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Fusion of splicing factor genes *PSF* and *NonO* (*p54^{nrb}*) to the *TFE3* gene in papillary renal cell carcinoma

Jeremy Clark[1], Yong-Jie Lu[2], Sanjiv K Sidhar[1], Chris Parker[1], Sandra Gill[1], Damian Smedley[1,2], Rifat Hamoudi[3], W Marston Linehan[4], Janet Shipley[2] and Colin S Cooper[1]

Sections of [1]Molecular Carcinogenesis, [2]Cell Biology and Experimental Pathology and [3]Cancer Gene Cloning Laboratory, Institute of Cancer Research, Haddow Laboratories, Belmont, Sutton, Surrey, SM2 5NG, UK; [4]Urologic Oncology Section, National Cancer Institute, Bethesda, Maryland 20892, USA

We demonstrate that the cytogenetically defined translocation t(X;1)(p11.2;p34) observed in papillary renal cell carcinomas results in the fusion of the splicing factor gene *PSF* located at 1p34 to the *TFE3* helix–loop–helix transcription factor gene at Xp11.2. In addition we define an X chromosome inversion inv(X)(p11.2;q12) that results in the fusion of the *NonO* (*p54^{nrb}*) gene to *TFE3*. *NonO* (*p54^{nrb}*), the human homologue of the *Drosophila* gene *NonA^{diss}* which controls the male courtship song, is closely related to *PSF* and also believed to be involved in RNA splicing. In each case the rearrangement results in the fusion of almost the entire splicing factor protein to the TFE3 DNA-binding domain. These observations suggest the possibility of intriguing links between the processes of RNA splicing, DNA transcription and oncogenesis.

Keywords: renal cell carcinoma; PSF splicing factor; *NonO* (*p54^{nrb}*) splicing factor; chromosome translocations; TFE3 transcription factor

Introduction

Renal cell carcinoma (RCC), the most common cancer of kidneys, can be divided into several tumor subtypes including, clear cell, granular cell, sarcomatoid and papillary based on histological appearance (Murphy *et al*., 1994; Savage, 1994). The clear cell carcinomas are characterized in cytogenetic and molecular studies by frequent abnormalities of the short arm of chromosome 3, including loss of heterozygosity. The von Hippel-Lindau (VHL) disease, a dominantly inherited cancer syndrome in which family members develop multiple bilateral clear cell renal carcinomas, as well as tumors of the brain, spine and eye, has been mapped to this chromosome region. The *VHL* gene has now been cloned and mutations of this gene are found in a high proportion of sporadic clear cell, granular cell and sarcomatoid tumors (Gnarra *et al*., 1994; Herman *et al*., 1994).

By comparison, papillary renal cell carcinomas, which account for 10–20% of renal cell tumors, do not exhibit *VHL* gene loss or mutation (Gnarra *et al*., 1994). A high frequency of loss of chromosome Y and trisomy of chromosome 7 has, however, been reported in papillary RCC (Kovacs *et al*., 1987, 1991; Kovacs, 1993; van den Berg *et al*., 1993; Elfving *et al*., 1995) and a specific translocation between chromosomes X and 1, t(X;1)(p11.2;q21.2), has been identified in cytogenetic studies (de Jong *et al*., 1986; Meloni *et al*., 1993; Mitelman, 1994; Shipley *et al*., 1995; Tonk *et al*., 1995). It has recently been shown (Sidhar *et al*., 1996; Weterman *et al*., 1996a, b) that this translocation results in the fusion of a novel chromosome 1 gene, called *PRCC*, to the chromosome X gene *TFE3*, which encodes a member of the basic-helix–loop–helix family of transcription factors originally identified by its ability to bind to μE3 elements in the immunoglobin heavy chain intronic enhancer (Beckmann *et al*., 1990). The *PRCC-TFE3* chimaeric gene encodes a fusion protein in which N-terminal PRCC sequences containing a proline-rich domain, become fused to the TFE3 DNA-binding domain (Sidhar *et al*., 1996; Weterman *et al*., 1996a).

Cytogenetic studies have also identified other abnormalities of the Xp11 region in renal cell carcinomas. Cases containing t(X;17)(p11.2;q25), del(X)(p11) and t(X;10)(p11.2;q23) have been reported in papillary RCC (Tomlinson *et al*., 1991; Ohjimi *et al*., 1993; Dijkuizen *et al*., 1995) while translocations involving Xp11 and 1p34, t(X;1)(p11.2;p34), have been identified in a papillary RCC and two RCCs of unspecified type (Kovacs *et al*., 1987; Dijkuizen *et al*., 1995; Yoshida *et al*., 1995). These observations raise the possibility that in a proportion of papillary RCC the *TFE3* gene at Xp11.2 may be involved in fusions with genes other than *PRCC*. In agreement with this idea in the present study we report that the *TFE3* gene may also become fused to the splicing factor genes *PSF* and *NonO*(*p54^{nrb}*) in papillary renal cell carcinoma.

Results

Abnormalities of the TFE3 gene in the UOK109 and UOK145 cell lines

Southern blot analysis using a probe corresponding to the 5′ end of the *TFE3* gene was used to screen for rearrangements of this gene in papillary renal cell carcinoma cell lines. Rearrangements were detected in the UOK109 and UOK145 cell lines but not in the UOK112 and UOK132 cell lines (Figure 1a). Additional evidence for alteration of the *TFE3* gene was provided by the results of Northern analyses which showed abnormal patterns of *TFE3* transcripts in the UOK109 and UOK145 cell lines (Sidhar *et al*., 1996). However, in parallel RT–PCR experiments using 5′

Correspondence: J Clark
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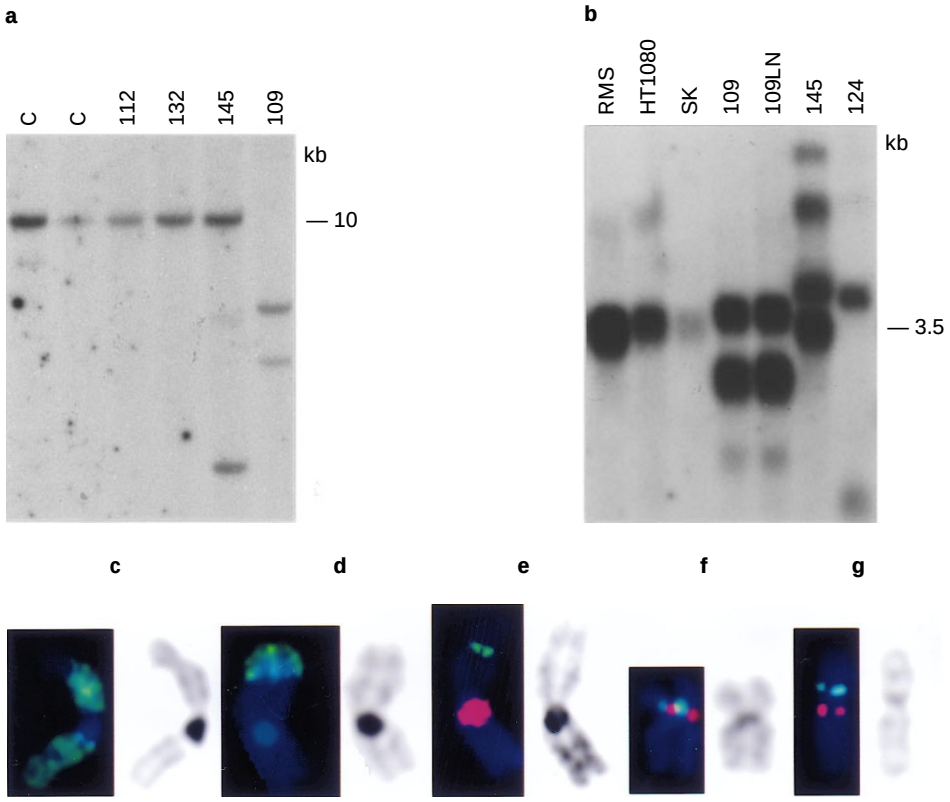

Figure 1 Rearrangements of the *TFE3* locus in papillary renal cell carcinomas. (**a**) Southern blot analysis of DNA from papillary renal tumor cell lines UOK112, UOK132, UOK145 and UOK109. DNAs digested with *Hind*III were hybridized to a *TFE3* probe that corresponded to positions 899–1342 of the *TFE3* cDNA sequence (Accession No. X96717). Lanes labeled C contain control DNA from cell lines that did not harbour the abnormalities of Xp11.2. (**b**) Northern blot analysis of RNA from human cell lines probed with a *TFE3* probe that spanned from positions 232-1513 of the *TFE3* cDNA sequence (Accession Number X96717). RNAs were from: RMS, rhabdomyosarcoma; HT1080, fibrosarcoma; SK melanoma; and UOK109, UOK109LN, UOK145 and UOK124 papillary renal carcinoma cell lines. UOK109 and UOK109LN were derived from primary and lymph node tumors from the same patient. Representative images from the FISH studies are shown in (**c–g**). The derivative 1 chromosome, der(1)t(X;1)(p11.2;p34), from UOK145 is shown following hybridization to (**c**) a chromosome 1 paint, (**d**) a chromosome X paint and (**e**) the marker GATA (green), normally localising telomeric to *TFE3* at Xp11 and a probe for the pericentric region of chromosome 1 (red). (**f**) shows the markers GATA (green) and pTAK8 (red), which lie telomeric and centromeric to *TFE3* in the Xp11 region, respectively, hybridizing to the normal X chromosome in UOK145. The inv(X)(p11.2;q12) in UOK109 is demonstrated in (**g**) with the pTAK8 YAC (red), which is normally at Xp11 but centromeric to GATA (green), localising below the centromere on this chromosome

PRCC primers and 3′ *TFE3* primers we failed to detect fusion of the *PRCC* and *TFE3* genes (results not shown).

The karyotype of the UOK145 cell line was as follows: 41,X,-X,der(1)t(X;1) (p11.2;p34),−6,−8,+14,i (17)(q10),−18,−20,−21,−22, +mar [5] /41,X,-X,der(1) t (X;1) (p11.2; p34), add (2) (q37),−3, −6,−8,+14, i (17) (q10),−18,−21, −22,+mar[3]. Notably the resulting rearrangement t(X;1)(p11.2;p34), was identical to that previously reported in three renal tumors (Kovacs *et al*., 1987; Dijkuizen *et al*., 1995; Yoshida *et al*., 1995) suggesting that this is a recurrent abnormality in renal cell cancers. The translocation involving chromosomes X and 1 was confirmed by FISH analysis and disruption of the markers flanking the TFE3 gene at Xp11.2 identified (Figure 1c, d and e). The karyotype of the cell line UOK109 was determined as 47,XY, +add (3) (q10),−5,−6, del (6) (q21),+add (7) (q32), −9, −14,+16,+2mar. This analysis failed to identify any abnormalities of the X chromosome. However, when FISH studies were performed using probes that flanked the *TFE3* gene at Xp11.2 it became apparent that a pericentric inversion of the X chromosome had occurred described as inv(X)(p11.2;q12) (Figure 1f and g). When taken together these observations suggested that in the UOK109 and UOK145 cell lines the *TFE3* gene may have translocation partners other than PRCC which is involved in the t(X;1)(p11.2;q21.2) (Sidhar *et al*., 1996; Weterman *et al*., 1996a).

Fusion to the PSF gene in UOK145

To identify sequences fused to the *TFE3* gene in the UOK145 cell line, 5′-RACE was performed using a *TFE3* gene exon 4 primer (Sidhar *et al*., 1996). The sequence of the product obtained (Figure 2a) diverged from the normal *TFE3* sequence 5′ to exon 4. Database searches of the unique 5′ sequences revealed that they were identical to the human *PSF* splicing factor gene (Patton *et al*., 1993). The splicing reaction involves two steps. In step I unspliced pre-mRNA is converted into separate exon 1 and lariat-intron-exon 2 structures. In step II exons 1 and exons 2 are joined and an excised lariat-intron is formed. PSF is a component of the spliceosome complex which catalyses these reactions and has been shown to be required for catalytic step II of the splicing reaction (Gozani *et al*., 1994). The 712aa PSF protein contains several distinct regions (Figure 3)

including (i) an N-terminal domain rich in proline and glutamine and (ii) two conserved RNA-binding domains (Patton *et al*., 1993). Notably the *PSF-TFE3* hybrid transcript detected in UOK145 is predicted to encode a fusion protein containing almost the entire PSF protein fused to the C-terminal TFE3 DNA-binding domain (Figure 3).

Fusion to the NonO (p54^{nrb}) gene in UOK109

To identify sequences fused to the *TFE3* gene in the UOK109 cell line, *TFE3* 5′-RACE and 3′-RACE products were obtained. For both 5′ and 3′-RACE, sequences were obtained that diverged from known *TFE3* sequences at the exon 3-exon 4 junction (eg, the 5′-RACE product is shown in Figure 2b). Database searches of the new sequences present in the 5′-RACE and 3′-RACE products revealed that they exactly matched adjacent regions of a gene designated *NonO* (*p54^{nrb}*) that is closely related to *PSF* (Dong *et al*., 1993; Yang *et al*., 1993).

The 471 amino acid NonO (p54^{nrb}) protein contains several distinct domains (Figure 3) including (i) a short N-terminal sequence composed entirely of histidine, glutamine and proline residues, (ii) a helix–turn–helix domain flanked by charged amino acids that is responsible for binding to the octamer sequence in double stranded DNA and (iii) a short C-terminal proline-rich region. The NonO (p54^{nrb}) protein has a region of 320 amino acids with 71% identity and 7% similarity to a 320aa region of PSF (Patton *et al*., 1993; Dong *et al*., 1993; Yang *et al*., 1993). This same region of NonO (p54^{nrb}) exhibits 42% identity and 7% similarity to a 321aa region of the 700aa Drosophila NonA^{diss} protein. *NonA* mutants in Drosophila show abnormal phototaxis and optomotor response and this gene is also the locus of the *dissonance* (*diss*) allele which results in an altered male courtship song. p54^{nrb}, PSF and NonA^{diss} are all members of a family of proteins called DBHS (for *D*rosophila *b*ehaviour and *h*uman *s*plicing) that are believed to have an important role in controlling splicing of pre-mRNA species. As observed for the *PSF-TFE3* fusion the *NonO* (*p54^{nrb}*)-*TFE3* hybrid transcript is predicted to encode a fusion protein in which almost the entire p54^{nrb} protein is fused to the C-terminal DNA-binding domain of TFE3. Interestingly both the PSF-TFE3 and NonO (p54^{nrb})-TFE3 protein fusions (Figure 3) lack the TFE3 acidic activation domain (AAD) indicating that this sequence is not essential for transformation.

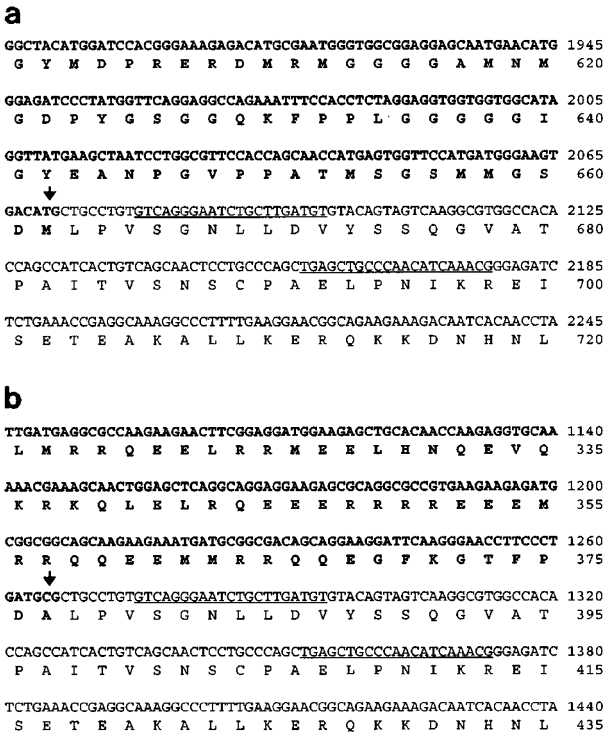

Figure 2 Nucleotide sequences of the *TFE3* 5′-RACE products obtained from the UOK145 (**a**) and UOK109 (**b**) cell lines together with predicted amino acid sequences. The sequences have been extended 3′ to show the position of the *TFE3* 5′-RACE primers which are marked by the lines under the sequence. Sequences that did not match the *TFE3* sequence are shown in bold. The vertical arrows show the positions of the breakpoints between the *PSF*, *NonO* and *TFE3* found in lines UOK109 and UOK145

RT–PCR detection of hybrid transcripts

The presence of a *PSF-TFE3* hybrid transcript encoded by the der(1) chromosome in the UOK145 cell line was confirmed by RT-PCR. Using 5′-*PSF* and 3′-*TFE3* primers a product of the predicted size was observed for UOK145 but not in other renal cell lines examined (Figure 4). The der(X) chromosome resulting from the t(X;1)(p11.2;p34) translocation is absent in UOK145 and indeed we failed to detect the reciprocal *TFE3-PSF* hybrid transcript that would be encoded by the der(X) in this cell line (results not shown). Using a similar approach we confirmed the presence of both the *NonO* (*p54^{nrb}*)-*TFE3* (Figure 4) and *TFE3-NonO* (*p54^{nrb}*) (result not shown) transcript in the UOK109 cell line. As expected hybrid transcripts involving the *PSF* and *NonO* (*p54^{nrb}*) genes were not detected in other renal carcinoma cell lines. Analysis of the presence of normal *PSF*, *NonO* and *TFE3* transcripts using pairs of primers that flanked the position of the translocations (Figure 4) demonstrated that fusion of the X chromosome genes *NonO* and *TFE3* in the male UOK109 cell line was associated with the loss of both normal *NonO* and normal *TFE3* transcripts.

Discussion

In these studies we report that the UOK145 papillary renal cell carcinoma cell line contains a t(X;1)(p11.2;p34) translocation that results in the fusion of the *PSF* gene located on chromosome arm 1p to the *TFE3* transcription factor gene. This translocation has been observed previously in renal tumors (Kovacs *et al*., 1987; Dijkuizen *et al*., 1995; Yoshida *et al*., 1995) and therefore represents a recurrent abnormality in this tumor type. We also demonstrated that in the UOK109 cell line an inv(X)(p11.2;q12) results in the fusion of the *NonO* (*p54^{nrb}*) gene to the *TFE3* gene. The abnormality inv(X)(p11.2;q12) has not been cytogenetically detected previously in renal carcinoma but this subtle alteration is not evident by standard cytogenetic examination. In previous studies we showed that a novel gene called *PRCC* becomes fused to the *TFE3* gene in papillary renal carcinomas containing the t(X;1)(p11.2;q21.2)

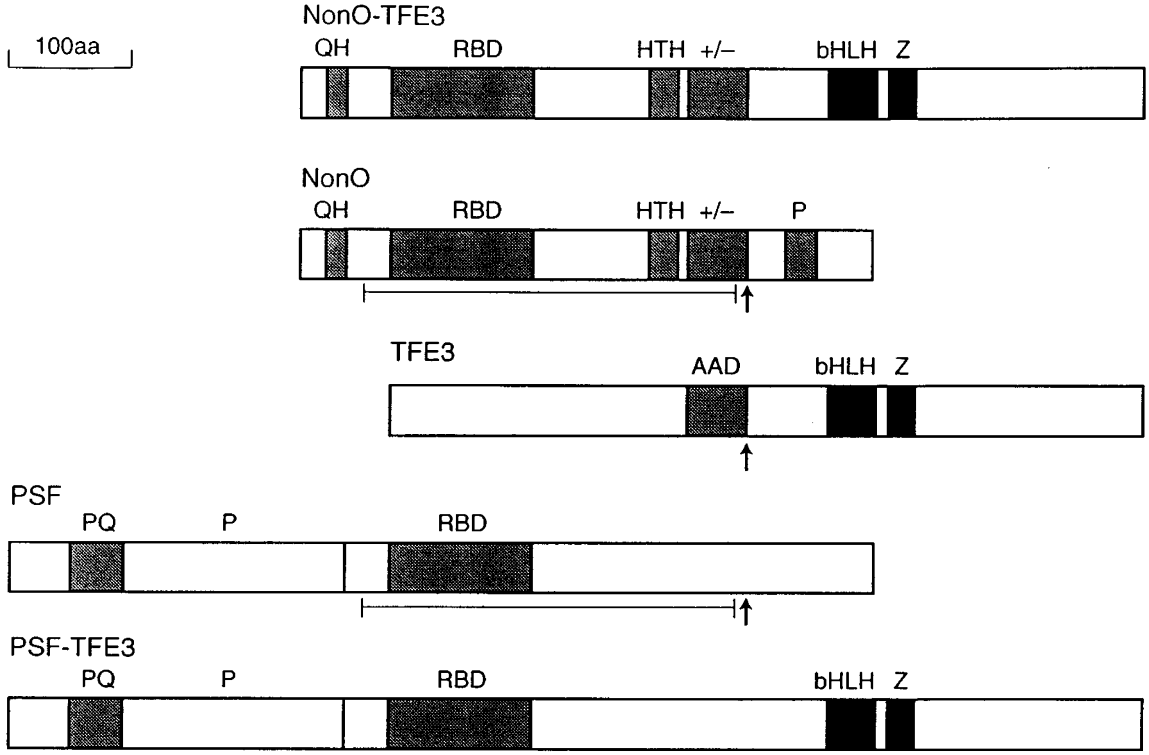

Figure 3 Schematic representation of wild type PSF, NonO ($p54^{nrb}$) and TFE3 proteins and of the PSF-TFE3 and NonO-TFE3 chimaeric proteins present in respectively UOK145 and UOK109. The PSF protein is 712 amino acids in length and contains an N-terminal domain rich in proline and glutamine (PQ), a region rich in proline (P) and tandem RNA-binding domains (RBD). The 471 amino acid NonO protein contains regions rich in glutamine and histidine (QH) and proline (P), tandem RNA-binding domains (RBD), and a helix-turn-helix-charged (HTH, +/−) region implicated in DNA binding. The regions of conservation between PSF and NonO which encompasses the RBD region and NonO HTH, +/− region is shown by the bar. The TFE3 protein contains acidic activation (AAD) and DNA binding domains (bHLH;Z) and has an overall length of 575 amino acids. To check the structure of the TFE3 protein we isolated and sequenced normal *TFE3* cDNA clones. The predicted structure, which is shown in this figure, is in agreement with that reported by Weterman *et al.* (1996a). RT–PCR of RNA from the UOK109 cell line has been used to confirm the entire structure shown in this figure. The arrows represent the positions of fusion

translocation (Sidhar *et al.*, 1996). Taken together, our observations, therefore, demonstrate consistent alteration of the *TFE3* gene in papillary renal cell carcinoma but show that *TFE3* may have a variety of fusion partners. Notably, a t(X;17)(p11.2;q25) translocation (Tomlinson *et al.*, 1991) and a t(X;10)(p11.2;q23) translocation (Dijkuizen *et al.*, 1995) have also been reported in papillary renal cell carcinoma suggesting that the *TFE3* gene may have partners in addition to those currently identified.

Fusion of a single gene to several distinct partners has also been observed for translocations found in sarcomas and haematopoietic cancers. For example, in acute myelocytic leukemia the homeobox gene *MLL* at 11q23 can have a large variety of fusion partners (Rabbitts, 1994). Similarly in Ewing's sarcoma, the 5′ region of the *EWS* gene at 22q12 can become fused to the *FLI1*, *ERG* and *ETV1* genes, three members of the family of transcription factor genes located respectively on chromosomes 11, 21 and 7 (Zucman *et al.*, 1993; Jeon *et al.*, 1995). In alveolar rhabdomyosarcoma 3′ *FKHR* sequences on chromosome 13 can become joined to either 5′ *PAX3* sequences located on chromosome 2 or 5′ *PAX7* sequences on chromosome 1 (Galili *et al.*, 1993; Davis *et al.*, 1994).

Previous analysis of the fusion between the *PRCC* and *TFE3* genes indicated that it is the generation of the *PRCC-TFE3* hybrid transcript, rather than the formation of the *TFE3-PRCC* hybrid transcript, that is a consistent feature associated with the t(X;1)(p11.2;q21.2) translocation. By analogy it would be expected that the generation of the *NonO* ($p54^{nrb}$)*-TFE3* and *PSF-TFE3* hybrid transcripts are the key events in transformation. In agreement with this observation we failed to detect the reciprocal *TFE3-PSF* transcript in UOK145 cells.

Since work on the identification of fusions of the *TFE3* gene to *PRCC*, *NonO* and *PSF* genes has all been carried out on immortal cell lines it could be proposed that this fusion could be an artefact of *in vitro* selection. However this possibility seems unlikely since the t(X;1)(p11.2;q21.2) and t(X;1)(p11.2;p34) translocations that result in these fusions were originally observed in cultures of primary tumor material (Kovacs *et al.*, 1987; de Jong *et al.*, 1986; Meloni *et al.*, 1993; Mitelman, 1994; Dijkuizen *et al.*, 1995; Tonk *et al.*, 1995; Yoshida *et al.*, 1995). In addition, we have directly detected alteration of the *TFE3* gene in primary renal tumor material (DS and JS unpublished).

Several lines of circumstantial evidence have suggested links between RNA processing, transcription factors and oncogenesis. For example, WT1 protein which has been demonstrated to act as a transcriptional regulator (Hastie, 1994) was shown to be able to associate with spliceosomes and to coiled-bodies, nuclear organelles of unknown function that contain components of the spliceosome (Larsson *et al.*, 1995). In Friend spleen focus forming virus-induced

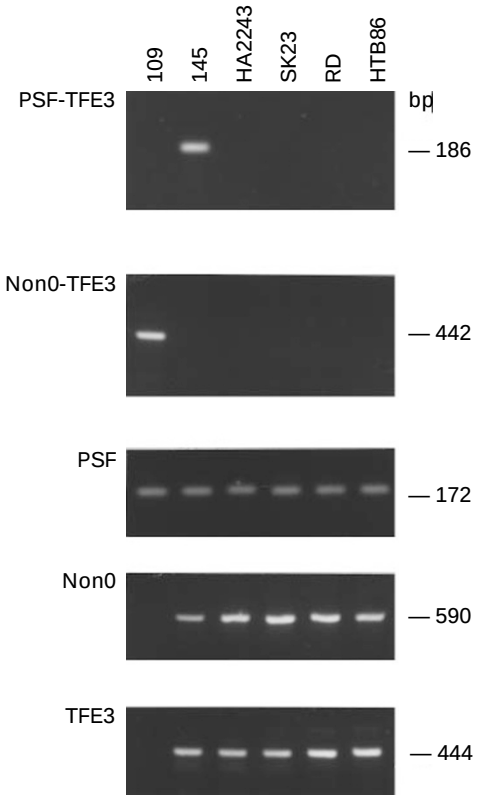

Figure 4 Detection of *PSF-TFE3* and *NonO-TFE3* hybrid transcripts by RT–PCR (upper two panels). RT–PCR was performed using 5′ *PSF* or 5′-*NonO* primers together with 3′ *TFE3* primers. Normal *PSF*, *NonO* and *TFE3* transcripts were detected by RT–PCR using pairs of primers that flanked the position of translocations within each of these genes. For primers sequences see the Materials and methods section. RNAs used for these experiments were from the UOK109 and UOK145 papillary renal cell carcinoma cell lines and the following human tumor samples; the HA2243 synovial sarcoma cell line; the SK23 melanoma cell line; the RD rhabdomyosarcoma cell lines; and the HTB86 Ewings sarcoma cell line

erythroleukemia, insertional mutagenesis can occur adjacent to the *Spi-1* gene resulting in overexpression of the normal Spi-1/PU1 protein, a member of the Ets-family of DNA-binding transcription factors. Recent evidence has shown that the 55 kDa Spi-1/PU1 protein can also bind to the NonO ($p54^{nrb}$) protein and affect the splicing process *in vitro* (Hallier *et al*., 1996).

In the present study we provide a more direct link between the splicing process, transcription factors and oncogenesis by demonstrating fusion of the splicing factor genes *PSF* and *NonO* (*$p54^{nrb}$*) to the *TFE3* transcription factor gene in papillary renal cell carcinomas. A notable feature of this fusion is that in each case it results in fusion of almost the entire splicing factor to the DNA binding domain of TFE3. It is therefore possible that the PSF-TFE3 and NonO($p54^{nrb}$)-TFE3 fusions may retain activity in both splicing and DNA transcriptional control and it is interesting to speculate that the resulting interference between these two processes may represent a new mechanism of tumor development. This may provide a fascinating area of investigation for future studies.

Previous studies on the t(X;1)(p11.2;q11.2) translocation indicated that generation of the PRCC-TFE3 fusion protein was invariably associated with the loss of transcription of the normal X chromosome *TFE3* gene. This led to the proposal that the fusion may have a dual function in both generating a fusion protein and in removing the product of the normal gene. In the current study we did, however, detect a normal *TFE3* transcript in the UOK145 cell line in addition to the hybrid *PSF-TFE3* transcript (as shown in figure 4). This observation would be consistent with the view that removal of normal *TFE3* transcript is not an essential step in the transformation process.

It has been proposed that renal cell carcinomas bearing abnormalities of Xp11.2 may constitute a subgroup distinct from the classic histologically defined papillary carcinoma (Meloni *et al*., 1993). This idea was based on initial cytogenetic observations showing that tumors containing alterations of Xp11.2 were found entirely in male patients and arose mainly in children and young adults, although sporadic renal cell carcinoma is a disease that is most common in the fifth to seventh decades of life. We have now found alterations of the *TFE3* gene in five of seven papillary renal cell carcinoma cell lines examined. In agreement with these earlier conclusions the average age of onset of these five tumors (27 years) was lower than that generally observed for renal cell carcinoma. In addition, three tumors harbouring *TFE3* abnormalities arose in females demonstrating that this alteration is not restricted to male patients. The advances reported should now allow analysis of larger series of tumors to determine its true age and sex distribution of patients with *TFE3* abnormalities and to assess whether this translocation can be used as a diagnostic or prognostic marker. It may also be interesting to see, in studies of larger tumor series, whether fusion of the *TFE3* gene to different partners correlates with particular histological and biological phenotypes.

Materials and methods

Cell lines

The UOK145, UOK109, UOK112 and UOK132 cell lines were derived from primary papillary renal cell carcinoma specimens as described (Anglard *et al*., 1992). The cell lines were derived respectively from tumors arising in an 18 year old female, a 39 year old male, a 67 year old male and a 63 year old female.

Cytogenetics and FISH analysis

Cytogenetic analysis of UOK109 and UOK145 was carried out using standard procedures. Chromosome 1 and X paints and a probe for the pericentric region of chromosome 1 were used singly or in combination with differentially labeled YAC probes as previously described in order to confirm the origin of the derivative 1 and X chromosomes (Shipley *et al*., 1993, 1995). YAC clones for GATA and pTAK8 (Shipley *et al*., 1995) lie telomeric and centromeric to *TFE3* at Xp11 and were labeled with green and red fluorochromes, respectively. These YACs were co-hybridised to chromosome preparations of the two cell lines and analysed as detailed previously (Shipley *et al*., 1993, 1995).

Analysis of DNA and RNA

Preparation of genomic DNA and cytoplasmic RNA were carried out as described (Clark *et al*., 1994). Restriction

endonuclease digestions and Southern blot analysis were carried out as described previously. (Clark *et al*., 1994).

RT–PCR analysis

To detect *PSF-TFE3* hybrid transcripts reverse transcribed RNA was subject to amplification using the *PSF* primer 5′-TGGTGGTGGCATAGGTTATG-3′ (forward primer) and *TFE3* primer 5′-CGTTTGATGTTGGGCAGCTC-3′ (reverse primer). *NonO (p54^{nrb})-TFE3* hybrid transcripts were detected using the *NonO (p54^{nrb})* primer 5′-GAGAAACTAGACACAGCAAC-3′ (forward primer) and the *TFE3* primer 5′-CTTTCTTCTGCCGTTCCTTC-3′ (reverse primer). *PRCC-TFE3* hybrid transcripts were detected using the *PRCC* primer 5′CCAAGCCAAAGAAGAGGA-3′ (forward primer) and the *TFE3* primer 5′-AGTGTGGTGGACAGGTACTG-3′ (reverse primer). Normal *PSF* transcripts were detected using the forward *PSF* primer described above together with the reverse *PSF* primer 5′-TCTACCATATCCTGCTGGAG-3′. Normal *NonO* transcripts were detected using the forward *NonO* primer described above and the *NonO* reverse primer 5′-CAAGTGGACCGCAACATCAAG-3′. Normal *TFE3* transcripts were detected using the primers 5′-CCCGCAAGTGCCCAGCCACTG-3′ (forward primer, exon 3) and 5′-CAGTTCCTTGATCCTGTCG-3′ (reverse primer, exon 4). As positive controls to confirm that each RNA sample could yield products RT–PCR amplification was carried out with actin primers as described previously (Sidhar *et al*., 1996). In these analyses all reverse transcribed samples generated an actin PCR product of the expected size. Amplification conditions were 93°C for 20 s, 59°C for 30 s (cycles 7–36) and 68°C for 30 s for 30 cycles using Expand High Fidelity DNA polymerase (Boehringer Mannheim) in a final volume of 25 μl. For the first six cycles touch down annealing temperatures were 61°C ($\times$2) and 59°C ($\times$2) and 57°C ($\times$2). The products were separated by electrophoresis in agarose gels followed by staining with ethidium bromide.

5′RACE and 3′RACE

For 5′RACE 1 μg of RNA was reverse transcribed using Superscript II reverse transcriptase using the *TFE3* specific primer CAGTTCCTTGATCCTGTCGA and then tailed with 5′-polyC as described (Sidhar *et al*., 1996). Amplification of cDNA 5′ ends was then performed using the first round PCR primers 5′-CTTTCTTCTGCCGTTCCTTC-3′ (*TFE3* reverse primer) and the GI tag primer 5′-GACTCGAGTCGACATCGGGIIGGGIIGGGIIG-3′ where I is inosine. Aliquots of the reaction were then subject to nested PCR using the reverse primer 5′-CGTTTGATGTTGGGCAGCTC-3′ (*TFE3* reverse primer) and tag primer 5′GACTCGAGTCGACATC-3′. For 3′-RACE reverse transcription was performed using the tagged random hexamer primer 5′-GACTCGAGTCGACATCNNNNNN-3′. 3′-RACE was then performed using the *TFE3* forward primer 5′-CCCGCAAGTGCCCAGCCACTG-3′ together with the tag primer 5′-GACTCGAGTCGACATC-3′. Nested PCR was then performed using the *TFE3* forward primer 5′-TCACCATCGGGTCCAGCTCA-3′ together with the tag primer. PCR conditions for both 5′ PCR and 3′RACE were 93°C for 15 s; 57°C for 30 s and 68°C for 30 s for 30 cycles.

cDNA libraries

A cDNA library made from human placental DNA in the pcDM8 vector were kindly provided by the Sanger Centre, Cambridge, UK.

DNA sequencing

DNA sequencing was performed exactly as described previously (Sidhar *et al*., 1996).

Abbreviations

RCC, Renal cell carcinoma; RT–PCR, reverse transcription-polymerase chain reaction; VHL, von Hippel-Lindau; AAD, acidic activation domain.

Accession No. 5′-TFE3: X96717.

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