Human Molecular Genetics, 1998, Vol. 7, No. 4 637–642

ARTICLE

The t(8;13)(p11;q11–12) rearrangement associated with an atypical myeloproliferative disorder fuses the fibroblast growth factor receptor 1 gene to a novel gene *RAMP*

Damian Smedley[1,3,*], Rifat Hamoudi[2], Jeremy Clark[3], William Warren[3], Munah Abdul-Rauf[4], Gino Somers[5], Deon Venter[5], Kerry Fagan[6], Colin Cooper[3] and Janet Shipley[1]

[1]Cell Biology and Experimental Pathology Section, [2]Cancer Gene Cloning Laboratory, [3]Molecular Carcinogenesis Section and [4]Academic Haematology and Cytogenetics Section, Institute of Cancer Research, Haddow Laboratories, Belmont, Surrey SM2 5NG, UK, [5]Department of Pathology, Peter MacCallum Cancer Institute, East Melbourne, Victoria, Australia and [6]Hunter Area Pathology Service, Mater Hospital, Edith Street, Waratah, New South Wales, Australia

Received December 19, 1997; Revised and Accepted January 27, 1998 DDBJ/EMBL/GenBank accession no. AF035374

A recently described atypical myeloproliferative disorder is invariably associated with reciprocal translocations involving 8p11–12. The most common rearrangement is a t(8;13)(p11;q11–12). Here we determine that this translocation results in the fusion of the fibroblast growth factor receptor 1 gene (*FGFR1*), a member of the receptor tyrosine kinase family at 8p11, to a novel gene at 13q11–12 designated *RAMP*. The predicted RAMP protein exhibits strong homology to the product of a recently cloned candidate gene for X-linked mental retardation, *DXS6673E*. We also provide the first report of a novel, putative metal-binding motif, present as five tandem repeats in both RAMP and DXS6673E. RT-PCR detected only one of the two possible fusion transcripts, encoding a product in which the N-terminal 641 amino acids of RAMP become joined to the tyrosine kinase domain of FGFR1. Receptor tyrosine kinases are not commonly involved in the formation of tumour-specific fusion proteins. However, the previous reports of involvement of receptor tyrosine kinases in fusion proteins in non-Hodgkin's lymphoma, chronic myelomonocytic leukaemia and papillary thyroid carcinoma described similar rearrangements. By analogy with these, we propose that the RAMP–FGFR1 fusion product will contribute to progression of this myeloproliferative disorder by constitutive activation of tyrosine kinase function.

INTRODUCTION

An atypical myeloproliferative disorder involving eosinophilia, a high incidence of T-cell non-Hodgkin's lymphoma (NHL) and rapid progression to either chronic or acute myeloid leukaemia (CML/AML) has been described (1–3). In each case, reciprocal translocations involving 8p11–12 were reported, often as the only detectable cytogenetic change. The most common rearrangement is t(8;13)(p11;q11–12), but rarer t(8;9)(p11–12;q32–34) and t(6;8)(q27;p12) were also reported (1–3).

We have shown previously that yeast artificial chromosome (YAC) 899e2 (1.6 Mb) spans the chromosome 8 breakpoint of the t(8;13)(p11;q11–12) rearrangement (3). Our localization is consistent with reports that the 8p11 breakpoint in the t(8;13) rearrangement is flanked by YACs 782h11 and 847b12 (4) and is spanned by YAC 959a4 (5).

In the present study, we demonstrate that the t(8;13)(p11;q11–12) results in disruption of the fibroblast growth factor receptor 1 (*FGFR1*) gene at 8p11–12. Further characterization led to the discovery that the *FGFR1* gene becomes fused to a novel gene designated *RAMP* (for rearranged in an atypical myeloproliferative disorder). The predicted RAMP protein exhibits strong homology to DXS6673E, the predicted product of a recently cloned candidate gene for X-linked mental retardation at Xq13.1 (6), and we describe a novel, putative metal-binding motif in both proteins.

*To whom correspondence should be addressed. Tel: +44 181 643 8901; Fax: +44 181 643 0238; Email: damian@icr.ac.uk

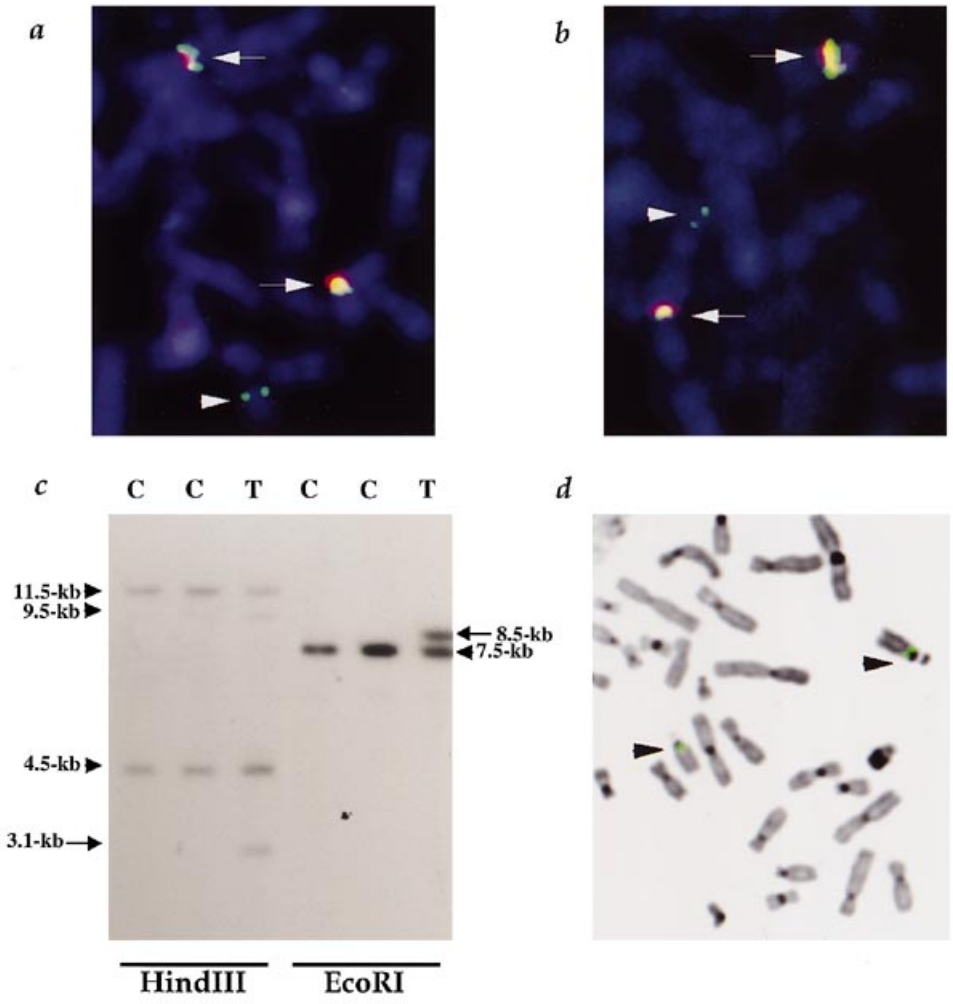

Figure 1. (**a** and **b**) FISH analysis of the 8p11 breakpoint in case 1 (a) and 2 (b). PAC 364p11 with a green signal hybridizes to both the normal and derivative 8 chromosomes (arrows) and the derivative 13 chromosome (arrowhead). The chromosome 8 centromere-specific probe gave a red signal. (**c**) Southern blot analysis of patient DNA with the t(8;13)(p11;q11–12) rearrangement (T) and control DNA from cell lines that do not harbour the t(8;13) translocation (C). DNAs digested with either *Eco*RI or *Hin*dIII were hybridized to a clone isolated during cDNA selection containing sequence corresponding to exons 5, 7 and 8 of *FGFR1*. Normal bands are indicated by arrowheads and rearranged bands by arrows. (**d**) FISH on a partial normal metaphase with the PAC clone 11n11 which contains sequence from the novel gene *RAMP*. Arrows indicate hybridization to 13q11–12.

RESULTS

Identification of a PAC spanning the chromosome 8 breakpoint

To map the chromosome 8 breakpoint in the t(8;13)(p11;q11–12) more precisely, a human genomic phage P1-derived artificial chromosome (PAC) library was screened with an inter-Alu PCR product generated from YAC 899e2 which spans this breakpoint (3). The 37 positive clones were characterized by fluorescent *in situ* hybridization (FISH) on metaphase chromosomes from two cases of the disorder. For each case, PAC 364p11 hybridized to both the derivative 8 and 13 chromosomes (Fig. 1a and b) and therefore appeared to span the chromosome 8 breakpoint.

Involvement of the *FGFR1* gene

cDNA selection as well as shotgun cloning and sequencing were used to identify genes encoded within PAC 364p11. Several novel genes and one known gene encoding the receptor tyrosine kinase (RTK), FGFR1, were identified. DNA and RNA for further characterization of the rearrangement were only available for case 1. Southern blot analysis, using a probe corresponding to exons 5, 7 and 8 of the *FGFR1* gene, revealed a rearrangement in this case for DNA digested with *Eco*RI, *Hin*dIII (Fig. 1c) or *Bam*HI (data not shown).

Fusion of *FGFR1* to a novel gene, *RAMP*, at 13q11–12

The breakpoints in previously described rearrangements involving RTKs are invariably located just before or after the transmembrane

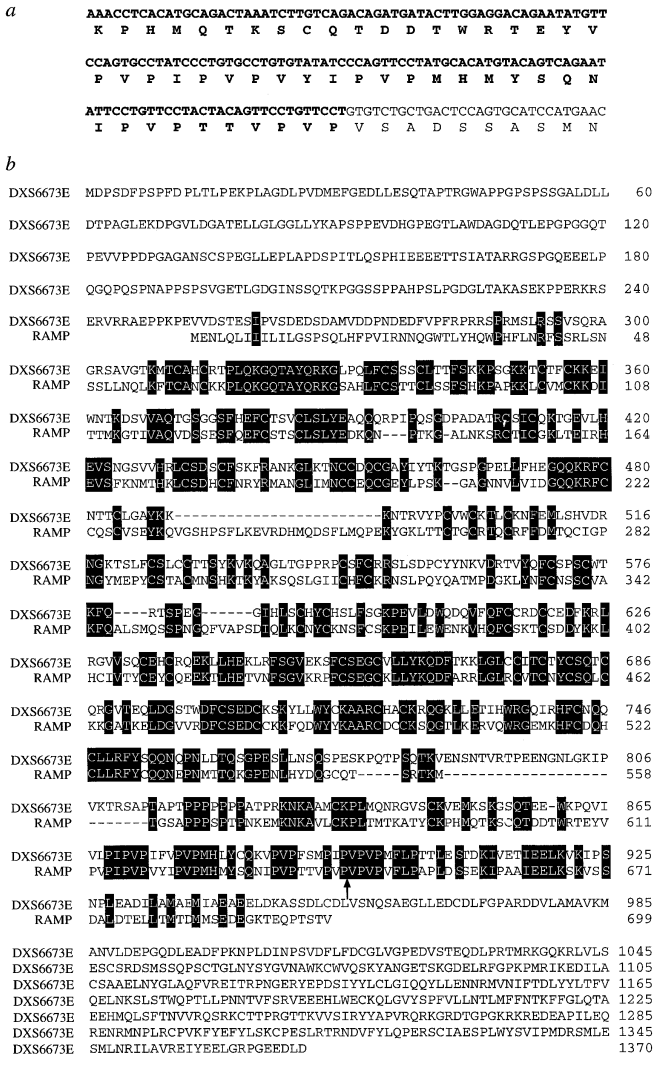

Figure 2. (**a**) Nucleotide sequences of the 5′ RACE product together with its predicted amino acid sequence. Novel sequences from the *RAMP* gene that did not match the published *FGFR1* sequence are shown in bold. (**b**) Predicted amino acid sequence of wild-type RAMP aligned with the predicted product of a candidate gene for X-linked mental retardation in Xq13.1 (DXS6673E). The vertical arrow shows the position of the breakpoint in *RAMP*.

segment (7–11). In order to identify the fusion partner, 5′ RACE was performed using primers designed from *FGFR1* exon 9 located close to the sequence encoding the transmembrane segment in exon 8. The sequence from the 5′ RACE product diverged from the normal *FGFR1* sequence at the known junction between exon 8 and exon 9 (12) (Fig. 2a). PAC 11n11 was isolated by screening a human genomic library with a probe containing the novel sequence, and was shown to map to 13q11–12 as predicted (Fig. 1d).

To obtain the full coding sequence of the new gene, further 5′ and 3′ RACE reactions were carried out using primers designed from the novel sequence. Sequencing of the RACE products generated a continuous sequence of 2553 bp (GenBank accession no. AF035374). This gene, designated *RAMP*, contained an open reading frame of 699 amino acids (Fig. 2b). RAMP exhibited strong homology (43% identity) to the products of a recently cloned candidate gene for X-linked mental retardation at Xq13.1, *DXS6673E* (6) (Fig. 2b), and a cDNA clone KIAA0385 (GenBank accession no. AB002383) which is identical to *DXS6673E* except for a deletion of residues 793–804. *DXS6673E* was shown to be X-inactivated and disrupted in the 5′-untranslated region by a balanced t(X;13)(q13.1;q32) in a mentally retarded female (6).

Identification of a novel, putative metal-binding motif in RAMP

A large, cysteine-rich region in RAMP (59–523) is highly conserved in DXS6673E (311–747) and consists of five tandem repeats of a Cys-X_2-Cys-$X_{19–22}$-Cys-X_3-Cys-$X_{13–17}$-Cys-X_2-Cys-$X_{19–25}$-Cys-X_3-Cys motif where X is any amino acid, although at certain positions there are distinct preferences for particular amino acids (Fig. 3a). This motif closely resembles zinc-binding sequences (Fig. 3b), and BLAST searches with the DXS6673E and RAMP sequences failed to identify the motif in any other known protein sequences in the GenBank, PDB, SwissProt and PIR databases. Therefore RAMP and DXS6673E contain a novel, putative metal-binding motif.

PCR detection of *RAMP–FGFR1* transcripts

The *RAMP–FGFR1* hybrid transcript was detected in case 1 by RT-PCR (Fig. 4a), and sequencing of this PCR product confirmed the 5′ and 3′ RACE sequences. However, the reciprocal *FGFR1–RAMP* fusion transcript was not observed, despite detection of normal *FGFR1* and *RAMP* transcripts by RT-PCR reactions which utilized the same primers (Fig. 4a). The expressed fusion transcript is comprised of the N-terminal 641 amino acids of RAMP fused to the tyrosine kinase (TK) domain of FGFR1 (Fig. 4b).

DISCUSSION

Here we report that a reciprocal translocation, t(8;13)(p11;q11–12), associated with an atypical myeloproliferative disorder, results in expression of a fusion gene resulting from disruption of a novel chromosome 13 gene called *RAMP* and the *FGFR1* receptor tyrosine kinase gene at 8p11–12. All four members of the FGFR family are RTKs composed of three immunoglobulin-like domains in the extracellular receptor component, a transmembrane segment and a cytoplasmic tyrosine kinase domain (13–15). Activating mutations in FGFR1, FGFR2 and FGFR3 have been reported as the cause of several human craniosynostosis and dwarfism syndromes (16–18). Recently a t(4;14)(p16.3;q32.3) rearrangement in multiple myeloma was shown to be associated with increased expression and activating mutations of FGFR3 (19). This dysregulation of expression was proposed to result from fusion of the IgH locus at 14q32 to one of a cluster of breakpoints 70 kb from *FGFR3* at 4p16.3.

The majority of tumour-specific fusion proteins involve transcription factors, although involvement of RTKs has been demonstrated (20). In papillary thyroid carcinomas, fusions of N-terminal regions of various proteins to the TK domains of the RTKs, RET and NTRK1, have been demonstrated (7–9). In addition, the N-terminal 154 amino acids of the transcription factor TEL, including the helix–loop–helix motif predicted to be involved in protein–protein interactions, becomes joined with the TK domain of the RTK, platelet-derived growth factor receptor (PDGFRβ), in chronic myelomonocytic leukaemia (10). In NHL,

a

RAMP	rpt 1 (55-137)	LKPTCANCKKPLQKGQTAY QRKGSAHLPCSTTCLSSFSHKP APKKLCVMCKKDITTMKG TIVAQVDSSESFQEFCSTSCLSLY
DXS6673E	rpt 1 (307-389)	TKMTCAHCRTPLQKGQTAY QRKGLPQLPCSSSCLTTFSKKP SGKKTCTFCKKEIWNTKD SVVAQTGSGGSFHEFCTSVCLSLY
RAMP	rpt 2 (149-230)	NKSRCTICGKLTEIRHEVS FKN MTHKLCSDHCFNRYRMANG LIMNCCEQCGEYLPSK G AGNNVLVIDGQQKRFCCQSCVSEY
DXS6673E	rpt 2 (405-488)	DATRCSICQKTGEVLHEVS NGS VVHRLCSDSCPSKFRANKG LKTNCCDQCGAYIYTKTGSPGPELLFHEGQQKRFCNFTCLGAY
RAMP	rpt 3 (260-344)	KLTTCTVCR TQCRFFDMTQCIGPNGYMEPYCSTACMNSHKTKYAKSQSLGIICHFCKRNSLPQ YQATMPDGKLYNFCNSSCVAKF
DXS6673E	rpt 3 (494-578)	RVYPCVWCK TLCKNFEMLSHVDRNGKTSLPCSLCCTTSYKVKQAGLTGPPRPCSFCRRSLSDP CYYNKVDRTVYQFCSPSCWTKF
RAMP	rpt 4 (363-440)	IQLKCNYCKNSFCSKPEIL EWENKVHQFCSKTCSDDYKKLH CIVTYCEYCQEEKTLH ETVNFSGVKRPFCSEGCKLLY
DXS6673E	rpt 4 (587-664)	IHLSCHYCHSLFSGKPEVL DWQDQVFQFCCRDCCEDFKRLR GVVSQCEHCRQEKLLH EKLRFSGVEKSFCSEGCVLLY
RAMP	rpt 5 (451-527)	RCVTCNYCS QLCKKGATK ELDCVVRDFCSEDCCKKFQDWY YKAARCDCCKSQGTLK ERVQWRGEMKHFCDQHCLLRF
DXS6673E	rpt 5 (675-751)	CCITCTYCS QTCQRGVTE QLDGSTWDFCSEDCKSKYLLWY CKAARCHACKRQGKLL ETIHWRGQIRHFCNQQCLLRF

b

NOVEL MOTIF: Cys-X_2-Cys-X_{19-22}-Cys-X_3-Cys-X_{13-17}-Cys-X_2-Cys-X_{19-25}-Cys-X_3-Cys

RING: Cys-X_2-Cys-X_{9-39}-Cys-X_{1-3}-His-X_{2-3}-Cys-X_2-Cys-X_{4-48}-Cys-X_2-Cys

PHD/LAP: Cys-X_2-Cys-X_{8-21}-Cys-X_{2-4}-Cys-X_{4-5}-His-X_2-Cys-X_{12-46}-Cys-X_2-Cys

LIM: Cys-X_2-Cys-X_{16-23}-His-X_2-Cys-X_2-Cys-X_2-Cys-X_{16-23}-Cys-X_2-Cys

Figure 3. (**a**) Multiple sequence alignment of the five repeats of the novel, putative metal-binding motif seen in RAMP and DXS6673E. The absolutely conserved cysteine residues with a potential role as metal ligands are shown in bold. The numbering in parentheses refers to the amino acid positions in the full-length protein sequences. (**b**) Comparison of the novel motif with RING, PHD/LAP and LIM sequence motifs where X refers to any amino acid, although for each motif there are distinct preferences at particular positions (25).

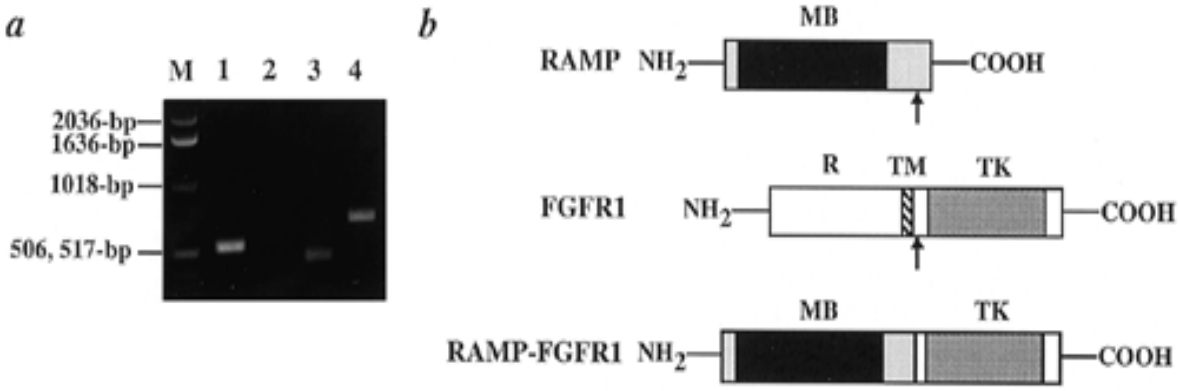

Figure 4. (**a**) RT-PCR reactions to detect *RAMP–FGFR1* (lane 1), *FGFR1–RAMP* (lane 2), *RAMP* (lane 3) and *FGFR1* (lane 4) transcripts in case 1. M, 1 kb ladder (Gibco BRL). RT-PCR was performed as described in Materials and Methods using primers A and B (lane 1), C and D (lane 2), A and D (lane 3) and B and C (lane 4) which can generate potential products of 525, 610, 454 and 681 bp respectively. (**b**) Schematic representation of wild-type RAMP and FGFR1 proteins and the RAMP–FGFR1 fusion protein. RAMP consists of 699 amino acid residues and includes a cysteine-rich region with novel, putative metal-binding motifs (MB). FGFR1 is composed of an extracellular receptor domain (R), transmembrane segment (TM) and cytoplasmic tyrosine kinase (TK) domain. The t(8;13)(p11;q11–12) results in fusion of the N-terminal 641 amino acids of RAMP to the TK domain of FGFR1. Arrows indicate the breakpoints in each protein.

the N-terminal 117 amino acids of the nucleolar protein NPM, including a putative metal-binding motif, fuses with the TK domain of the RTK, ALK (11). All these chromosomal rearrangements produce strikingly similar fusion products in which N-terminal regions of various proteins become joined to RTKs truncated either just before or straight after the transmembrane region. In each case, motifs in the N-terminal fusion partner result in dimerization or higher order oligomerization and constitutive activation of TK function (9,21–23). This is analogous to activation of the full-length RTK which follows ligand-dependent receptor dimerization (24). Based on these studies, we propose that the expressed RAMP–FGFR1 fusion transcript with the N-terminal 641 amino acids of RAMP fused to the TK domain of FGFR1 (Fig. 4b) will also oligomerize, due to motifs in RAMP, and exhibit constitutively activated TK function.

The novel Cys-X_2-Cys-$X_{19–22}$-Cys-X_3-Cys-$X_{13–17}$-Cys-X_2-Cys-$X_{19–25}$-Cys-X_3-Cys motif, present as five tandem repeats in RAMP and DXS6673E (Fig. 3a), closely resembles zinc-binding sequences, especially the RING, PHD/LAP and LIM motifs (25) (Fig. 3b). However, the lack of a conserved histidine residue and the alternate spacing of two and then three residues between the pairs of cysteines distinguishes this motif from known zinc-binding sequences. BLAST searches with the DXS6673E and RAMP sequences failed to identify the motif in any other known protein sequences in the GenBank, PDB, SwissProt and PIR databases. However searches on the expressed sequence tag (EST) databases identified several ESTs encoding predicted protein sequences which showed strong homology to RAMP and DXS6673E, and contained the novel motif (ESTs AA251795, W01125, N93066, R70433, AA280676, AA494557, AA280677 and AA001379). Cloning of the genes corresponding to these ESTs will refine the motif further and facilitate investigation of its functional significance. Future studies will assess if the motif binds zinc or other metal atoms and determine the structure of the protein–metal complex.

All the localization data are consistent with *FGFR1* at 8p11–12 being involved in each t(8;13) rearrangement (3–5). However, as previously reported, the 13q11–12 breakpoint in case 1 mapped several megabases telomeric to those observed in three other cases with the t(8;13) rearrangement (3,5,26), and the chromosome 13 breakpoint for case 2 has been localized as centromeric to all of these cases (data not shown). The distinct 13q11–12 breakpoints suggest that more than one gene at 13q11–12 can become fused

to *FGFR1*. Future cloning of the alternative fusion partners at 13q11–12, as well as those involved in the variant t(8;9) and t(6;8) rearrangements, will establish if they share common elements with *RAMP* and fuse with *FGFR1* to produce a similarly altered function.

MATERIALS AND METHODS

Patient samples

Two previously reported cases with the t(8;13)(p11;q11–12) rearrangement were studied: case 1 (3) and case 2 (27). Fixed material from the original cytogenetic analysis of both cases was used for FISH. Snap-frozen lymph node material, shown to be infiltrated by tumour cells with the translocation, was available from case 1.

Isolation of PAC clones

To generate probe for the first screen of the PAC human genomic library, YAC 899e2, which spans the 8p11 breakpoint, was used as a template in an inter-Alu PCR reaction using the primers 5′-GGATTACAGGCGTGAGCCAC-3′ and 5′-GATCGCGCC-ACTGCACTCC-3′ which amplify regions between Alu repeats as described previously (28). The second screen utilized a PCR product containing novel sequence from the 5′ RACE product. This was generated from normal genomic DNA by 5′-GGAG-GACAGAATATGTTCCAG-3′ and 5′-CAGGAACAGGAAC-TGTAGTAG-3′.

Analysis of DNA and RNA

Preparation of genomic and recombinant clone DNA, extraction of cytoplasmic RNA, restriction endonuclease digestion, agarose gel electrophoresis, Southern and northern transfer, hybridization, washes and autoradiography were all carried out as described previously (29).

Fluorescence *in situ* hybridization (FISH)

FISH was performed on metaphase material as described for the previous localization study (3).

Shotgun cloning and sequencing

PAC 364p11 was digested with either *Alu*I, *Rsa*I or *Hae*III and shotgun-cloned with the pCR-Script Cloning kit (Stratagene) following the manufacturer's instructions. Inserts were isolated from 800 colonies by PCR with the T7 (5′-GTAATACGACTCAC-TATAGGGC-3′) and T3 (5′-AATTAACCCTCACTAAAGGG-3′) primers and sequenced in both directions with these same primers.

cDNA selection

Capture of cDNAs was carried out using a previously described method (30) with minor modifications: (i) *Rsa*I alone and *Rsa*I–*Hae*III digests of the PAC were combined to generate selector DNA fragments; (ii) streptavidin-coated magnetic beads were washed four times in 0.1× SSC/0.1% SDS for 5 min each at room temperature and four times in 0.1× SSC/0.1% SDS for 10 min each at 65°C. Target cDNA for selection was produced from human testis poly(A) RNA (Clontech) using the Superscript Choice kit (Gibco BRL). Selected cDNA was subcloned into pCR-Script and inserts generated and sequenced from colonies using T7 and T3 primers.

5′ RACE

One µg of RNA was reverse transcribed (Superscript II reverse transcriptase, Gibco BRL) using the *FGFR1* exon 9 reverse primer 5′-TCTTCGGGAAGCTCATACTC-3′. Newly synthesized cDNA was tailed at its 5′ end using terminal transferase (Boehringer Mannheim). Amplification of cDNA ends was then performed. The first round PCR primers were 5′-TACTCAGA-GACCCCTGCTAG-3′ (*FGFR1* reverse primer) and 5′-GACTC-GAGTCGACATCGGGIIGGGIIGGGIIG-3′ where I is inosine. An aliquot of this reaction was then subjected to nested PCR using 5′-CAGAAGAACCCCAGAGTTC-3′ (*FGFR1* reverse primer) and 5′-GACTCGAGTCGACATCG-3′ (forward primer) followed by a further nested PCR reaction using 5′-AGTTCATGGATG-CACTGGAG-3′ (*FGFR1* reverse primer) and the same forward primer. 5′ RACE from the novel *RAMP* sequence was carried out with the same protocol except that the *RAMP* reverse primer 5′-CTGACTGTACATGTGCATAGG-3′ was used for the reverse transcription reaction, and two *RAMP* reverse primers (5′-AG-GAACTGGGATATACACAGG-3′ and 5′-CTGGAACATATTCT-GTCCTCC-3′) replaced the *FGFR1* reverse primers in the next two PCR rounds. Further 5′ RACE to obtain the whole coding sequence of *RAMP* was carried out using the Marathon cDNA amplification kit (Clontech) with human thymus cDNA (Clontech) as described by the manufacturer using *RAMP*-specific primers 5′-GACAGTAGAAACGCAGTAAG-3′, 5′-TAGGACCTATAC-ACTGAGTC-3′ and 5′-GGAAGAAAGGCAGGTGGTAG-3′.

3′ RACE

One µg of RNA was reverse transcribed (Superscript II reverse transcriptase, Gibco BRL) using a random primer 5′-GACTCG-AGTCGACATCGIIINNNNNN-3′. Amplification of cDNA ends was then performed. The first round PCR primers were 5′-TGCCTGTGTATATCCCAGTTC-3′ (*RAMP* forward primer) and the reverse primer 5′-GACTCGAGTCGACATCG-3′. An aliquot of this reaction was then subject to nested PCR using 5′-CCTATGCACATGTACAGTCAG-3′ (*RAMP* forward primer) and the same reverse primer.

RT-PCR

One µg of RNA was reverse transcribed as described above for 3′ RACE except for the use of a random 6mer primer. In order to observe the *RAMP–FGFR1* fusion transcript, PCR reactions were carried out with 5′ *RAMP* primer A (5′-TAAAGAGCGAG-TTCAGTGGC-3′) and 3′ *FGFR1* primer B (5′-TCTTCGGGA-AGCTCATACTC-3′). To attempt detection of the *FGFR1–RAMP* hybrid transcript, PCR reactions were carried out with 5′ *FGFR1* primer C (5′-ACACATACCAGCTGGATGTC-3′) and 3′ *RAMP* primer D (5′-TTCTCACTGCTGTCCAATGG-3′). Detection of normal transcripts was carried out using primer pairs B and C for *FGFR1* and primer pairs A and D for *RAMP*. The amplification conditions were 94°C for 30 s, 56°C for 1 min and 72°C for 1 min for 35 cycles in a final volume of 25 µl. The products were separated by electrophoresis in agarose gels followed by staining with ethidium bromide.

DNA sequencing

For sequence analysis, PCR products were either subcloned with the pCR-Script Cloning kit following the manufacturer's instructions or sequenced directly from PCR products which had been gel purified using the QIAquick gel extraction kit (QIAGEN). All sequencing was carried out on an ABI 377 sequencer using a TaqFS Dye Terminator Sequencing kit (ABI, Foster City, CA).

ACKNOWLEDGEMENTS

The authors thank Howard Slater at the Murdoch Institute, Royal Children's Hospital, Parkville, Australia for providing the fixed metaphase material and Anthony Baldini at the Baylor College of Medicine, Houston, Texas for making available the chromosome 8-specific centromere probe, p4.4. Sandra Gill, Sandra Birdsall, Jane Renshaw, Rachel Hunter, Susan McGuire, Bina Desai, Samantha Dibley and Rachel Jackson are acknowledged for their excellent technical assistance. We are also grateful to the Sanger Centre, Cambridge, UK for supplying the PAC library and clones. This work included support from the Medical Research Council and Cancer Research Campaign.

REFERENCES

1. Macdonald, D., Aguiar, R.C.T., Mason, P.J., Goldman, J.M. and Cross, N.C.P. (1995) A new myeloproliferative disorder associated with chromosomal translocations involving 8p11: a review. *Leukemia*, **9**, 1628–1630.
2. Michaux, L., Mecucci, C., Velloso, E.R.P., Dierlamm, J., Criel, A., Louwagie, A., Van Orshoven, A. and Van den Berghe, H. (1996) About the t(8;13)(p11;q12) clinico-pathologic entity. *Blood*, **87**, 1658–1659.
3. Smedley, D., Somers, G., Venter, D., Chow, C.W., Cooper, C. and Shipley, J. (1998) Characterization of a t(8;13)(p11;q11–12) in an atypical myeloproliferative disorder. *Genes Chromosomes Cancer*, **21**, 70–73.
4. Aguiar, R.C.T., Chase, A., Coulthard, S., Macdonald, D., Swirsky, D., Goldman, J.M. and Cross, N.C.P. (1997) Abnormalities of chromosome band 8p11 in leukemia: two clinical syndromes can be distinguished on the basis of MOZ involvement. *Blood*, **90**, 3130–3135.
5. Still, I.H., Chernova, O., Hurd, D., Stone, R.M. and Cowell, J.K. (1997) Molecular characterization of the t(8;13)(p11;q12) translocation associated with an atypical myeloproliferative disorder: evidence for three distinct loci involved in myeloid leukaemias on 8p11. *Blood*, **90**, 3136–3141.
6. van der Maarel, S.M., Scholten, I.H.J.M., Huber, I., Philippe, C., Suijkerbuijk, R.F., Gilgenkrantz, S., Kere, J., Cremers, F.P.M. and Ropers, H.-H. (1996) Cloning and characterization of *DXS6673E*, a candidate gene for X-linked mental retardation in Xq13.1. *Hum. Mol. Genet.*, **6**, 887–897.
7. Grieco, M., Santoro, M., Berlingieri, M.T., Melillo, R.M., Donghi, R., Bongarzone, I., Pierotti, M.A., Della Porta, G., Fusco, A. and Vecchio, G. (1990) PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. *Cell*, **60**, 557–563.
8. Martin-Zanca, D., Hughes, S.H. and Barbacid, M. (1986) A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. *Nature*, **319**, 743–748.
9. Pierotti, M.A., Bongarzone, I., Borrello, M.G., Greco, A., Pilotti, S. and Sozzi, G. (1996) Cytogenetics and molecular genetics of carcinomas arising from thyroid epithelial follicular cells. *Genes Chromosomes Cancer*, **16**, 1–14.
10. Golub, T.R., Barker, G.F., Lovett, M. and Gilliland, D.G. (1994) Fusion of PDGF receptor β to a novel *ets*-like gene, *tel*, in chronic myelomonocytic leukaemia with t(5;12) chromosomal translocation. *Cell*, **77**, 307–316.
11. Morris, S.W., Kirstein, M.N., Valentine, M.B., Dittmer, K.G., Shapiro, D.N., Saltman, D.L. and Look, A.T. (1994) Fusion of a kinase gene, *ALK*, to a nucleolar protein gene, *NPM*, in non-Hodgkin's lymphoma. *Science*, **263**, 1281–1284.
12. Johnson, D.E., Lu, J., Chen, H., Werner, S. and Williams, L.T. (1991) The human fibroblast growth factor receptor genes: a common structural arrangement underlies the mechanisms for generating receptor forms that differ in their third immunoglobulin domain. *Mol. Cell. Biol.*, **11**, 4627–4634.
13. Dionne, C.A., Crumley, G., Bellot, F., Kaplow, J.M., Searfross, G., Ruta, M., Burgess, W.H., Jaye, M. and Schlessinger, J. (1990) Cloning and expression of two distinct high-affinity receptors cross-reacting with acidic and basic fibroblast growth factors. *EMBO J.*, **9**, 2685–2692.
14. Keegan, K., Johnson, D.E., Williams, L.T. and Hayman, M.J. (1991) Isolation of an additional member of the fibroblast growth factor receptor family, FGFR-3. *Proc. Natl Acad. Sci. USA*, **88**, 1095–1099.
15. Partanen, J., Makela, T.P., Eerola, E., Korhonen, J., Hirvonen, H., Claessonwelsh, L. and Alitalo, K. (1991) FGFR-4, a novel acidic fibroblast growth-factor receptor with a distinct expression pattern. *EMBO J.*, **10**, 1347–1354.
16. Muenke, M., Schell, U., Hehr, A., Robin, N.H., Losken, H.W., Schinzel, A., Pulleyn, L.J., Rutland, P., Reardon, W., Malcolm, S. and Winter, R.M. (1994) A common mutation in the fibroblast growth factor receptor 1 gene in Pfeiffer syndrome. *Nature Genet.*, **8**, 269–274.
17. Reardon, W., Winter, R.M., Rutland, P., Pulleyn, L.J., Jones, B.M. and Malcolm, S. (1994) Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. *Nature Genet.*, **8**, 98–103.
18. Shiang, R., Thompson, L.M., Zhu, Y.Z., Church, D.M., Fielder, T.J., Bocian, M., Winokur, S.T. and Wasmuth, J.J. (1994) Mutations in the transmembrane domain of FGFR3 cause the most common genetic form of dwarfism, achondroplasia. *Cell*, **78**, 335–342.
19. Chesi, M., Nardini, E., Brents, L.A., Schrock, E., Ried, T., Kuehl, W.M. and Bergsagel, P.L. (1997) Frequent translocation t(4;14)(p16.3;q32.2) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nature Genet.*, **16**, 260–264.
20. Rabbitts, T.H. (1994) Chromosomal translocations in human cancer. *Nature*, **372**, 143–149.
21. Jousset, C., Carron, C., Boureux, A., Quang, C.T., Oury, C., Dusanter-Fourt, I., Charon, M., Levin, J., Bernard, O. and Ghysdael, J. (1997) A domain of TEL conserved in a subset of ETS proteins defines a specific oligomerization interface essential to the mitogenic properties of the TEL-PDGFRβ oncoprotein. *EMBO J.*, **16**, 69–82.
22. Carroll, M., Tomasson, M.H., Barker, G.F., Golub, T.R. and Gilliland, D.G. (1996) The TEL/platelet-derived growth factor β receptor (PDGFβR) fusion in chronic myelomonocytic leukemia is a transforming protein that self-associates and activates PDGFβR kinase-dependent signaling pathways. *Proc. Natl Acad. Sci. USA*, **93**, 14845–14850.
23. Bischof, D., Pulford, K., Mason, D.Y. and Morris, S.W. (1997) Role of the nucleophosmin (NPM) portion of the non-Hodgkin's lymphoma-associated NPM-anaplastic lymphoma kinase fusion protein in oncogenesis. *Mol. Cell. Biol.*, **17**, 2312–2325 .
24. Heldin, C.H. (1995) Dimerization of cell surface receptors in signal transduction. *Cell*, **80**, 213–223.
25. Saurin, A.J., Borden, K.L.B., Boddy, M.N. and Freemont, P.S. (1996) Does this have a familiar RING? *Trends Biochem. Sci.*, **21**, 208–214.
26. Kempski, H., Macdonald, D., Michalski, A.J., Roberts, T., Goldman, J.M., Cross, N.C.P. and Cowell, J.K. (1995) Localization of the 8;13 translocation breakpoint associated with myeloproliferative disease to a 1.5 Mbp region of chromosome 13. *Genes Chromosomes Cancer*, **12**, 283–287.
27. Fagan, K., Hyde, S. and Harrison, P. (1993) Translocation (8;13) and T-cell lymphoma. A case report. *Cancer Genet. Cytogenet.*, **65**, 71–73.
28. Kass, D.H. and Batzer, M.A. (1995) Inter-Alu polymerase chain reaction—advancements and applications. *Anal. Biochem.*, **228**, 185–193.
29. Clark, J., Rocques, P.J., Crew, A.J., Gill, S., Shipley, J., Chan, A.M., Gusterson, B.A. and Cooper, C.S. (1994) Identification of novel genes *SYT* and *SSX* involved in the t(X;18)(p11.2;q11.2) translocation found in human synovial sarcoma. *Nature Genet.*, **7**, 502–508.
30. Futreal, P.A., Cochran, C., Rosenthal, J., Miki, Y., Swenson, J., Hobbs, M., Bennett, L.M., Haugenstrano, A., Marks, J., Barrett, J.C., Tavtigian, S.V., Shattuckeidens, D., Kamb, A., Skolnick, M. and Wiseman, R.W. (1994) Isolation of a diverged homeobox gene, MOX1, from the BRCA1 region on 17q21 by solution hybrid capture. *Hum. Mol. Genet.*, **3**, 1359–1364.