Characterization of the t(4;14)(p16.3;q32) in the KMS-18 multiple myeloma cell line

TO THE EDITOR

Chromosomal translocations involving the immunoglobulin heavy chain (*IGH*) locus at chromosome 14q32 represent a frequent event in multiple myeloma (MM). We and others have identified a new recurring t(4;14)(p16.3;q32) chromosomal translocation, which involves the most telomeric region of chromosome 4 in which the *FGFR3* gene (a member of the tyrosine-kinase receptor family FGFR1–4) and the novel gene *WHSC1/MMSET* (a putative transcription factor) are located.[1–3]

Otsuki *et al*[4] have recently characterized the KMS-18 cell line established from an MM patient, which shows several numerical and structural chromosomal abnormalities including a putative, non-karyotypically detectable t(4;14)(p16.3;q32) that may involve the *FGFR3* locus. In order to characterize this lesion further, we performed a FISH analysis as we previously described.[5] Using painting probes specific for chromosomes 4 and 14, we detected two apparently normal chromosomes 4 (data not shown) and two unidentified chromosomes bearing sequences from chromosome 14 (Figure 1a1). One of the structurally altered chromosomes was recognized by the probes specific for the Cα region of the *IGH* locus and the *FGFR3* locus (cosmid clone 7.1), which colocalized in its telomeric region (Figure 1a2). In addition, the clone 7.1 recognized one of the two apparently normal chromosomes 4 (Figure 1a2). Finally, co-hybridization with the JH region and the cosmid A1 specific for the 3′ region of the *MMSET* locus revealed associated signals on the putative der(4) (Figure 1a3). These data indicate that the t(4;14)(p16.3;q32) in the KMS-18 cell line leads to a complex chromosomal rearrangement, and that the 4p16.3 breakpoint is located between *MMSET* and *FGFR3* loci.

Molecular analyses of the 4p16.3 breakpoints cloned so far indicate that they are dispersed 50–100 kb centromeric to the *FGFR3* gene and within the 5′ regions of the *MMSET* gene (Figure 2a).[1–3] In order to characterize the KMS-18 breakpoints at molecular level, we performed a Southern blot analysis based on our previously described assay.[1] Using probes specific for the *IGH* constant and joining regions, two non-comigrating rearranged DNA fragments were identified by JH and Cγ probes (Figure 1b). Since our attempt to clone the Cγ fragment was unsuccessful (probably because of the length of the fragment), we isolated the JH-rearranged DNA fragment. The restriction map and sequence analysis shown in Figure 1c indicate that the 4p16.3 breakpoint is located within intron 3 of the *MMSET* gene 402 bp upstream of exon 4,[6] and therefore involves the same region as that containing previously cloned breakpoints (Figure 2a).

The t(4;14) translocation leads to the overexpression of the *FGFR3* gene, as well as to the generation of *IGH-MMSET* hybrid transcripts, the presence of which has been proposed as a molecular marker for the translocation.[3,7] In the KMS-18 cell line we detected the expression of *FGFR3* gene as recently reported by Nakazawa *et al*,[8] and the presence of *IGH-MMSET* hybrid transcripts that lack exons 1–3 of the *MMSET* gene as expected from the MB4–2 type breakpoint (Figure 2b and c).[7] These data support the hypothesis that the two genes play a role in myelomagenesis.

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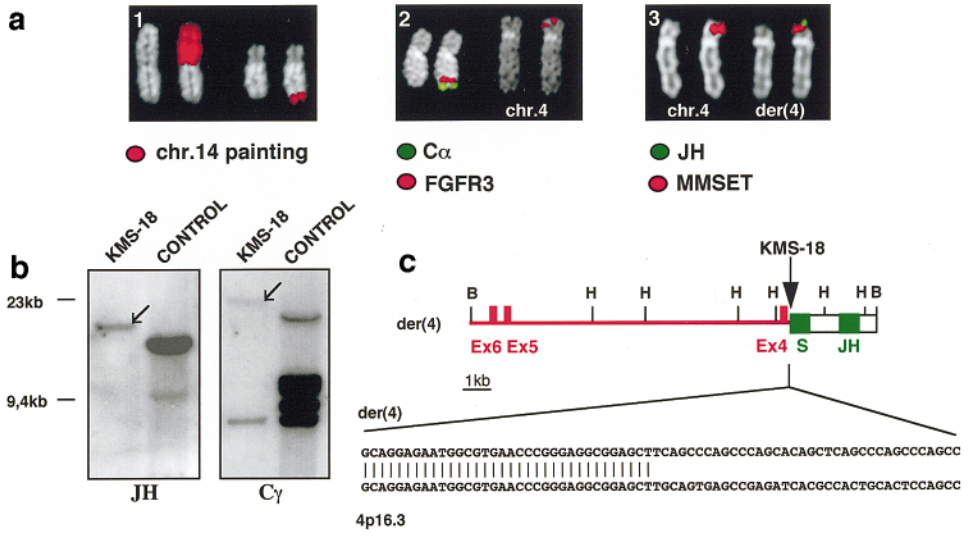

Figure 1 Cloning of the t(4;14)(p16.3;q32) chromosomal breakpoint in the KMS-18 MM cell line. (a) FISH analyses of the KMS-18 cell line; panels 1, 2 and 3 show partial KMS-18 metaphases hybridized with the probes specified below. The cosmid clones 7.1 (*FGFR3*) and A.1 (*MMSET*) were isolated from a human placenta cosmid library (Clontech, Palo Alto, CA, USA) using as probes FGFR3 cDNA and a PCR-generated fragment located about 60 kb centromeric to the 3′ end of the *MMSET* gene. DAPI counterstaining is shown for each chromosome. (b) Southern blot analysis: DNAs from KMS-18 line cell and placenta (control) were digested with the *Bam*HI restriction enzyme and hybridized first with the JH and then with the Cγ probe; aberrant DNA fragments are indicated by arrows, and the molecular weight marker is indicated in kilobases (kb). (c) Schematic representation of the cloned t(4;14)(p16.3;q32) breakpoint. Diagram of the phage clone that contains the aberrant rearranged fragment on der(4) chromosome: chromosome 14 sequences are indicated by a white box with the switch (S) and joining (JH) regions specified by green boxes; the chromosome 4 regions are shown as a solid line with red boxes representing exons 4, 5 and 6 of the *MMSET* gene. The position of the breakpoint between exon 3 and 4 of the *MMSET* gene is indicated by a vertical arrow; the restriction enzymes *Hin*dIII (H) and *Bam*HI (B) are shown. Below is the nucleotide sequence analysis of the breakpoint region in the KMS-18 cell line and its alignment with the corresponding 4p16.3 germline one. The typical IGH switch repeat sequences are underlined.

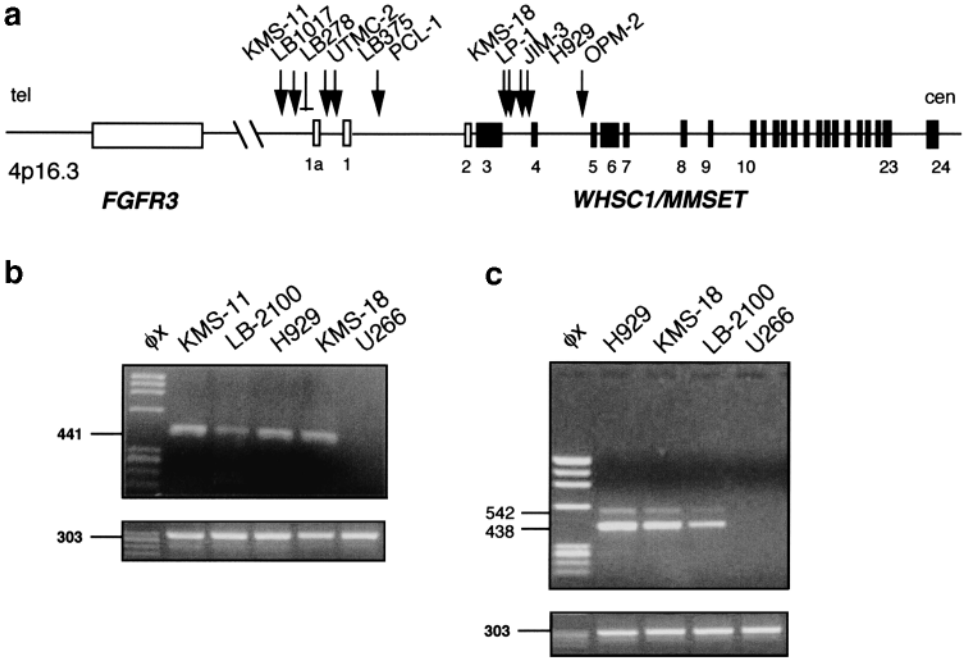

Figure 2 (a) Schematic representation of the cloned 4p16.3 MM breakpoints (indicated by arrows).[1–3] (b) RT-PCR analysis of *FGFR3* expression in MM cell lines using primers UF2 5′-TGGAGTTCCACTGCAAGGTGTA-3′ and TD3 5′-GGAGATCTTGTGCACGGGTGG-3′;[1] the length of the FGFR3 amplified fragment is indicated in bp. The U266 MM cell line was used as a negative control; case LB-2100 has been previously described.[7] (c) RT-PCR detection of the *IGH-MMSET* hybrid transcripts using primers Iμ2 and ms6r as in the previously described assay;[7] the length of the amplified fragments is indicated in bp (the one at the top contains the putative exon 4a).