger fragment resembles an acrocentric chromosome by painting and most likely represents the 3p14→cen region; the smaller fragment is attached to a hamster chromosome.

Anchor probes were mapped to each of the hybrid lines, and the results were integrated to form a hybrid-mapping panel; see bold face markers in Table 3. The nine hybrids separate the short arm of chromosome 3 into eight intervals designated A1, A2, B1a, B1b, B2, B3, B4, and B5. Interval A1 contains the region 3pter to distal 3p21 and includes five of the nine loci (bold face, Table 3) previously mapped to band p21. A2, centromeric to A1 and telomeric to the t(3;8) break, contains the remaining four 3p21-linked loci, including D3S2, which has been mapped to 3p21 (52) or to distal 3p14 (53). Region B1a is directly centromeric to the t(3;8) translocation breakpoint and contains only one anchor marker, the *PTPRG* locus, whose location has been refined to 3p14.2 by FISH on normal and t(3;8) lymphocyte metaphases (see Fig. 1 for an example). Thus, the A2 and B1a intervals represent the regions flanking the t(3;8) translocation breakpoint. Region B1b is defined by the hybrid SLF67 and carries the anchor locus D3S6 which maps by FISH centromeric to *PTPRG* (data not shown). The remaining intervals B2, B3, and B4 each contain one of three anchor markers previously mapped to 3p13–14. B2 contains the D3S30 locus and is most telomeric of the three intervals; B3 contains D3S3 and lies between B2 and B4; B4 contains the D3S4 locus. The relationships of probes and regions are detailed in Table 3 and Fig. 2.

Physical Mapping of 3p Probes on the 3p Hybrid Panel. The hybrid-mapping panel was then used to localize new 3p markers to the intervals defined. Using Southern blotting or PCR amplification, we mapped probes generated by us and others. Since we were particularly interested in saturating intervals surrounding the t(3;8) breakpoint,

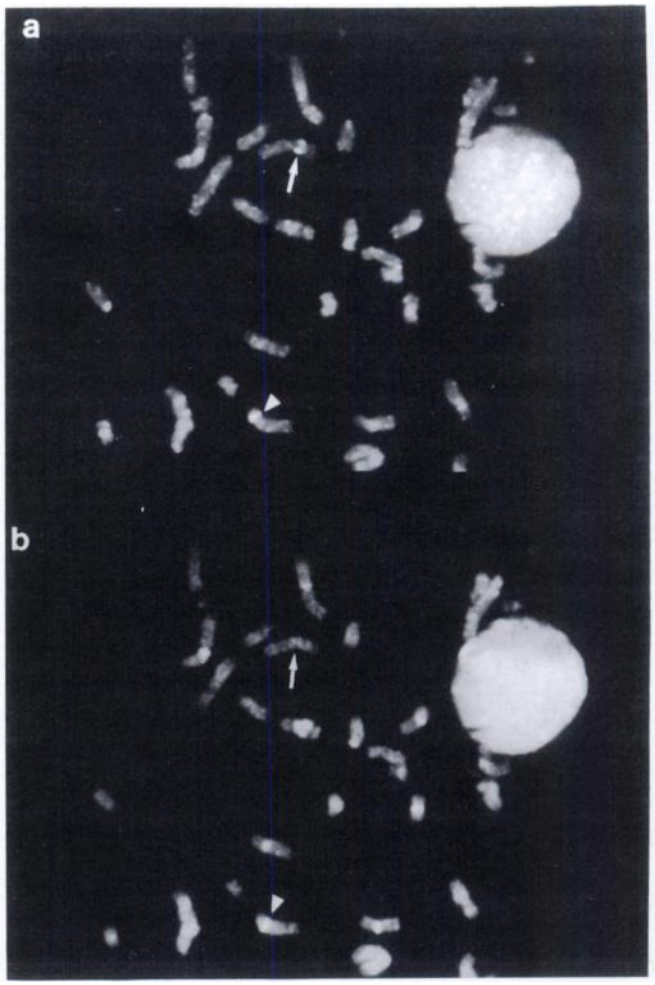

Fig. 1. Fluorescent *in situ* localization of the *PTPRG* locus in 3p14 centromeric to the t(3;8) break. a, partial metaphase of the lymphoblastoid cell 9944, derived from a member of the RCC family carrying the t(3;8) reciprocal translocation. Arrow, fluorescent spots on the normal chromosome 3 at 3p14; arrowhead, *PTPRG* signal on the der3 near the translocation break. b, same metaphase stained with DAPI to illustrate chromosome banding. The biotin-labeled probe was prepared by nick translation of pooled genomic clones from the *PTPRG* locus.

markers were chosen based on preliminary localization to the region 3p12-p21 (25, 26, 51, 54, 55). The majority of markers (designated Lib) were isolated from a chromosome 3 flow-sorted phage library (Los Alamos) and previously localized using an independent hybrid-mapping panel (26). The mapping of the 117 markers are presented partially in Table 3 and entirely in Fig. 2 and shows the highest density of markers in the 3p14.1→3p14.2 region. The mapping by Southern blotting was complemented by FISH analysis of selected probes on metaphases of normal and t(3;8) lymphocytes. D3S1187 mapped to 3p14.3-p21.1 and to the der 8 chromosome; D3S1188 mapped to 3p14.2–14.1 and the der 3 chromosome; probes 25–14, D3S6, and D3S642 mapped to 3p13–14.1 and the der 3 chromosome. Both FISH and hybrid analysis results have been combined in summary form in Fig. 2.

Size of Intervals Adjacent to the t(3;8) Break. By combining the physical mapping data with genetic linkage data, size in megabases of intervals between loci can be estimated. It has recently been reported that D3S30 is 2.6 cM centromeric of D3S6 (56). This can be roughly translated into a physical distance of 2.6 megabases (Fig. 3). This genetic order is consistent with our physical mapping of D3S30 in B2 and D3S6 in B1b as shown in Table 3 and Fig. 3. The D3S6 and D3S642 markers in region B1b are very close together by pulse-field

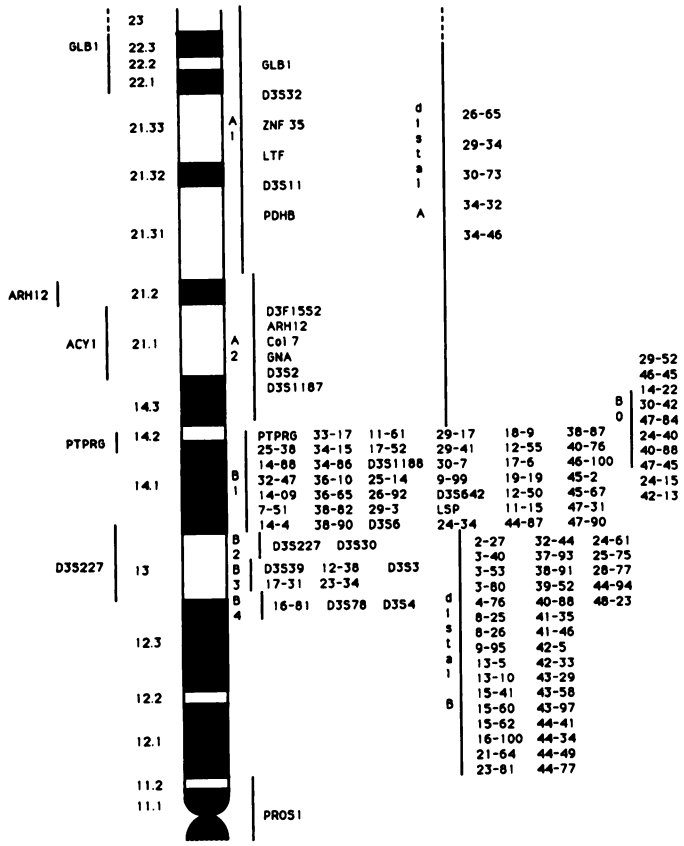

Fig. 2. Positions of genes and probes on chromosome 3p. In order to place regions A1 through B4 from Table 2 into correct positions on 3p, anchor probes were positioned as shown on the 3p ideogram, *left*. The intervals defined by the hybrid panel are shown in correct order and approximate position on the ideogram, *right*; order of markers within the regions was not determined. *Far right, top* (distal A), *middle* (B0), and *bottom* (distal B), groups of markers which were mapped using subsets of the 3p hybrids; these markers have not yet been tested for presence in the full hybrid panel. Distal A is defined as the fragment retained in hybrid 5L.8, distal B is the 3p fragment not retained in hybrid SLF4, and B0 is a region present in hybrid 297 cl3 which may overlap with a region present in hybrid 5L.8.

gel analysis and are within a single YAC clone[5]; thus, the distance between D3S6/D3S642 and D3S1188 can be estimated from earlier genetic maps to be ~15 cM (55). Additionally, the genetic distance between D3S1187 and D3S1188 is ~4.4 cM with *PTPRG* slightly closer to D3S1188 (57), as summarized in Fig. 3. If the distance between D3S1187 and *PTPRG* is ~2.2 cM, then the t(3;8) is within ~1 cM or 1 megabase of either *PTPRG* or D3S1187 and could be much closer to one of them. These size estimates are diagramed in Fig. 3.

Within region B1 are about 50 probes, including *PTPRG* and D3S1188 (Fig. 2); region B1 represents approximately all of band p14.1 and may be about 20 cM, thus representing a marker every 0.5 cM or one marker for approximately every 3–400 kilobases.

This is similar to the density (~150 kilobases) predicted when the probes were originally isolated (26).

Position of the t(3;8) Relative to Other Critical Regions. The placement of the *PTPRG* locus within the B1a region adjacent to the t(3;8) breakpoint and narrowing of that region to ~2 megabases emphasizes the fact that this gene remains a candidate for a tumor suppressor gene affected by the translocation, as previously suggested (38). At the same time, the more precise localization of the *PTPRG* locus demonstrates that it cannot be directly involved in the homozygous deletion of the LC-derived cell line U2020. The U2020 deletion, which was discovered when it was observed that the D3S3 probe was entirely deleted in the U2020 SCLC line (35), is known to be at least 5 megabases (59, 60) in size and is at least partially within our region B3 (Table 3), since D3S3 maps in B3. The *PTPRG* locus is separated from the U2020 deletion by region B2 and by numerous intervening loci and many cM as shown in Table 3 and Fig. 3.

The t(3;8) break is also separate from the 3p21 region most frequently deleted in SCLCs and recently shown to suppress tumorigenicity *in vivo* (61), since at least a portion of region A2 intervenes between the t(3;8) and the 3p21 region of LOH, as diagrammed in Fig. 3. Thus, including the VHL locus (at 3p25.3, not shown), there are at least 4 regions on 3p involved in tumorigenicity.

Order of Markers. In order to interpret and compare the various LOH studies, well-localized, physically, and genetically mapped markers which are commonly available must be included in the study. We have localized a set of commonly available markers (Table 2) to seven linearly ordered intervals on 3p as shown in Table 3. The following order was obtained for some commonly used probes:

D3S1, Cen, PROS1→D3S4→D3S3→D3S30→D3S6→*PTPRG*→D3S2, D3F15S2, *ARH12*→*PDHB*, D3S32, *LTF*, *ZNF35*, D3S11→tel

where arrows are between markers that are separable on the hybrid panel and commas are between markers that have not been relatively ordered.

DISCUSSION

RCC is the most common kidney malignancy to strike adults (31). There are approximately 25,000 cases of RCC and more than 9000

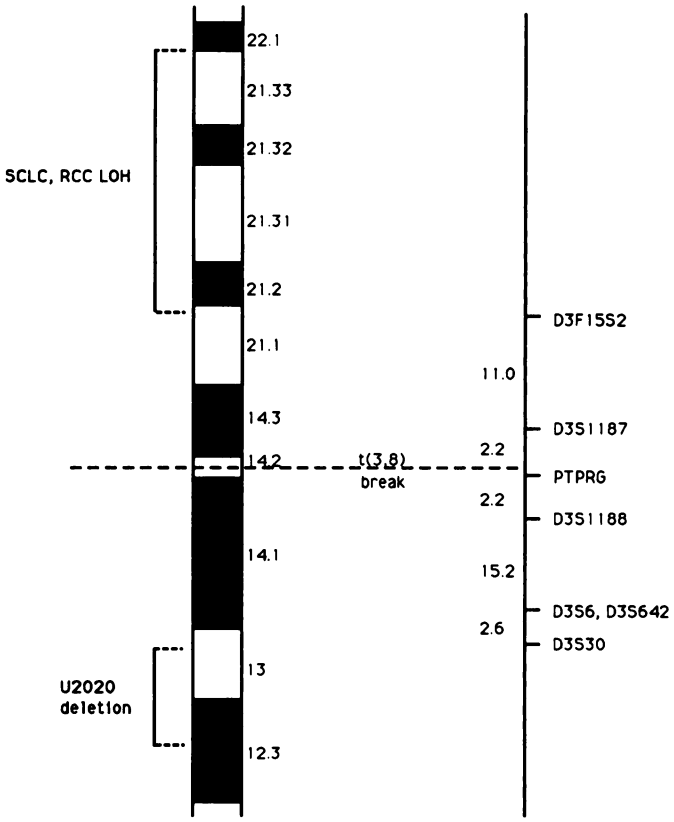

Fig. 3. Position of putative tumor suppressor loci on 3p. The ideogram illustrates the relative positions of putative tumor suppressor loci on 3p in parallel with a partial genetic map. The genetic distances between markers (55–58) can be used to estimate physical distances as discussed in the text. The estimated physical length of chromosome 3 is 214 megabases with an estimated genetic length of 237 cM (58); thus, for chromosome 3, we estimate that 1 cM = ~0.9 megabases.

[5] K. Kastury, M. Ohta, and K. Huebner, unpublished results.

deaths each year in the United States alone. Fundamental problems of late diagnosis and poor response to currently available treatment modalities contribute to this high mortality rate. The development of tumor-specific markers and insight into the molecular events involved in tumor pathogenesis are needed for future advances in early diagnosis and treatment options.

To gain insight into the pathogenesis of RCC one goal is to isolate, by positional cloning, the breakpoint of the t(3;8) translocation associated with hereditary RCC. In order to focus on a small region surrounding the t(3;8) break, we developed and characterized rodent-human hybrid cells retaining portions of chromosome 3p. This hybrid panel created small intervals on each side of the chromosome 3 translocation breakpoint. The t(3;8) break falls between D3S1187 and *PTPRG*, an interval of ~2.2 cM. Thus, the break must be within 1 megabase of one of these markers. We are currently using these markers to isolate YACs, with which contigs will be established, for the fine mapping of the region; FISH will be used to determine which YACs cross the breakpoint.

As we proceed in our cloning efforts, further assessment of *PTPRG* involvement will be possible. Tsukamoto *et al.* (62) recently reported the detection of aberrant *PTPRG* transcripts in two tumor cell lines. We recently cloned and sequenced the smaller of the two major transcripts for *PTPRG* (63, 64); the locus for this 5.3-kilobase cDNA falls entirely on the centromeric side of the 3p14.2 break.[6] Concurrently, we have observed a nucleotide difference within the carbonic anhydrase-like domain of *PTPRG* in an RCC cell line, which results in an amino acid substitution; the other *PTPRG* allele from the same RCC is missing.[7] We have also characterized an homozygous deletion within the *PTPRG* carbonic anhydrase-like domain in murine L-cell lines (64).

The order of commonly available markers developed in this report is largely in agreement with previous genetic and physical maps. Two areas of disagreement exist. Some investigators place D3S4 centromeric to both D3S3 and D3S30 (26, 65), in agreement with our order, while others place D3S4 telomeric to D3S30 which is telomeric to D3S3 (53). Placement of D3S32 with respect to D3F15S2 also remains controversial. Some reports place D3S32 centromeric to D3F15S2 (53), while others agree with our telomeric placement (65). Thus, a lack of consensus exists at both ends of this region. Exact physical order is important for definition of the four separate regions on 3p that play a role in tumorigenesis.

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Detailed Genetic and Physical Map of the 3p Chromosome Region Surrounding the Familial Renal Cell Carcinoma Chromosome Translocation, t(3;8)(p14.2;q24.1)

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