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The *DIRC1* gene at chromosome 2q33 spans a familial RCC-associated t(2;3)(q33;q21) chromosome translocation

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Abstract A reciprocal, balanced, constitutional chromosome translocation, t(2;3)(q33;q21), which is associated with familial clear cell renal cancer, has been described and the genomic regions surrounding the 2q and 3q breakpoints have been characterized. Based on the genomic map of the 2q break, EST AI468595 was positioned near the 2q33 translocation and the full-length gene and cDNA were isolated. This 57-kb gene, designated the *DIRC1* gene, was disrupted between exons 1 and 2 by the familial translocation. The 1.5-kb mRNA encodes an 11-kDa predicted protein of 104 amino acids. Low-level expression of *DIRC1* was detected by reverse transcriptase-polymerase chain reaction amplification in adult placenta, testis, ovary, and prostate and in fetal kidney, spleen, and skeletal muscle. A GFP-Dirc1 fusion protein was expressed in vitro and a polyclonal anti-Dirc1 peptide serum was prepared. A panel of cancer and cancer-derived cell line DNAs was examined for *DIRC1* mutations, but only a rare polymorphism was observed. Two familial tumors showed loss of the derivative 3 chromosome, as observed in a Dutch kindred with t(2;3)-associated renal cancers. Mutations in the second *DIRC1* allele were not detected. Further studies will be required to determine if disruption of the *DIRC1* gene contributed to development of the associated familial clear cell renal cancers.

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Introduction

Two families have been described with similar balanced constitutional chromosome translocations that segregate with renal cell cancer (RCC) (Koolen et al. 1998; Zajaczek et al. 1999). Podolski et al. (2001) describe in more detail a Polish family with a balanced constitutional chromosome translocation, t(2;3)(q33;q21). Two living brothers presented with early-onset clear cell renal cancer; kidney cancer was also noted in two earlier generations. A Dutch family with a similar constitutional translocation, t(2,3)(q35;q21), had four family members in three generations with RCCs (Koolen et al. 1998; Bodmer et al. 1998).

In 1979 Cohen et al. identified a large three-generation family in which clear cell renal carcinoma was associated with inheritance of a balanced t(3;8)(p14.2;q24) chromosome translocation. It was proposed that dysregulation of a gene at the 3p translocation breakpoint might contribute to development of the bilateral, multifocal RCCs. Ohta et al. (1996) identified and characterized the *FHIT* gene at 3p14.2, which encompassed the 3p14.2 translocation break, as well as the most active common fragile site and numerous other cancer-associated chromosome alterations. The gene was proposed as a tumor suppressor gene and experimental support for the proposal continues to accumulate (Ishii et al. 2001; Dumon et al. 2001). At the same time, a number of investigators suggested that chromosomal translocations can contribute to kidney cancer initiation or progression, in the sporadic or familial setting, through facilitation of tumor-specific loss of derivative chromosomes carrying large portions of the short arm of chromosome 3 (Gnarra et al. 1994; Bodmer et al. 1998; Eleveld et al. 2001), the locus of several proposed renal cancer tumor suppressor genes, including the *VHL* gene.

Consistent with this hypothesis, DNA from several tumors from the Dutch familial RCC kindred had lost the derivative 3 (der3) chromosome. Very recently a constitutional t(1;3)(q32;q13.3) associated with RCC in a Japanese kindred was described (Kanayama et al. 2001), and several familial tumors had lost the der3. Also, consistent with this hypothesis, the chromosome breakpoints in the Polish family differed from those of the Dutch family (Podolski et al. 2001), eliminating the possibility of a recurrent translocation break that might have pointed to a specific kidney cancer gene. The Japanese 3q13.3 break was not characterized in enough detail to determine if the breakpoint is similar to the Dutch or Polish 3q break.

It is important to determine if there are altered genes at translocation breakpoints in the familial cancers, so that a possible contribution to cancer development can be explored. We have used standard positional cloning strategies to identify the genomic regions encompassing the breakpoints on chromosomes 2q and 3q, followed by sequencing and database searches for markers, ESTs, and genes surrounding the breaks. A full-length cDNA for EST AI468595 was cloned and the gene, designated *DIRC1* (Disrupted in Renal Cancer 1), was characterized and shown to be disrupted by the 2q33 translocation. To determine if disruption of this gene might have contributed to development of the familial cancers, we initiated experiments to characterize the biological activity of the gene and to assess the integrity of the *DIRC1* locus in a panel of cancers.

Materials and methods

Cells and tissues

An Epstein-Barr virus-transformed lymphoblast cell line was established from an affected member of the t(2;3) family. Also, paraffin blocks of tissue samples from three affected family members were available for microdissection and other analyses. Informed consent from family members was obtained for inclusion of these materials in the study. DNAs from normal and cancer tissue from sporadic renal cancer cases were obtained from the Kimmel Cancer Center Pathology Core shared resource. Collection, storage, and use of human tissues from the facility has been reviewed and approved by the appropriate Institutional Review Board.

Rapid amplification of cDNA ends (RACE)

5′ and 3′ RACE products were generated from testis Marathon-Ready cDNA (Clontech, Palo Alto, CA, USA) with AP1 primer (Clontech) and primers specific for the AI468595 EST sequence. To increase the specificity of the product, a second polymerase chain reaction (PCR) was carried out using nested gene-specific primers and the AP2 primer (Clontech). PCR cycling was performed at 94°C for 5s and 72°C for 5min for 5 cycles, 94°C for 5s and 70°C for 5min for 5 cycles, and 94°C for 5s and 68°C for 5min for 25 cycles. RACE products were electrophoresed and sequenced. Similar methods were used to look for chimeric transcripts in familial lymphoblast RNA.

Reverse transcriptase-PCR

Multiple tissue cDNA (MTC) panels (Clontech) were used as templates in standard PCR reactions with ~10pmol of each gene-specific primer (Table 1) for 35 cycles of 95°C for 30s, 60°C for 30s, and 72°C for 1min. Products were electrophoresed and sometimes isolated and sequenced, or cloned and sequenced.

Single-strand conformation polymorphism (SSCP)

The procedure for PCR-SSCP was described previously (Druck et al. 1995). Samples were amplified by PCR with the appropriate oligonucleotides flanking each exon (Table 1). PCR product (1μl) was mixed with 10μl of loading buffer (95% formamide, 10mM NaOH, 0.05% bromphenol blue, 0.05% xylene cyanol FF) and denatured at 94°C for 5min. Samples were electrophoresed on a 0.5 X Hydrolink MDE Gel (J.T. Baker, Phillipsburg, NJ, USA) at 5W for ~18h.

Loss of heterozygosity

Primers that amplify polymorphic microsatellite-containing alleles were used for loci *D3S1581* (3p21.2), *D3S1262* (3q27), *D2S103* (2q33, centromeric to translocation), and

Table 1. *DIRC1* primers for SSCP and RTPCR

Name	Sequence 5′ → 3′	Use
5UTRF1RACE	TGA CAT GGG CAG AGT TTC TCT TGC C	Exon 1 SSCP
sscp5utrinR	AAT AGA GTA GAC TGT CCT GG	Exon 1 SSCP
sscpi1F	CCC TGG TAG ACA TCA ATT TT	Exon 2a SSCP
5RACER	GGA AGG CTC CAA TGT TGC AGC AGC A	Exon 2a SSCP
3RACEF2	CAC CAC AGA CCA CGG AAG CAA GAA G	Exon 2b SSCP
3UTRR2	AAC GAA TAA AGG GAG ATG AG	Exon 2b SSCP
JP5raceF	TTT CTC TTG CCC TTA AAG TC	RTPCR
3UTRR	GGA GTA CAT TAT GGA ATG GA	RTPCR

SSCP, single-strand conformation polymorphism; RT-PCR, reverse transcriptase polymerase chain reaction

D2S115 (2q33, telomeric to translocation). These loci were amplified from normal and tumor DNA from kidney tissue after microdissection and extraction from fixed tissue obtained from surgical procedures of affected family members. The PCR amplification and electrophoresis procedures were previously described (Druck et al. 1995).

A region surrounding the 3q21 breakpoint was analyzed for allelic imbalance in a group of 21 primary clear cell RCCs. DNA was extracted from matched normal and cancerous kidney tissue as described previously (Lubinski et al. 1994). Primer pairs that amplify polymorphic microsatellites were used for the following loci: *D3S1552* (3p11–p12), *D3S3634* (3q11), *D3S1603* (3q11–q12), *D3S1271* (3q11–q13), *D3S1278* (3q13), *D3S1303* (3q13–q21), *D3S1258* (3q25–q26), *D3S1589* (3q21). Band positions were estimated from genetic and physical maps that are not in complete agreement with the cytogenetic location of our 3q21 breakpoint, which is between *D3S1271* (3q11–q13) and *D3S1278* (3q13), although the breakpoint placement is in agreement with Whitehead yeast artificial chromosome (YAC) contigs. Allelic imbalance of a marker was scored if the signal of one allele was at least 50% reduced in the tumor DNA when compared with its corresponding allele in the normal DNA. Tumors displaying microsatellite instability were not scored for imbalance at unstable loci.

Expression vectors

The *DIRC1* cDNA was amplified from a fetal kidney cDNA template (Clontech) using a forward primer containing an *Eco*RI site, a Kozak sequence, a FLAG octapeptide coding sequence, and the first few bases of the *DIRC1* sequence, and a reverse primer with the last few bases of the *DIRC1* sequence, including the stop and an *Xho*I restriction site. The PCR product was cloned into the *Eco*RI and *Xho*I sites of the pcDNA3 vector (Invitrogen, Rockville, MD, USA). *DIRC1* cDNA was also cloned into the *Xho*I and *Eco*RI sites of the pEGFP-C3 vector (Clontech).

Transfection

NIH3T3 and 293 cells were transfected with 1 μg plasmid vector DNA, with or without *DIRC1* insert, using the procedure recommended for the Fugene (Roche, Indianapolis, IN, USA) transfection reagent. For protein analysis after transient transfection, cells were lysed 24 or 48 h after transfection. Stably transfected 293 clones were selected in the presence of 600 μg/ml G418. Colonies were counted after methanol/acetone fixation and Giemsa staining.

Immunoblotting

Cell lysates and Western blots were prepared as described previously (Druck et al. 1997). Dirc1 protein was detected by using anti-FLAG, anti-green fluorescent protein (GFP), or rabbit polyclonal anti-Dirc1 antibody. Signal was detected with Super Signal chemiluminescent substrate (Pierce, Rockford, IL, USA). The rabbit polyclonal anti-Dirc1 antiserum was commercially prepared (Zymed, San Francisco, CA, USA) against Dirc1 peptide YKPITKDQLSSRSE (amino acids 67–80).

Results

Strategy for identification of breakpoint genes

Because the break on chromosome 3 in both the Dutch and Polish families was identified as 3q21 by cytogenetic analysis, we initiated our search for a gene near the 3q21 break, although it became clear early in our experiments that the 3q breaks in the two families were far apart (Podolski et al. 2001). We identified and sequenced bacterial artificial chromosome (BAC) 181L24 that, by fluorescent in situ hybridization (FISH) analysis, crossed the Polish 3q translocation. Various methods were used to identify cloned and uncloned genes within this BAC, but the sequence is highly repetitive and apparently gene poor. The region around the 2q33 break was also examined for genes. FISH analyses identified BAC 470J8 as crossing this translocation break and methods of finding genes revealed a candidate, EST AI468595. We obtained the full-length cDNA by RACE and characterized the genomic locus, studied its expression in tissues, examined cancer DNAs for alterations of the locus, and began protein studies.

DIRC1 cDNA sequence and genomic structure

EST AI468595 was determined to be near the 2q translocation by homology with our BAC 470J8 sequence, which crosses the 2q breakpoint. We obtained the full-length *DIRC1* cDNA sequence by extending the EST sequence with 5′ and 3′ RACE reactions using human testis cDNA as a template. The RACE products were sequenced and compared with the genomic sequence of BAC 470J8. The ~1.5 kb *DIRC1* cDNA sequence (accession #AY039011) comprised two exons, with exon 1 encoding the 5′ untranslated region (UTR) (accession #AY039012) and exon 2 encoding the amino acids and the 3′ UTR (accession #AY039013). The intron between the two exons is ~55 kb, and exon/intron boundary consensus sequences are conserved. Exon 1 is located on the telomeric side of the 2q translocation and exon 2 on the centromeric side; therefore in the familial RCC cases, exon 2 is on the der2 and exon 1 is on the der3. The predicted Dirc1 protein is 104 amino acids and does not share homology with known proteins. The cDNA and amino acid sequences are shown in Fig. 1.

Expression of endogenous *DIRC1*

To determine expression levels in tissues, tumors, and tumor cell lines, we performed RT-PCR and Northern analysis. On a Northern blot we were able to detect a faint ~1.5-kb band in Poly(A)$^+$ human tissue RNA samples from adult heart, skeletal muscle, kidney, and liver (not shown).

Fig. 1A,B. *DIRC1* sequence and genomic locus. **A** The nucleotide and predicted amino acid sequences of the *DIRC1* gene are shown. The first base of exon 2 is *underlined*. Amino acid 51, which can change from S → A because of a polymorphism, is *bold*. **B** The *DIRC1* genomic locus is drawn to scale, with *DIRC1* exons, sequence tagged sites (STSs), and the translocation breakpoint shown *above the line*. The restriction sites useful for localizing the translocation breakpoint are shown *under the line*

A

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   1 TGACATGGGCAGAGTTTCTCTTGCCCTTAAAGTCTTACTTTCCACTTTCTAAATACCTTC
  61 CAAGATGTTTTCCTCCACAAACACAATATCTTCCTTAAAAGATAGACCCTGCATCAGTTG
 121 TTAAGCTAACACATCTTTCTCCTCACTACAAATGTATCACTATTGAATTTCTCTCCACAA
 181 GTGTGAAGATGGCTGGAGTAGAAATTCTGGCATTGCTTCTCATCACCAGAAGGAAAAATT
 241 CAGAGAAACAGCCCGACCACCCACCCTGACAAATGCCTGAGGCCCACATGCAGCCTGCAA
                                   1  M  P  E  A  H  M  Q  P  A  K
 301 AACTGCAAACATCACTGCCCACCACAGACCACGGAAGCAAGAAGCCTGTTTCCTGTTACT
  11  L  Q  T  S  L  P  T  T  D  H  G  S  K  K  P  V  S  C  Y  L
 361 TGCCACCACTCAGCAATGCCCACCCCATGTGTATAGAGGTCCAGAATGCTCAAAATTGTT
  31  P  P  L  S  N  A  H  P  M  C  I  E  V  Q  N  A  Q  N  C  S
 421 CTTCTGCTGCTGCAACATTGGAGCCTTCCATCATCTCAGACACATGCTTTTATAAACCAA
  51  S  A  A  A  T  L  E  P  S  I  I  S  D  T  C  F  Y  K  P  I
 481 TAACAAAGGATCAACTTTCTTCTAGGTCTGAATTGAATACTGTGAGGCTAAAGTGTCTTA
  71  T  K  D  Q  L  S  S  R  S  E  L  N  T  V  R  L  K  C  L  N
 541 ACTCATTGAGGGGGTGGAAAATATTAAACCAACTAAGCCTTACCTAATTACAATGCCCTG
  91  S  L  R  G  W  K  I  L  N  Q  L  S  L  T
 601 CTGCTGCTCATCTCCCTTTATTCGTTTGTACTCATACTCAAAAGAGGTTGTGTTTTTTTC
 661 CCTTCATTCAATAACGATTTTCCTTGATTCTTAGATAAGTCCATTCCATAATGTACTCCT
 721 TAACTGTTCTGAATTTCTTTCAGAGAAAAACTGGAAAAGACATTAAATCATGTTGAAATA
 781 TTTGACTGACTTTCATCTAGGAAAAAATATAAAGTTAAATCTCTACACTAAAATATAAAC
 841 AAAATAAAAAGAAATTTCAGTAAGACCAAAACATTTACTTTTAAAAACCTATATCATGTT
 901 ATATGTATAACTGAGGAATGTAATCATAATTTTGATTTGTAGAACTGAAGACAATATCAA
 961 GTAACCCTCAAAATGTAAATTCAAGAAACAAGGTAGTTACGTTAGCCTGCATTAAAATAA
1021 AAATATCTGTGTGACAATATATGCTGTAGAGCAAATTAAAAAACAAGTGAAAAGGTTGGG
1081 TAAATATTTGCAATACATATTGATACGGTTTGGATTTGTGTCCCTGCCCAAATCTTATGG
1141 CAAATTGGAGGAGGGGCCTGGTGGGAGGTGATTGGATCATAGGAGCGGATTTCCCCCTTG
1201 CTGTTCTCATGATAGTGAGTGAGTTCTCACAATATCTGATGGATTAAAAGTGTGTGCACC
1261 TCCCCTCTTGCACTTTCTCTCCAGTCACCATGTGAAGTTCCTTGCTTCCCCTTCTGCCAT
1321 GATTGTAAGTTTCCTGAGGCCTCCCAGTCATGCTTCTTATTAAGCCTGTGGAATTGTGAG
1381 TCAATTAAGCCTTTTTATTCATAAATTATCCAGTCTCAGGTAGATCTTTATAGAGTGTGA
1441 AAACAGACTAATGC
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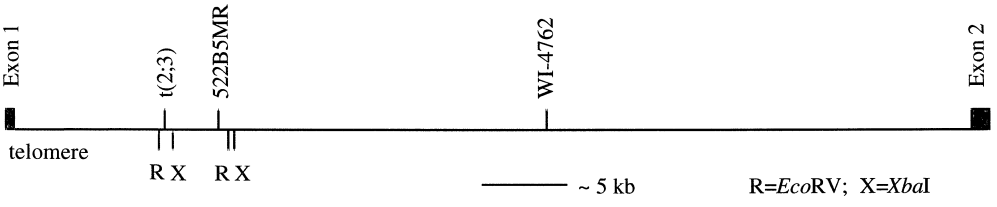
B



By RT-PCR analysis, we were able to detect expression in adult ovary (Fig. 2A), fetal kidney, and fetal skeletal muscle after 50 cycles (Fig. 2B), as well as in the proband lymphoblasts. In tumor cell lines and primary tumors we did not detect *DIRC1* transcription even by this very sensitive method.

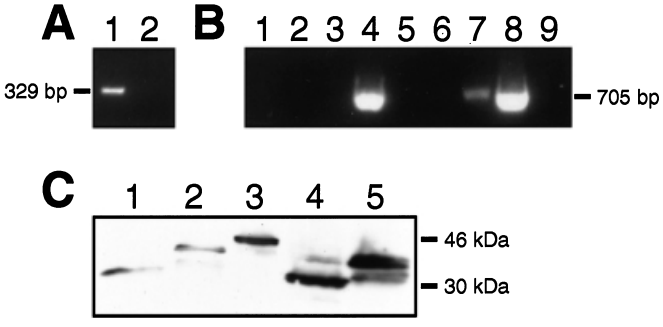


Fig. 2A–C. Expression of *DIRC1* in normal tissue. **A** Nested reverse transcriptase (RT)-PCR for 35 cycles per round was performed and products from ovary (*lane 1*) and no DNA (*lane 2*) were run in a gel. **B** RT-PCR for one round of 50 cycles is shown for fetal tissues from brain (*lane 1*), lung (*lane 2*), liver (*lane 3*), kidney (*lane 4*), heart (*lane 5*), spleen (*lane 6*), thymus (*lane 7*), skeletal muscle (*lane 8*), and no DNA control (*lane 9*). **C** 3T3 (*lanes 1–3*) and 293 (*lanes 4* and *5*) cells were transfected with green fluorescent protein (GFP) (*lanes 1* and *4*), GFP/Dirc1 (*lanes 2* and *5*), or GFP/Fhit (*lane 3*). Cells were lysed and the protein lysates run on a 12% acrylamide gel, transferred to nitrocellulose, and GFP and GFP fusion proteins detected with a GFP monoclonal antibody

Evaluation of the *DIRC1* gene in familial tissues and in tumors

Because the gene, although not the predicted protein coding exon, is interrupted by the translocation, expression of the allele on the der2 may be dysregulated; in addition, the 5′ UTR or novel control regions juxtaposed to *DIRC1* exon 2 could conceivably control expression of a chimeric transcript. To investigate these possibilities, we performed 3′ RACE from the 5′ UTR and 5′ RACE from exon 2, using the familial lymphoblast RNA as template. We found no evidence of a chimeric transcript. Thus, it is likely that *DIRC1* locus products are not expressed from the der2 or the der3. Next, we carried out sequencing and SSCP analysis of the *DIRC1* gene from tumor DNA of affected family members, to look for mutations of the intact *DIRC1* allele. Three sets of primers (Table 1) were designed to amplify *DIRC1* exons from genomic DNA. We did not find mutations in these cases, although complete deletion of an exon or exons would not be detected by this method. Additionally, analysis of DNA from more than 35 primary kidney tumors; 10 peripheral blood leukocytes (PBLs); and

9 kidney, 6 cervical, 12 liver, 4 prostate, 3 pancreas, 5 nasopharyngeal, 6 esophagus, 4 lung, 4 breast, and 4 gastric cancer-derived cell lines showed a single, low-frequency *DIRC1* variant. The variant SSCP band was detected for NPC cell line Fadu (Fig. 3A), one PBL, and one primary kidney matched pair. The sequenced product revealed a T → G base pair substitution that results in a Ser to Ala change at amino acid 51. This polymorphism may be more frequent in the Hindu ethnic group, to which Fadu and the PBL with the variant belonged.

Analysis of allelic imbalance in RCCs

To determine if the der3, der2, or chromosomes 2 or 3 were retained in the familial tumors, we chose four markers, one from each side of the breakpoints on both chromosomes, and analyzed normal and tumor DNA from two affected family members for allelic imbalance in the tumors (Fig. 3B). Markers *D3S1581* and *D2S115* were reduced in the tumors, *D2S103* was retained, and *D3S1262* was noninformative, consistent with loss of the der3 chromosome and retention of the der2 and normal chromosome 2, and inconclusive for the normal chromosome 3.

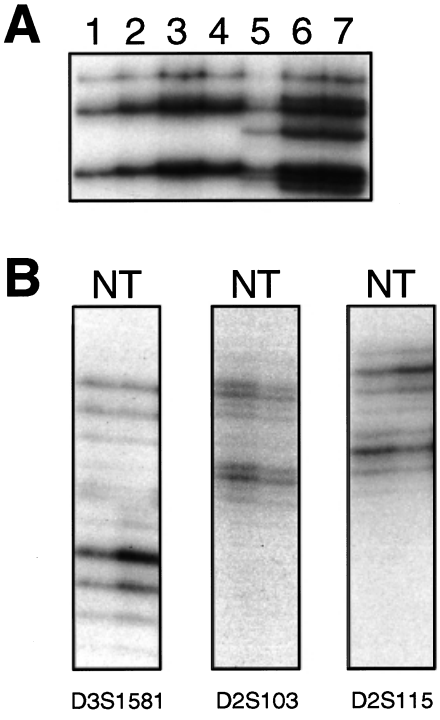


Fig. 3A,B. Single-strand conformation polymorphism and loss-of-heterozygosity (LOH) analysis. **A** Exon 2 was amplified in two pieces before separation on a Hydrolink MDE gel. Polymerase chain reaction (PCR) products from peripheral blood leukocyte DNAs (*lanes 1–5*) and paired normal (*lane 6*) and tumor (*lane 7*) kidney DNAs for the portion called exon 2a are shown. Note the shifted bands in lanes *5–7*; they were sequenced and revealed a T → G base-pair substitution in the coding region, resulting in a serine → alanine amino acid change. **B** Paired normal (*N*) and tumor (*T*) kidney DNA from an affected individual analyzed for LOH with markers *D3S1581*, *D2S103*, and *D2S115*

Eight microsatellite markers on chromosome 3 flanking the 3q breakpoint, *D3S1552*, *D3S3634*, *D3S1603*, and *D3S1271* centromeric to the break and *D3S1278*, *D3S1303*, *D3S1258*, and *D3S1589* telomeric to the break, were amplified from 21 normal/clear cell RCC matched DNA pairs. The most centromeric markers, *D3S1552* and *D3S3634*, showed allelic imbalance in 25% and 31% of cancers, respectively, whereas the markers flanking the 3q break showed imbalance in only 5%–10% of the kidney cancers.

A microsatellite marker, *D2S103*, within the YAC contig that encompasses the 2q break showed allelic imbalance in 2 of 17 (12%) informative RCCs. Thus, allelic imbalance, indicating gain or loss of a locus, at the 2q33 or 3q21 translocation regions is not a frequent event in clear cell RCCs.

Expression of Dirc1 protein in transfected clones

To preliminarily assess a possible growth suppressor effect of Dirc1 overexpression, we transfected murine 3T3 fibroblasts and 293 transformed human kidney cells with pcDNA3 control vector and pcDNA3/*DIRC1*. The number of colonies counted after stable transfection of 293 cells with pcDNA3 and pcDNA3/*DIRC1* was nearly equal (294 and 275, respectively). However, we were unable to detect Dirc1 protein expression using anti-FLAG mAb after transient transfection of both cell types or after isolation of single clones following selection with G418. Because the protein is small and may be easily degraded, we also transfected with the GFP/*DIRC1* construct. Expression was detected both transiently after 24 and 48h, and in an isolated clone that grew well and did not show obvious morphological changes (Fig. 2C). Only 1 in 12 clones from a 293 transfection was expressing GFP-Dirc1, as detected with anti-GFP mAb and rabbit anti-Dirc1 sera produced against Dirc1 peptides 67–80. Thus, results suggest that Dirc1 protein was not expressed in the majority of transfected cells and colonies. This result is consistent with a possible negative growth effect of overexpression of Dirc1. Alternatively, exogenously expressed Dirc1 may be unstable. The Dirc1 antiserum, although suitable for detecting exogenous Dirc1, has not detected endogenous Dirc1 by immunoblot of protein lysates from numerous tissues and cell lines thus far tested. Thus, we have been unable to compare levels of Dirc1 protein in normal and cancerous kidney tissues.

Discussion

Alterations of loci on the short arm of chromosome 3 are involved in initiation and progression of renal cancers (Zbar et al. 1987; Lubinski et al. 1994; Hadaczek et al. 1996). Constitutional mutations in the *VHL* gene at 3p25 are the cause of von Hippel–Lindau familial clear cell renal cancer, and, as is true for many tumor suppressor genes, somatic mutations of this familial cancer gene, including allelic losses, contribute to a large fraction of sporadic renal cancers (Gnarra et al. 1994; Foster et al. 1994; Shuin et al.

1994). A locus at 3p21 is also involved in allelic loss in renal cancers and the recently identified *RASSF1A* isoform (Dammann et al. 2000; Burbee et al. 2001) is a candidate renal cancer suppressor gene at this locus. The *FHIT* gene at 3p14.2 is interrupted and inactivated by a familial RCC-associated translocation, and Fhit protein is reduced or absent in the majority of sporadic RCCs (Hadaczek et al. 1998; Hadaczek et al. 1999; Eyzaguirre et al. 1999), although the mechanism of inactivation has been characterized only for a few RCC-derived cell lines (Druck et al. 1997). Translocations involving breakpoints at various 3p bands have also been observed frequently in sporadic renal cancers (Wilhelm et al. 1995), and a few years ago Gnarra et al. (1994) proposed that the function of these translocations, including the familial 3p14.2 translocation within the *FHIT* gene, may be to facilitate cancer progression-associated loss of the translocated portion of 3p. Thus, there would not necessarily be a cancer-related function for specific genes altered by translocation breaks. This hypothesis was consistent with the observed loss of the der8 (8pter → 8q24::3p14.2 → 3pter) chromosome in several of the familial RCCs from the family carrying the 3p14.2 translocation break.

More recently, RCC-associated constitutional translocations involving breakpoints on 3q have been observed and characterized and several of the RCCs have been shown to have lost the der3. These tumors have frequently lost at least one copy of the short arm of chromosome 3 with its associated tumor suppressor genes (Geurts van Kessel et al. 1999). Thus far, genes at the familial chromosome breaks in these families have not been reported.

We have characterized the breakpoint regions at 3q13–21 and 2q32–33 (Podolski et al. 2001) and have isolated the *DIRC1* gene, which is interrupted between exons 1 and 2 by the 2q breakpoint. *DIRC1* mRNA is expressed at a low steady-state level in a restricted set of tissues, which includes kidney, although there may be conditions under which expression is induced to a higher level. Endogenous protein levels are likely to be low as well, because our anti-Dirc1 peptide serum does not detect endogenous protein in the tissues and cell lines tested.

A translocated gene might contribute to familial cancers through overexpression, as a result of juxtaposition to an inappropriate regulatory region, or through inactivation of the translocated gene and silencing of the other allele through loss, mutation, or methylation. In addition, it might be expected that some sporadic RCCs would also show either over- or underexpression of the gene, with concomitant amplification or allelic loss of the locus encompassing the familial gene. Thus, we examined a panel of RCCs for allelic imbalance in the regions near the 3q and 2q breakpoints of our t(2;3) translocation. A low frequency of imbalance (~25%) was observed in the region centromeric to the 3q break, possibly reflecting the frequent loss of 3p in RCCs. Very few RCCs exhibited allelic imbalance at a locus within the 2q breakpoint contig. Thus, the *DIRC1* gene is unlikely to contribute to a large fraction of RCCs through allelic imbalance, although it is possible that it may be involved in allelic imbalance in other types of tumors, such as lung or ovarian, which have been shown to exhibit loss of heterozygosity on 2q (Shiseki et al. 1996). In addition, we did not observe mutations in *DIRC1* in a large panel of cancer cell lines.

We also wanted to determine the status of the derivative and normal chromosomes 2 and 3 and the *DIRC1* gene in the t(2;3) familial tumors to determine if *DIRC1* alteration may have contributed to their development. We were able to show that the der3 was lost in two tumors from two affected family members and that Fhit protein was not expressed in at least one familial tumor (the other was unsuitable for analysis, unpublished work). Point mutations in the *DIRC1* gene were not observed in DNA microdissected from two familial tumors, although the methods used would not have detected deletion of an entire exon or methylation of the promoter region. Several web-based CpG island searches failed to find any regions from the chromosome 2 sequence surrounding *DIRC1* exon 1 for methylation study.

In conclusion, we have characterized a novel gene at 2q32–33 that is interrupted by the familial t(2;3) translocation, but further studies will be required to determine if inactivation of the *DIRC1* locus contributed to development of the familial cancers.

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References

Bodmer D, Eleveld MJ, Ligtenberg MJL, Weterman MAJ, Janssen BAP, Smeets DFCM, deWit PEJ, van den Berg A, van den Berg E, Koolen MI, Geurts van Kessel A (1998) An alternative route for multistep tumorigenesis in a novel case of hereditary renal cell cancer and a t(2;3)(q35;q21) chromosome translocation. Am J Hum Genet 62:1475–1483

Burbee DG, Forgacs E, Zöchbauer-Müller S, Shivakumar L, Fong KM, Gao B, Randle D, Kondo M, Virmani A, Bader S, Sekido Y, Latif F, Milchgrub S, Tayooka S, Gazdar AF, Lerman MI, Zabarovsky E, White M, Minna JD (2001) Epigenetic inactivation of *RASSF1A* in lung and breast cancers and malignant phenotype suppression. J Natl Cancer Inst 93:691–699

Cohen AJ, Li FP, Berg S, Marchetto DJ, Tsai S, Jacobs SC, Brown RS (1979) Hereditary renal-cell carcinoma associated with a chromosomal translocation. N Engl J Med 301:592–595

Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP (2000) Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. Nat Genet 35:315–319

Druck T, Kastury K, Hadaczek P, Podolski J, Toloczko A, Sikorski A, Ohta M, LaForgia S, Lasota J, McCue P, Lubinski J, Huebner K (1995) Loss of heterozygosity at the familial RCC t(3;8) locus in most clear cell renal carcinomas. Cancer Res 55:5348–5353

Druck T, Hadaczek P, Fu T-B, Ohta M, Siprashvili Z, Baffa R, Negrini M, Kastury K, Veronese ML, Rosen D, Rothstein J, McCue P, Cotticelli MG, Inoue H, Croce CM, Huebner K (1997) Structure and expression of the human *FHIT* gene in normal and tumor cells. Cancer Res 57:504–512

Dumon KR, Ishii H, Vecchione A, Trapasso F, Baldassarre G, Chakrani F, Druck T, Rosato EF, Williams NN, Baffa R, During MJ,

Huebner K, Croce CM (2001) *FHIT* expression delays tumor development and induces apoptosis in human pancreatic cancer. Cancer Res 61:4827–4836

Eleveld MJ, Bodmer D, Merkx G, Siepman A, Sprenger SHE, Weterman MAJ, Ligtenber MJ, Kamp J, Stapper W, Jeuken JWM, Smeets D, Smits A, Geurts van Kessel A (2001) Molecular analysis of a familial case of renal cell cancer and a t(3;6)(q12;q15). Genes Chromosomes Cancer 31:23–32

Eyzaguirre EJ, Miettinen M, Norris BA, Gatalica Z (1999) Different immunohistochemical patterns of Fhit protein expression in renal neoplasms. Mod Pathol 12:979–983

Foster K, Prowse A, van den Berg A, Fleming S, Hulsbeck MMF, Crossey PA, Richards FM, Cairns P, Affara NA, Ferguson-Smith MA, Buys CHCM, Maher ER (1994) Somatic mutations of the von Hippel-Lindau disease tumour suppressor gene in nonfamilial clear cell renal carcinoma. Hum Mol Genet 3:2169–2173

Geurts van Kessel A, Wijnhovan H, Bodmer D, Eleveld M, Kiemeney L, Mulders P, Weterman M, Ligtenberg M, Smeets D, Smits A (1999) Renal cell cancer: chromosome 3 translocations as risk factors. J Natl Cancer Inst 91:1159–1160

Gnarra JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, Latif F, Liu S, Chen F, Duh F-M, Lubensky I, Duan DR, Florence C, Pozzatti R, Walther MM, Bander NH, Grossman HB, Brauch H, Pomer S, Brooks JD, Isaacs WB, Lerman MI, Zbar B, Linehan WM (1994) Mutations of the VHL tumour suppressor gene in renal carcinoma. Nat Genet 7:85–90

Hadaczek P, Podolski J, Toloczko A, Kurzawski G, Sikorski A, Rabbitts P, Huebner K, Lubinski J (1996) Accumulation of losses at 3p common deletion sites is characteristic of clear cell renal cell carcinoma. Virchows Arch 429:37–42

Hadaczek P, Siprashvili Z, Markiewski M, Domagala W, Druck T, McCue PA, Pekarsky Y, Ohta M, Huebner K, Lubinski J (1998) Absence or reduction of Fhit expression in most clear cell renal carcinomas. Cancer Res 58:2946–2951

Hadaczek P, Kovatich A, Gronwald J, Lubinski J, Huebner K, McCue PA (1999) Loss of Fhit expression in renal neoplasias: correlation with histogenic class. Hum Pathol 30:1276–1283

Ishii H, Dumon KR, Vecchione A, Trapasso F, Mimori K, Alder H, Mori M, Sozzi G, Baffa R, Huebner K, Croce CM (2001) Effect of adenoviral transduction of FHIT into esophageal cancer cells. Cancer Res 61:1578–1589

Kanayama H, Lui W-O, Takahashi M, Naroda T, Kedra D, Wong FK, Kuroki Y, Nakahori Y, Larsson C, Kagawa S, Teh BT (2001) Association of a novel constitutional translocation t(1q;3q) with familial renal cell carcinoma. J Med Genet 38:165–170

Koolen MI, van dear Meyden AP, Bodmer D, Eleveld M, van dear Looij E, Brunner H, Smits A, van den Berg E, Smeets D, Geurts van Kessel A (1998) A familial case of renal cell carcinoma and a t(2;3) chromosome translocation. Kidney Int 53:273–275

Lubinski J, Hadaczek P, Podolski J, Toloczko A, Sikorski A, McCue P, Druck T, Huebner K (1994) Common regions of deletion in chromosome regions 3p12 and 3p14.2 in primary clear cell renal carcinomas. Cancer Res 54:3710–3713

Ohta M, Inoue H, Cotticelli MG, Kastury K, Baffa R, Palazzo J, Siprashvili Z, Mori M, McCue P, Druck T, Croce CM, Huebner K (1996) The FHIT gene, spanning the chromosome 3p14.2 fragile site and renal carcinoma-associated t(3;8) breakpoint, is abnormal in digestive tract cancers. Cell 84:587–597

Podolski J, Zajaczek S, Byrski T, Druck T, Zimonjic DB, Popescu NC, Lubinski J, Huebner K (2001) Characterization of a familial RCC-associated t(2;3)(q33;q21) chromosome translocation. J Hum Genet (in press)

Shiseki M, Kohno T, Adachi J, Okazaki T, Otsuka T, Mizoguchi H, Noguchi M, Hirohasi S, Yokota J (1996) Comparative allelotype of early and advanced stage non-small cell lung carcinomas. Genes Chromosomes Cancer 117:71–77

Shuin T, Kondo K, Torigoe S, Kishida T, Kubota Y, Hosaka M, Nagashima Y, Kitamura H, Latif F, Zbar B, Lerman MI, Yao M (1994) Frequent somatic mutations and loss of heterozygosity of the von Hippel-Lindau tumor suppressor gene in primary human renal cell carcinomas. Cancer Res 54:2852–2855

Wilhelm M, Bugert P, Kenck C, Staehler G, Kovacs G (1995) Terminal deletion of chromosome 3p sequences in nonpapillary renal cell carcinomas: a breakpoint cluster between loci D3S1285 and D3S1603. Cancer Res 55:5383–5385

Zajaczek S, Gronwald J, Kata G, Borowka A, Lubinski J (1999) Familial renal cell cancer (CRCC) associated with a constitutional reciprocal translocation t(2;3)(q33;q21). Cytogenet Cell Genet 85:172

Zbar B, Brauch H, Talmage C, Linehan M (1987) Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature 327:721–724