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Translocation of c-*myc* in the hereditary renal cell carcinoma associated with a t(3;8)(p14.2;q24.13) chromosomal translocation

(somatic cell hybrids/gene mapping/Gly⁻B mutant/acylase I)

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ABSTRACT **A translocation between chromosomes 3 and 8, t(3;8)(p14.2;q24.13), has been reported in a family with hereditary renal cell carcinoma. Using somatic cell hybrids, we have isolated, separately, both derivative chromosomes. We find that the c-*myc* oncogene (8q24.1) has been translocated to the derivative 3 [der(3)]. We have not detected a rearrangement within an ≈21-kilobase region around the c-*myc* gene using restriction enzyme digestion and Southern blot hybridization analysis. The translocated c-*myc* gene should provide a probe to the chromosome 3p14 region, which appears to be important not only in renal cell carcinoma but also in small cell carcinoma of the lung. These hybrids have also been useful for the regional mapping of the Chinese hamster ovary cell Gly⁻B defect to 8q22.1→q24.13 and support the regional assignment of acylase I to 3p21.**

Renal cell carcinoma is the most common malignant disease arising from the kidney (1). Although potentially curable when detected early, more frequently this disease is detected late and responds poorly to treatment. In 1979, a unique opportunity became available to investigate the genetic abnormalities that might be involved in renal cell carcinoma. A family with hereditary renal cell carcinoma was reported in which a germ-line reciprocal chromosome translocation, between nos. 3 and 8, was associated with an 87% cumulative probability of developing this disease by age 59 in those family members who inherited the translocation (2). In this translocation, the region of chromosome 8 specified as 8q24.13→8qter has been translocated to chromosome 3 at band 3p14.2, resulting in a derivative 3 [der(3)] chromosome, whereas the 3p14.2→3pter region of chromosome 3 has been translocated to chromosome 8 at band 8q24.13, resulting in a der(8) chromosome (2, 3). The high association of the chromosomal rearrangement with renal cell carcinoma in this family strongly implicates an alteration in expression of one or more genes on chromosome 3 or 8 with this disease. Pathak *et al.* (4) have described another familial occurrence of renal cell carcinoma over three generations, in which a 3;11 translocation was limited to the tumor tissue. Again, the breakpoint on chromosome 3 was at 3p13 or 14. Furthermore, abnormalities of the 3p region appear to be frequent in renal cell carcinomas from unrelated patients. The chromosome 3p14 region is also of interest because small cell carcinoma of the lung has been characteristically associated with a deletion of the 3p14→3p23 region (5). An inducible chromosome fragile site at 3p14 has been described (6, 7), and there is accumulating evidence that an association exists among fragile sites, the locations of certain oncogenes, and specific chromosome rearrangements in various malignant diseases (8, 9).

The chromosome 8 breakpoint occurs cytogenetically where the c-*myc* oncogene is located (10). Deregulation of c-*myc* expression as a result of the translocations involving this oncogene is a frequent, if not consistent, finding in Burkitt lymphoma (11, 12). Therefore, the 3;8 translocation associated with renal cell carcinoma appears to involve two chromosome regions highly implicated in the development of several malignant diseases.

We have isolated both derivative chromosomes from cells from family members carrying this 3;8 translocation in separate somatic cell hybrids that do not contain the normal chromosome 3 or 8 by cytogenetic, biochemical, or molecular analysis. We find that the c-*myc* oncogene (8q24.1), normally located at the cytogenetic region where the break occurs, has been translocated to the der(3).

MATERIAL AND METHODS

Chinese Hamster Ovary (CHO) Cell Mutants. The CHO cell mutant Gly⁻B is defective in glycine metabolism (13), which is complemented by a gene on chromosome 8 (14)—specifically, the region 8q22→qter (15). The Urd⁻C cell mutant requires exogenous uridine for growth, which is due to the lack of orotate phosphoribosyltransferase and orotidylate decarboxylase activities (16). The gene coding for these enzymatic activities has been assigned to the long arm of chromosome 3. Gly⁻B cells were grown in F12 medium (17) containing 6% (vol/vol) fetal calf serum, and Urd-C was grown in the same medium with the addition of 30 μM uridine.

Cell Fusion. Cell fusions were done by using UV-inactivated Sendai virus as described (18). The Gly⁻B hybrids were constructed from the t(3;8)-containing fibroblast line 2843T. The Urd⁻C hybrids were constructed from a t(3;8)-containing lymphoblastoid line, TL9542, kindly provided by Tom Glover. Hybrids were selected by growth in the appropriate deficient medium.

Production of Segregants That Have Lost the der(8). Segregants were produced by using the BrdUrd and visible light method as described by Puck and Kao (19). Briefly, after growth in complete medium to facilitate loss of the der(8), the cells were starved in deficient medium for 24 hr. BrdUrd, at 0.01 mM was added for 24 hr, followed by exposure of the cells to visible light. Those cells that had lost the der(8) ceased DNA synthesis and did not incorporate BrdUrd in deficient medium, thereby surviving the exposure to visible light.

Cytogenetic Analysis. Sequential Giemsa/trypsin banding and Giemsa-11 (G-11) staining have been described (20, 21).

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Abbreviation: CHO, Chinese hamster ovary.

With G-11 staining, human chromosomes appear blue, whereas the CHO chromosomes appear magenta.

Isozyme Analysis. Isozyme analysis was performed by using Cellogel electrophoresis according to procedures described by Meera Khan (22) and van Someren *et al.* (23). The acylase I isozyme analysis was performed by using the method of Voss *et al.* (24).

Molecular Probes. The molecular probes used were kindly provided by the following investigators: c-*mos*, George Vande Woude; c-*myc* (Ryc 7.4), Carlo Croce; c-*raf* 3′ flanking sequences, Tom Bonner; and UMP synthase, D. Parker Suttle.

DNA Isolation, Gel Electrophoresis, Southern Transfer, and Hybridization. High molecular weight DNA was prepared as described by Gusella *et al.* (25). DNA was digested with the indicated restriction endonuclease under conditions recommended by the manufacturer. Digested DNA was subjected to electrophoresis in a horizontal 0.75% agarose gel in a standard Tris/acetate buffer. Transfer of DNA to nitrocellulose paper was performed by using the method of Southern (26). *Hin*dIII-digested phage λ DNA was used for molecular weight markers. The nitrocellulose-bound DNA was then hybridized in a solution containing 50% formamide (vol/vol) and 0.75 M sodium chloride/75 mM sodium citrate, pH 7.0, at 42°C for 15–18 hr to the indicated ^{32}P-labeled probes that had been nick-translated to a specific activity of $\approx 10^8$ cpm/μg of DNA. The filters were washed with 0.3 M sodium chloride/30 mM sodium citrate, 0.1% (vol/vol) $NaDodSO_4$ at room temperature and followed by washing in 15 mM sodium chloride/1.5 mM sodium citrate at 55°C. Filters were air-dried and used to expose Kodak XAR-5 film for various periods.

Determination of the Human Transferrin Receptor. An immunofluorescent assay, similar to that used by Hoffman *et al.* (27), was utilized to determine the presence or absence of the human transferrin receptor on the cell surface. A mouse monoclonal antibody, 3a7.7.1.2, specific for the human transferrin receptor, was kindly provided by York Miller and Carol Jones. The human lymphoblastoid line TL9542 and a human–hamster hybrid, 314, containing only human chromosome 3 were used as positive controls. The CHO cell line K1 was used as a negative control.

RESULTS

Previously, we localized the CHO Gly$^-$B defect to the region 8q22.1→qter (15). Therefore, it was possible that either of the derivative chromosomes would be selectively retained in the Gly$^-$B–human hybrids. We therefore screened Gly$^-$B hybrids cytogenetically for both of the derivative chromosomes demonstrated in Fig. 1*A*. Hybrid 3;8/4-1, shown in Fig. 1*B*, was found to contain the der(8), in the absence of the der(3) and the normal nos. 3 and 8. In addition to the der(8) chromosome, which was identified in 23 of 24 mitoses examined, the human chromosomes 6, 12, 19, 20, and 21 were variably present as was a marker chromosome that presumably arose during the hybrid formation. This further defines the region of chromosome 8 that complements the glycine-requiring CHO mutant Gly$^-$B as 8q22.1→8q24.1.

The cytogenetic assessment of the chromosome breakpoints in t(3;8)(p14.2;q24.13) would predict that the following chromosome 3 and 8 markers should be present in hybrid 3;8/4-1: human glutathione reductase activity (8p21.1) (28), human c-*mos* (8q22) (29), and human c-*raf* (3p25) (ref. 30 and T. Bonner, personal communication). These markers were indeed present. Fig. 2 demonstrates the Southern blot hybridization experiments using the c-*mos* and c-*raf* probes, which confirm the presence of these DNA sequences in hybrid 3;8/4-1.

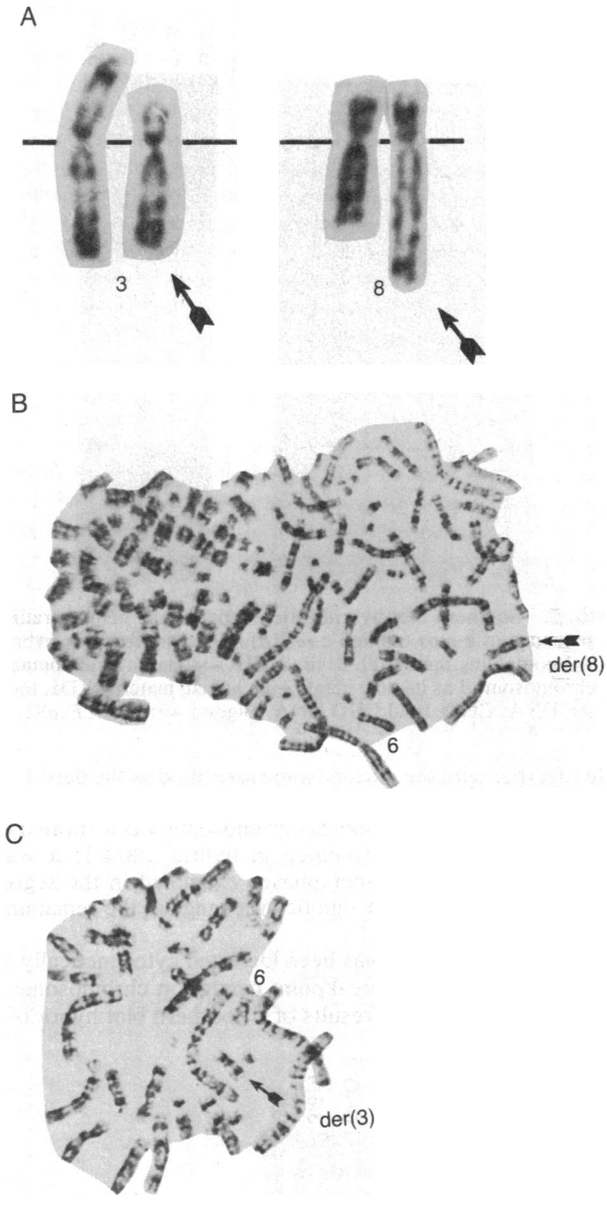

FIG. 1. Giemsa/trypsin/Giemsa-11 banding of metaphase cells. (*A*) Partial karyotype showing the chromosome pairs 3 and 8 from a patient with the 3;8 translocation. The rearranged chromosomes are indicated by arrows. (*B*) CHO–human hybrid 3;8/4-1 containing the der(8). Two adjacent metaphase spreads are seen. (*C*) CHO–human hybrid TL9542/UC2-1 containing the der(3).

The gene coding for human acylase I activity has been localized previously to chromosome 3, more specifically by the smallest region of overlap to 3p21 (31). Hybrid 3;8/4-1 contains human acylase I activity but does not contain UMP synthase DNA sequences (Fig. 3) and does not express the human transferrin receptor (data not shown). The genes for both UMP synthase and the transferrin receptor have been localized to the long arm of chromosome 3 (16, 32). These data further confirm the nature of the 3;8 translocation and the assignment of acylase I to 3p21.

Segregants of hybrid 3;8/4-1 were produced by the BrdUrd-visible light method. Eight separate clones were isolated. All clones had lost the ability to grow in glycine-deficient medium, as well as glutathione reductase and acylase I activities, demonstrating that these markers, originally located on chromosomes 8 and 3, respectively, segre-

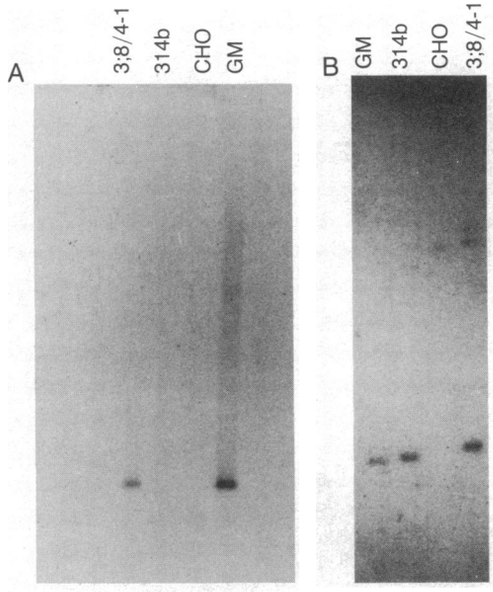

FIG. 2. Southern blot hybridization experiments demonstrating the presence of c-*mos* (*A*) and c-*raf* (*B*) DNA sequences in hybrid 3;8/4-1 containing the der(8). 314b, A CHO–human hybrid containing chromosome 3 as its only identifiable human material; GM, total human DNA; CHO, total CHO DNA. Digests were with *Eco*RI.

gate together with the chromosome identified as the der(8). A detailed cytogenetic analysis on one of these segregants was performed. Whereas the der(8) chromosome was identified in 23 of 24 metaphases examined in hybrid 3;8/4-1, it was uniformly absent in 21 metaphases examined in the segregant, while there was no significant change in the remaining human chromosomes.

The c-*myc* oncogene has been localized cytogenetically to band 8q24, where the breakpoint occurs on chromosome 8 (10). Fig. 4*A* shows the results of a Southern blot hybridization experiment to determine if c-*myc* has been translocated. The human c-*myc* DNA sequences are not present in hybrid 3;8/4-1, indicating that these sequences have been translocated to the der(3) chromosome. To confirm this finding, we isolated the der(3) in a separate hybrid by using the Urd$^-$C mutant. Hybrid TL9542/UC2-1 (Fig. 1*C*) contains, by cytogenetic analysis, the der(3) in the absence of the der(8) and the normal nos. 3 and 8. In contrast to hybrid 3;8/4-1, this hybrid lacks c-*raf* DNA sequences as well as human acylase I and glutathione reductase activities. However, the human c-*myc* DNA sequences are present in hybrid TL9542/UC2-1, as demonstrated in Fig. 4*B*. Moreover, this hybrid expresses the human transferrin receptor and contains the UMP synthase DNA sequences (data not shown). A summary of these results is given in Table 1.

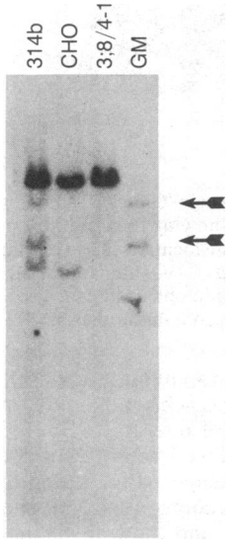

FIG. 3. Southern blot hybridization demonstrating the absence of UMP synthase DNA sequences, from the long arm of chromosome 3, in hybrid 3;8/4-1 containing the der(8). The probe used was isolated from a rat cDNA library. Digestion was with *Eco*RI. The human bands are indicated by arrows. GM and CHO, total human and hamster DNA, respectively; 314b, a CHO–human hybrid containing human chromosome 3. The lowest band seen in 314b and CHO is of hamster origin and appears to be polymorphic in various CHO lines examined.

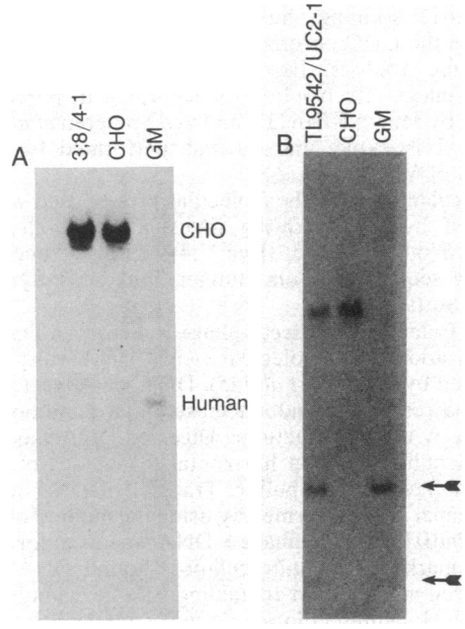

FIG. 4. Southern blot hybridization experiments demonstrating the translocation of c-*myc* DNA sequences. DNA was digested with *Sst* I and probed with c-*myc* (Ryc 7.4). The absence of c-*myc* in hybrid 3;8/4-1 containing the der(8) is shown in *A*. The presence of c-*myc* in hybrid TL9542/UC2-1 containing the der(3) in the absence of the normal chromosome 8 is shown in *B*. The human bands in *B* are indicated by arrows.

Table 1. Summary of molecular and biochemical analysis of hybrid 3;8/4-1, hybrid TL9542/UC2-1, and segregant of hybrid 3;8/4-1

	Hybrid 3;8/4-1 (der8)	Hybrid TL9542/UC2-1 (der3)	Segregant of hybrid 3;8/4-1
Complements Gly$^-$B (8q22.1→qter)	+	NA	−
Complements Urd$^-$C (3q)	NA	+	NA
Glutathione reductase (8p21.1)	+	−	−
c-*mos* (8q22)	+	−	−
c-*myc* (8q24)	−	+	NT
Acylase I (3p21)	+	−	−
c-*raf* (3p25)	+	−	NT
UMP synthase (3q)	−	+	NT
Transferrin receptor (3q23→qter)	−	+	NT

NA, not applicable; NT, not tested; +, activity or DNA sequence present; −, activity or DNA sequence absent.

We have not detected any rearrangement of c-*myc* in DNA from a patient with the 3;8 translocation, using the enzymes *Eco*RI, *Sst* I, and *Bam*HI and the c-*myc* probe Ryc 7.4, which cover ≈21 kilobases (33). In addition, we have not detected a rearrangement using a c-*myc* first exon probe and the enzymes *Eco*RI, *Pst* I, and *Sst* I.

DISCUSSION

We have isolated separately both derivative chromosomes found to be highly associated with a hereditary form of renal cell carcinoma in somatic cell hybrids. We have demonstrated that the c-*myc* oncogene located cytogenetically at the region on chromosome 8 where the breakpoint occurs has been translocated to the der(3). We have not detected a rearrangement in a 21-kilobase region surrounding c-*myc*, suggesting that the site of rearrangement occurs outside this region.

Most cases of Burkitt lymphoma are associated with an 8;14 translocation in which the c-*myc* gene is involved, although rearrangements of the c-*myc* locus detectable by using conventional electrophoretic techniques are not always seen. The translocation seen in Burkitt lymphoma seems to result in deregulation of transcription of the c-*myc* gene, whether or not the translocation breakpoint is within the gene (11). Altered transcription of c-*myc* has also been observed in other malignancies. Clearly, translocation of c-*myc* in this hereditary renal cell carcinoma is consistent with the possibility that altered transcription of this gene might be involved in this disease.

The chromosome 3 region that is involved in the 3;8 translocation is also of considerable interest. Pathak *et al.* have described a family with hereditary renal cell carcinoma in which a 3;11 translocation was found only in the tumor tissue (4). The chromosome 3 breakpoint was 3p13 or 14. Additionally, deletions in the short arm of chromosome 3 appear to be present in many nonfamilial renal cell carcinomas from unrelated patients (34). Thus, alterations in the 3p region may be critical for the development of renal cell carcinoma and possibly small cell lung cancer.

A constitutive inducible chromosomal fragile site has been identified at the 3p14 region (6, 7). This fragile site appears to be inducible to high levels by a variety of chemical agents (9, 35). The location of chromosomal fragile sites has been shown to be correlated with the locations of known oncogenes and with the locations of chromosomal abnormalities associated with various malignancies (8, 9). Clearly, the fragile site at 3p14 shows such a correlation. It is conceivable that this fragile site facilitates the 3p14 alterations seen in renal cell carcinoma and small cell lung cancer. Our hybrid containing the translocated c-*myc* gene at 3p14.2 should greatly facilitate the isolation of genes in this region. One possibility would be to utilize newly described electrophoretic techniques (36, 37) and *myc*-specific DNA probes to isolate the 3;8 junction region.

It is certainly possible, and perhaps even likely, that abnormalities involving sites on both chromosomes 3 and 8 are important for development of renal cell carcinoma in this family and perhaps also in general. The development of malignancy is felt to be a multistep process. Abnormalities resulting from the 3;8 translocation could provide one or even two of these steps along the pathway if both chromosomal regions participate in the pathogenesis of this disease. Clearly, alterations in the c-*myc* oncogene have been detected in a variety of tumors and the 3p14 region likewise appears important. Further research into the 3p14 region is likely to prove important not only for understanding renal cell carcinoma but also small cell lung cancer, chromosome fragile sites, and perhaps additional tumor types, such as malignant mesothelioma (38).

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