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The t(X;1)(p11.2;q21.2) translocation in papillary renal cell carcinoma fuses a novel gene *PRCC* to the *TFE3* transcription factor gene

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The specific chromosomal translocation t(X;1)(p11.2;q21.2) has been observed in human papillary renal cell carcinomas. In this study we demonstrated that this translocation results in the fusion of a novel gene designated *PRCC* at 1q21.2 to the *TFE3* gene at Xp11.2. *TFE3* encodes a member of the basic helix-loop-helix (bHLH) family of transcription factors originally identified by its ability to bind to μE3 elements in the immunoglobin heavy chain intronic enhancer. The translocation is predicted to result in the fusion of the N-terminal region of the PRCC protein, which includes a proline-rich domain, to the entire TFE3 protein. Notably the generation of the chimaeric *PRCC–TFE3* gene appears to be accompanied by complete loss of normal *TFE3* transcripts. This work establishes that the disruption of transcriptional control by chromosomal translocation is important in the development of kidney carcinoma in addition to its previously established role in the aetiology of sarcomas and leukaemias.

INTRODUCTION

Renal cell carcinoma (RCC) can be divided into papillary cell, clear cell, granular cell and sarcomatoid subgroups based on histological appearance (1,2). For the clear cell, granular cell and sarcomatoid tumours loss or inactivation of the von Hippel-Lindau (VHL) suppressor gene on chromosome arm 3p has been implicated in tumour development (3,4). By comparison papillary renal cell tumours, which account for around 15–20% of renal carcinomas (1), do not exhibit mutation of the VHL suppressor gene or loss of 3p (3). Recurrent numerical abnormalities of other chromosomes have been identified in papillary tumours including tetrasomy 7, trisomy 10,12,16,17 and 20 and loss of the Y chromosome (5–8). However, there is evidence that some of these alterations may also be present in the surrounding normal tissue and thus are not tumour specific (9).

Abnormalities of Xp11.2 region have often been observed in papillary RCC. A specific and recurrent translocation between chromosome X and 1, t(X;1)(p11.2;q21.2), has frequently been found (10–13) while a t(X;17)(p11.2;q25) and a del(X)(p11) have been found in two separate cases (14,15). In addition two other cases involving translocations between Xp11 and 1p34 have been documented in unspecified types of renal cell carcinomas (6,16). The *TFE3* gene, which encodes a member of the helix-loop-helix family of transcription factors (17) has recently been mapped adjacent to the position of the t(X;1) breakpoint (12). In the present study we initially demonstrate the disruption of the *TFE3* gene in papillary RCCs carrying the t(X;1) translocation. Further characterisation of the *TFE3* gene in these tumours led to the discovery that it becomes fused to a novel chromosomal gene designated *PRCC* (for papillary renal cell carcinoma).

RESULTS

Involvement of the *TFE3* gene

Southern blot analysis using a probe corresponding to the 5′ end of the *TFE3* gene detected rearrangements in three papillary renal tumour cell lines (UOK120, UOK124 and UOK146) shown in cytogenetic studies to contain the t(X;1)(p11.2;q21.2) translocation (Fig. 1a). Rearrangements in all three lines were detected following digestion of tumour DNA with *Hin*dIII and *Bgl*II. These results were consistent with fluorescence *in situ* hybridisation studies carried out on the UOK120 and UOK124 cell lines which demonstrated that probes prepared from individual cosmids spanning the 5′ end of the *TFE3* gene hybridised to both derivative X and derivative 1 chromosomes formed as a result of the t(X;1) translocation (results not shown).

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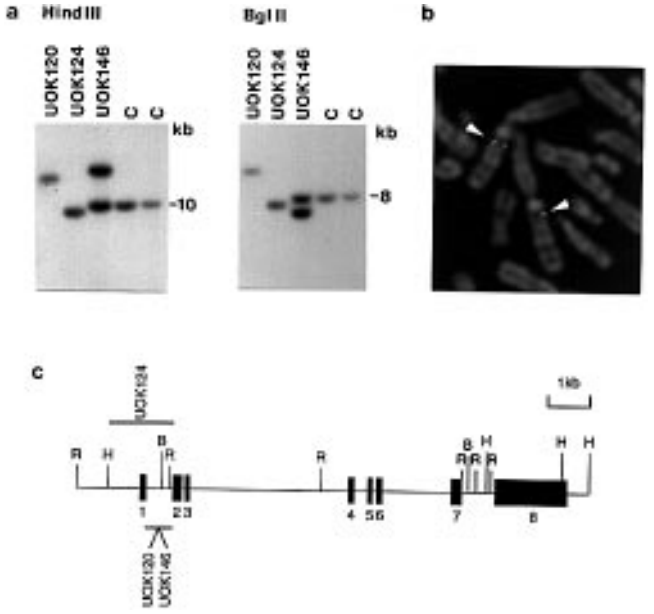

Figure 1. (**a**) Southern blot analysis of DNA from papillary renal tumour cell lines. DNAs digested with *Bgl*II and *Hin*dIII were hybridised to a *TFE3* probe that corresponded to a 379 bp *Eco*RI–*Bgl*II cDNA fragment at the 5′ end of the published human TFE3 cDNA sequence (17). Lanes labelled C contain control DNA from cell lines that did not harbour the t(X;1) translocation. Tumour UOK124, which arose in a female patient, has lost the untranslocated copy of the X chromosome (12). A *TFE3* gene rearrangement was also observed when the same probe was hybridised to UOK120 DNA digested with *Eco*RI. (**b**) Chromosomal localisation of the *PRCC* gene. Fluorescence *in situ* hybridisation was carried out using a 2.0 kb *PRCC* cDNA clone as a probe. The *PRCC* cDNA clone used in these experiments was isolated by screening a monocyte cDNA library with the unique 5′ sequence present in the *TFE3* 5′RACE product isolated from line UOK124. The arrows marks the position of the hybridisation signals. (**c**) Restriction map of the *TFE3* gene showing its exon–intron structure. Restriction sites are: R, *Eco*RI, B, *Bam*HI and H, *Hin*dIII. Genomic DNA sequence that spans exons 1–8 are available; exons 1, 2 and 3, accession number X97160; exons 4,5 and 6, accession number X97161; exons 7 and 8, accession number X97162. The position of the breakpoints in the UOK120, UOK124 and UOK146 cell lines are shown. For UOK120 and UOK146 *PRCC* sequences are joined to *TFE3* exon 2 in the *PRCC–TFE3* hybrid transcript while in the reciprocal *TFE3–PRCC* hybrid transcript the fusion involves joining of *TFE3* exon 1 to *PRCC*. These results can only be explained if the breakpoint in both of these cell lines occurs within *TFE3* intron 1.

Fusion of *TFE3* to the novel chromosome 1 gene *PRCC*

To determine whether the *TFE3* transcripts in UOK124 had been altered at their 5′ ends *TFE3* 5′RACE products were obtained from this cell line. The sequence of the 5′RACE product (Fig. 2a) diverged from the normal *TFE3* sequence (18) at its 5′ end. Construction of the exon–intron map of the *TFE3* gene (Fig. 1c) revealed that the position of divergence from the normal cDNA sequence corresponded exactly to the site of the junction between *TFE3* exon 1 and exon 2.

When a probe prepared from the unique 5′ sequences present in the 5′RACE product was used to isolate clones from human monocyte and foetal brain cDNA libraries, two clones of 1.5 kb and 2 kb were isolated. Use of the largest clone as a probe in FISH studies allowed the localisation of these sequences to chromosome band 1q21.2 (Fig. 1b). The assignment of these sequences to chromosome 1 was confirmed by Southern analysis and

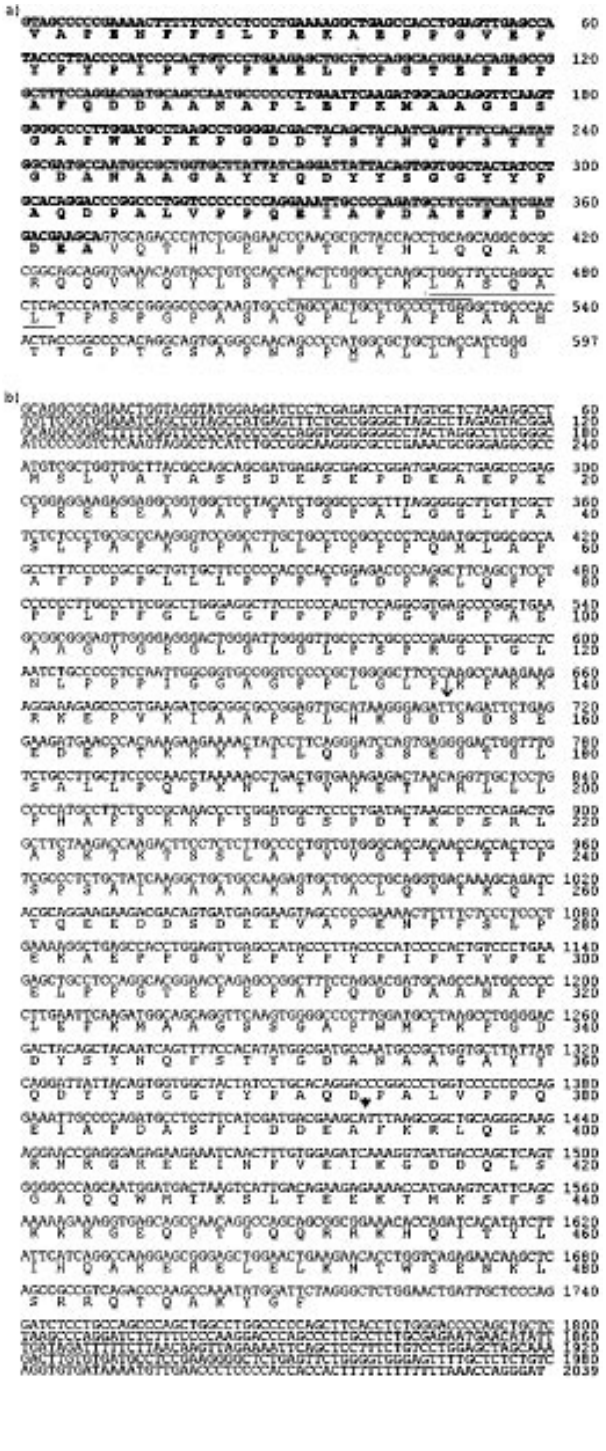

Figure 2. (**a**) Nucleotide sequences of the 5′RACE product obtained from the UOK124 cell line together with predicted amino acid sequence. The sequence has been extended 3′ to show the position of the TFE3 initiating methionine (M). Sequences that did not match the published mouse 5′*TFE3* sequence (17,18) or human *TFE3* 5′RACE product (accession number X96717) are shown in bold. These novel sequences represent an internal region of the *PRCC* cDNA sequence that although isolated by 5′RACE did not extend to the 5′ end of the *PRCC* transcript. The lines over the nucleotide sequence show the position of the *TFE3* primers used to isolate the 5′RACE product. (**b**) The human chromosome 1 *PRCC* cDNA nucleotide sequence and predicted amino acid sequence (assession number X97124). The vertical arrows shows the position of the breakpoints found in the UOK120 and UOK146 cell lines (↓)and in the UOK124 cell line (➧). The nucleotide sequences matched several EST sequences including R13902, R93849, T34683, T35480 and R93814 (Genbank).

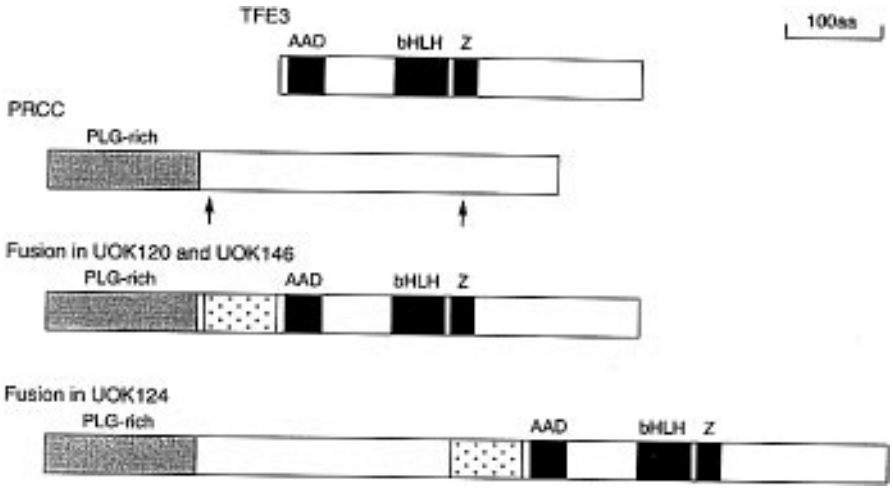

Figure 3. Schematic representation of wild type PRCC and TFE3 proteins and of the PRCC–TFE3 chimaeric proteins. The PRCC protein is 491 amino acids in length and contains an N-terminal domain of 150 amino acids that is rich in proline, leucine and glycine (PLD-rich). In UOK120 and UOK146 the 156 amino acids N-terminal region of PCC becomes fused to the entire TFE3 protein including the acidic activation domain (AAD), the central basic-helix-loop-helix (HLH) and the leucine zipper (Z) regions. In UOK124 393 amino acids of N-terminal PRCC sequences become fused to the same TFE3 sequences. The region of the fusion proteins that is encoded by TFE3 mRNA sequences immediately upstream of the TFE3 initiating methionine is shown (stippled box). RT–PCR of RNA from the three cell lines has been used to check the structures shown in this figure. The arrows represent the position of fusion of the PRCC protein to TFE3.

PCR-based analysis of human-rodent somatic cell lines that contained a single copy of chromosome 1 (results not shown). Sequencing of the cDNA clones generated a continuous sequence of 2039 bp (Fig. 2b). The size of the continuous cDNA sequence obtained in these studies was similar in size to normal transcripts of 2.0 kb detected in northern analyses of human sarcoma and melinoma cell lines (results not shown). This gene, designated *PRCC*, contained an open reading frame of 491 amino acids (Fig. 2b). The predicted PRCC protein possessed an N-terminal domain rich in proline (25%), leucine (13%) and glycine (13%) but failed to exhibit significant homology to known protein sequences and contained no motifs suggestive of biochemical function. Searches of the EMBL databases did, however, reveal several EST sequences that matched the *PRCC* cDNA sequence (Fig. 2b).

A comparison of normal *PRCC* and *TFE3* gene sequences with the *PRCC-TFE3* junction sequences isolated by 5′RACE allowed the position of the fusion of the *PRCC* to *TFE3* to be identified. Although the break occurs upstream of the *TFE3* initiating methionine fusion of *PRCC* to *TFE3* is nonetheless predicted to generate a fusion protein (Fig. 2a). Thus the new open reading frame extends from the *PRCC* sequences through sequences upstream of the *TFE3* initiating methionine and into the *TFE3* open reading frame. These analyses revealed that in UOK124 cells 393 amino of N-terminal PRCC sequences become fused to the entire TFE3 bHLH transcription factor, which includes an N-terminal acidic transcriptional activation domain (AAD), a control basic-helix-loop-helix-leucine zipper region implicated in DNA binding and dimerisation (17–22). Notably the structure of the TFE3 protein present in this fusion was consistent with that recently reported by Macchi *et al.* (22) but was not consistent with earlier reports indicating that TFE3 may have an extended proline-rich C-terminal domain (18).

PCR detection of *PRCC–TFE3* transcripts

The presence of a *PRCC–TFE3* hybrid transcript in all three papillary renal tumour cell lines was demonstrated by RT–PCR using 5′*PRCC* and 3′*TFE3* primers. A product of the predicted size (819 bp) was observed for cell line UOK124 (Fig. 4a). Much smaller (108 bp) products were obtained for the lines UOK120 and UOK146 (Fig. 4a). Analysis of the smaller products indicated that they corresponded to a fusion in which the 156 aa proline-rich N-terminal domain of PRCC becomes joined to the same TFE3 sequences (Fig. 3). In parallel experiments reciprocal *TFE3–PRCC* hybrid transcripts were detected in the UOK146 and UOK120 lines but not in the UOK124 cell line (result not shown) suggesting that it is the formation of the *PRCC–TFE3* transcript that is the consistent feature associated with this translocation. When considered together with Southern blot data showing *TFE3* rearrangements (Fig. 1a), these data allowed the mapping of the positions of the genomic breakpoint within all three tumours (Fig. 1c). Notably in UOK120 and UOK146 the breakpoint could be assigned to *TFE3* intron 1.

Loss of normal TFE3 transcripts

RT–PCR using a 5′ primer corresponding to *TFE3* exon 1 sequences and a 3′ primer corresponding to *TFE3* exon 2 sequences was used to detect intact *TFE3* transcripts. Transcription of the *TFE3* gene has been observed in all tissue examined (19). In agreement with this observation we found PCR products of the predicted size in sarcoma and melanoma lines that were examined. However we failed to detect expression of normal *TFE3* transcripts in the three renal tumour cell lines (Fig. 4b). This would be expected for t(X;1) translocations found in males and for translocations in females involving the active X chromosome

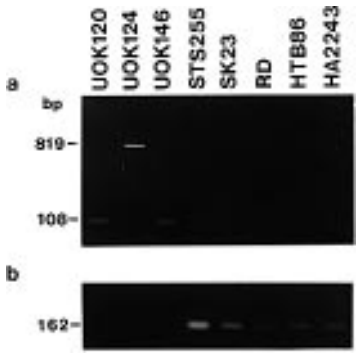

Figure 4. (**a**) Detection of *PRCC–TFE3* hybrid transcripts by RT–PCR. PCR was performed using a 5′*PRCC* primer and a 3′*TFE3* primer to amplify reverse transcribed RNA from papillary renal cell carcinomas (UOK120, UOK124, UOK146) and the following human tumour samples: STS255 and A2243, synovial sarcoma cell lines; RD, rhabdomyosarcoma cell line; HTB86 Ewings sarcoma cell line, and SK23 melanoma cell line. (**b**) Detection of normal *TFE3* transcripts in the same RNA samples was performed by RT–PCR using a forward primer, corresponding to *TFE3* exon 1 sequences and a reverse primer corresponding to *TFE3* exon 2 sequences. For primer sequences see the Materials and Methods Section.

(UOK120 arose in a male while UOK124 and UOK146 arose in females). This observation raises the intriguing possibility that the t(X;1) translocation may uniquely have a dual role in both generating a dominantly acting fusion protein and removing the activity of normal TFE3 proteins.

DISCUSSION

In these studies we report that the t(X;1)(p11.2q21.2) translocation found in papillary renal cell carcinoma results in the fusion of a novel chromosome 1 gene called *PRCC* to the *TFE3* transcription factor gene. There have been very few reports of recurrent translocations and their molecular characterisation in human carcinomas. An inversion within chromosome 10 fuses the *RET* gene to an unidentified gene in papillary thyroid carcinoma (23,24). In addition fusion of the *TPR* gene to the *TRK* gene has been found in a proportion of this same tumour type (25). Both *RET* and *TRK* encode transmembrane tyrosine kinase receptors. The current work therefore provides the first demonstration of a recurrent fusion involving a transcription factor gene in a human carcinoma.

By comparison the involvement of transcription factor genes in chromosomal translocation genes found in sarcoma and leukaemias has been well documented (26–27). Of particular note is the involvement of members of the bHLH gene family including *CMYC*, *LYL1*, *TAL1* and *TAL2* in translocations found in haemopoietic malignancies. As a consequence of the translocations the bHLH genes become juxtaposed to immunoglobin light or heavy chain genes or to T-cell receptor genes (26,27). Notably these translocations result in deregulation or ectopic expression of the bHLH gene and, in contrast to the situation observed for *TFE3*, are not associated with the formation of fusion proteins.

A frequent theme observed for translocations found in sarcomas is the fusion of a transcription factor activation domain to a transcription factor DNA-binding element. For example as a result of the t(11;22), t(21;22) and t(7;22) translocations found in Ewings sarcoma the N-terminal transcriptional activation domain of the EWS protein becomes fused to the DNA binding domain of respectively FLI1, ERG and ETV1, all members of ETS protein family (28,29). N-terminal EWS sequences also become fused to the DNA binding domains of the CHN-TEC steroid/thyroid receptor protein in myxoid chondrosarcomas (30,31), of the WT1 protein in desmoplastic small round cell tumours (32), and of the ATF1 protein in malignant melanoma of soft parts (33). Proline-rich regions have been identified as transcriptional activation domains in several proteins (for example see ref. 34). It is therefore possible that fusion of the N-terminal PRCC domain to TFE3 may act in a manner similar to that observed for the EWS fusions by creating a PRCC–TFE3 fusion protein that has an N-terminal protein-rich transcriptional activation domain adjacent to the TFE3 DNA-binding domain.

The TFE3 protein binds μE3 elements in the immunoglobin heavy chain, (IgH) intronic enhancer, in Ig kappa enhancers and in some IgH variable region promoters (17–22). However, since *TFE3* transcripts are found in all tissues examined including kidney, the encoded protein may have a much broader role in transcriptional control (19). The N-terminal transcriptional activation domain of TFE3, called AAD (Fig. 3), is encoded by the 105 nucleotide exon 3 of the *TFE3* gene (19). Removal of this exon by differential splicing produces a shortened isoform of the TFE3 protein (TFE3-S) that is expressed in kidney cells (19) and that has a dominant negative effect on TFE3 activity. We have found in analysis of each of the three papillary RCC that formation of the t(X;1) is accompanied by loss of expression of the normal *TFE3* transcripts. It is therefore interesting to consider the possibility that the transformation that results from t(X;1) formation may require both the generation of the PRCC–TFE3 fusion protein and the removal of the shortened inhibitory isoform TFE3-S. Alternatively it is possible that loss of normal TFE3 transcripts simply occurs because TFE3 is X-linked and may have no contribution to the overall transformation process. A more detailed analysis of these models of transformation may represent an interesting area for future studies.

Cytogenetic studies initially identified the t(X;1)(p11.2;q21.2) translocation solely in papillary renal cell tumours arising in male patients (10,11). More recently this translocation has been found in tumours from female patients (12, present study). It has also been noted that the t(X;1) appears to occur more frequently in tumours arising in young patients (13). The identification of the genes involved in this translocation will now allow us to undertake molecular diagnostic studies on larger series of tumours to determine the true age and sex distribution of patients with the t(X;1) translocation and to assess whether this translocation can be used as a diagnostic or prognostic marker.

MATERIALS AND METHODS

Cell lines

The UOK120, UOK124 and UOK146 cell lines were derived from primary papillary renal cell carcinoma specimens as described (35). The cell lines were derived respectively from tumours arising in a 30 year old male, a 21 year old female and a 45 year old female. Cytogenetic analyses of all three lines

identified the reciprocal translocation t(X;1)(p11.2;q21.2) translocation (ref. 6 and JS unpublished).

Analysis of DNA and RNA

Preparation of genomic DNA and cytoplasmic RNA were carried out as described (36). Restriction endonuclease digestions, agarose gel electrophoresis, Southern transfer, hybridisation, washes and autoradiography were also carried out as described previously (36).

RT–PCR analysis

One µg of RNA was reverse transcribed using Superscript II reverse transcriptase (GIBCO BRL). Most efficient reverse transcription was obtained when RNA was heated to 94°C with a random 6-mer primer and cooled rapidly on dry ice prior to addition of the reverse transcriptase and buffer. Incubation was at 17°C for 18 h. To detect *PRCC–TFE3* hybrid transcripts the resulting cDNA was subject to amplification with the *PRCC* primer 5′-CACTGAGCTGGTCATCAC-3′ (forward primer) and the exon 2 *TFE3* primer 5′-AGTGTGGTGGACAGGTACTG-3′ (reverse primer). The presence of intact normal *TFE3* transcripts was assessed using the *TFE3* exon 1 primer 5′-TGTGGTTGGC-GTCTCTGCTG-3′ (forward primer) in combination with the same *TFE3* exon 2 reverse primer. To detect *TFE3–PRCC* hybrid transcripts amplification was performed with the *TFE3* primer, 5′-CATCTCTGTGGTTGGCGT-3′ (forward primer) and 3′*PRCC* primer 5′-GTTCTCCAGATGGGTCTGC-3′ (reverse primer). For UOK124 the additional reverse primer 5′-ATGTTG-ATTCTCGCAGAGGC-3′ that lies 3′ to the end of the *PRCC* open reading frame was also used in combination with the *TFE3* forward primer in attempts to detect a *TFE3–PRCC* hybrid transcript. As a positive control to confirm that each RNA sample could yield products RT–PCR amplification was carried out with actin primers as described previously (36). In these analyses all reverse transcribed samples gave an actin PCR product of the expected size. The amplification conditions were 93°C for 20 s, 61°C for 40 s and 72°C for 40 s for 30 cycles in a final volume of 25 µl. The products were separated by electrophoresis in agarose gels followed by staining with ethidium bromide.

5′RACE

One µg of RNA was reverse transcribed using Superscript II reverse transcriptase (GIBCO BRL) as described above using the *TFE3* primer 5′-TGAGCTGGACCCGATGGTGA-3′. Newly synthesised cDNA was then tailed with polydC at its 5′ end using terminal transferase (Boehringer Mannheim) according to the manufacturers instructions. Amplification of cDNA ends was then performed. The first round PCR primers were oligonucleotide 5′-TAGTGTGGGCAGCCTCAG-3′ (*TFE3* reverse primer) and 5′-GACTCGAGTCGACATCGGGIIGGGIIGGGIIG-3′ where I is inosine. Aliquots of this reaction were then subject to nested PCR using the primer oligonucleotides 5′-CTCAGGGGCAGG-CAGTGGCTG-3′ (*TFE3* reverse primer) and 5′-GACTCGAG-TCGACATCG-3′.

Fluorescence *in situ* hybridisation (FISH)

FISH using the 2.0 kb *PRCC* cDNA clone 75MI8 as a probe was performed exactly as described previously (37).

cDNA libraries

A cDNA library made from the human monocyte cell line U937 in the pcDM8 vector and a human foetal brain cDNA library made in the pcDNA vector were kindly provided by the Sanger Centre, Cambridge, UK.

DNA sequencing

For sequence analysis PCR products were either subcloned with the TA Cloning kit (Invitrogen) following the manufacturers instructions or sequenced directly from PCR products which had been purified by electrophoresis through agarose gels and isolated using the Geneclean II (BIO101) kit. Both PCR products and subcloned cDNA fragments were sequenced by the dideoxy method using a TaqFS Dye Terminator Sequencing kit (ABI, Foster City, CA) and ABI 377 DNA sequencers. Sequencing of all RT–PCR products and both strands of the *PRCC* gene cDNA clones was completed using these methods.

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