

SHORT COMMUNICATION

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Assignment of the *ZIP kinase* gene to human chromosome 19p13.3 by somatic hybrid analysis and fluorescence in-situ hybridization

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Abstract A cDNA for a putative member of the serine/threonine kinase family was cloned from an adult human testis cDNA library. The predicted translation product was identical to ZIP kinase, which has been suggested to play an important role in the induction of apoptosis. The messenger RNA was ubiquitously expressed in various tissues. The chromosomal location of the gene was determined by fluorescence in-situ hybridization and polymerase chain reaction-based analyses with both a human/rodent monochromosomal hybrid cell panel and a radiation hybrid mapping panel. This gene was mapped on the q13.3 region of chromosome 19.

Introduction

We have systematically screened serine/threonine protein kinases by reverse transcription-polymerase chain reaction (PCR) amplification with degenerated primers corresponding to the conserved regions of the protein kinase domain (Saito et al. 1995), and we have identified several independent kinase genes (Saito et al. 1995,1997; Seki et al. 1997). One of them showed a striking sequence homology to ZIP kinase reported by another research group (Kawai et al. 1998; DDBJ/EMBL/GenBank accession no., AB007144). Kawai et al. (1998) reported that the ZIP kinase induced morphological changes in apoptosis in mammalian cells when overexpressed, suggesting that it plays an important

role in the induction of apoptosis. Thus, the malfunction of ZIP kinase may cause unexpected cell growth. Precise investigation of the expression profiles and chromosome mapping of ZIP kinase could be important in elucidating the role of the kinase in tumorigenesis.

Results and discussion

In the present study, chromosomal assignment of *ZIP kinase* gene was done by PCR analysis of human/rodent somatic cell hybrid panels and radiation hybrid panels. The specific amplified PCR product for human was detected only from the hybrid containing human chromosome 19 (Fig. 1a). We performed further mapping analysis using a PCR-based radiation hybrid panel (Genebridge 4; Research Genetics, Huntsville, AL, USA) with the same primers as those used in the assay for the human/rodent somatic cell hybrid panel.

Statistical analysis of the radiation hybrid data was performed using the RHMAPPER software package (<http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl>). The data vector for the *ZIP kinase* gene was 0010000001 1000000010 1000011110 0100000001 1010110100 1021010010 0000000010 1110001000 0001011000 000 and the consequent report indicated that the gene was mapped between markers WI-6480 and AFMA134XB9, both of which have been cytogenetically mapped to 19p13. The position of the gene is 8.56cR proximal from WI-6480.

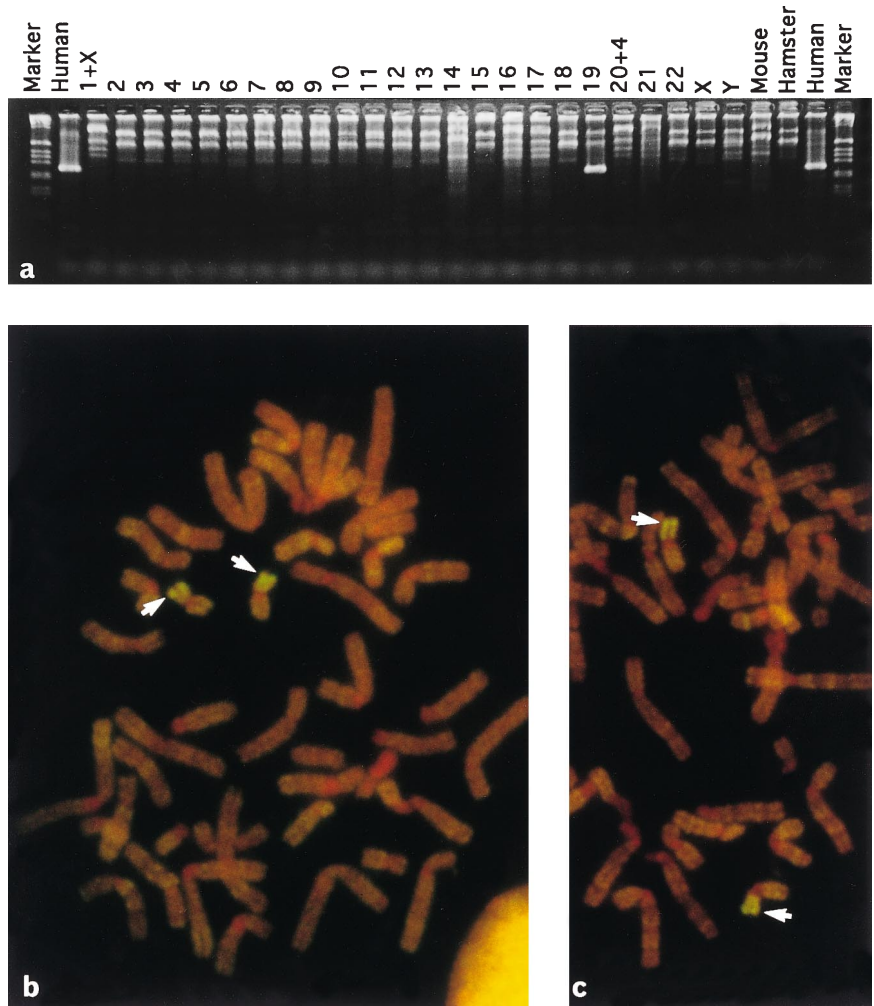
To confirm the PCR-based chromosome mapping by an independent approach, we performed R-banding fluorescence in-situ hybridization (FISH), using P1 phage DNA containing the *ZIP kinase* gene, as described previously (Saito et al. 1995,1997; Seki et al. 1997). The P1 clone was isolated by a method described previously (Ohira et al. 1997). Clear doublet signals were consistently demonstrated on the p13.3 position of chromosome 19. The typical pattern of the FISH experiment is represented in Fig. 1b,c. Thus, the gene was judged to be mapped on 19p13.3.

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Fig. 1a-c Chromosome mapping of the novel human *ZIP kinase* gene. **a** Polymerase chain reaction (PCR) analysis of human/rodent hybrid cell panels. A PCR screening of a human-rodent somatic cell hybrid panel was performed to map the *ZIP kinase* gene to human chromosome 19. DNA of the human-rodent somatic cell hybrid panel was purchased from the National Institute of General Medicine Service, Coriell Cell Repositories (Palo Alto, CA, USA). Human, mouse, and hamster genomic DNAs were also included as controls in the assay. Primers used for PCR amplification correspond to (5'-GCGGAGTGGGAGGA-GCTGGAG-3') and (5'-ACCCGCGGA-ATTGTCCGTGCA-3')(PCR product size, 260 bp). PCR was carried out in a final volume of 10 μ l containing 1 \times LA-PCR buffer (Takara, Kyoto, Japan), 2 μ M each primer, 200 μ M each dNTP, 50 ng template DNA, and 0.01 units of LA-Taq DNA polymerase (Takara). Temperature and time profiles were: 30 cycles at 95°C for 20s and at 66°C for 1 min. *Numbers at the top of each lane indicate the human chromosome contained in each somatic cell hybrid.* **b,c** Fluorescence in-situ hybridization (FISH) of the *ZIP kinase* gene. FISH was carried out using a biotinylated hybridization probe made from a P1 phage clone harboring the *ZIP kinase* gene. *Arrows indicate the hybridization signals on human chromosome 19p13.3.* The metaphase spreads were photographed with a Nikon (Tokyo, Japan) B-2A filter



We note here three candidate malignancies in which *ZIP kinase* might be involved. The genetic locus of Peutz-Jeghers (PJ) syndrome, an autosomal-dominant disorder characterized by melanocytic macules on the lips, multiple gastrointestinal hamartomatous polyps, and an increased risk of various neoplasms, was mapped to the p13.3 region of chromosome 19 by linkage analysis (Nakagawa et al. 1998; Olschwang et al. 1998). Recently, a novel serine/threonine kinase *STK11* was isolated from the region, and sequence analysis of the *STK11* revealed that germline mutations of the gene caused manifestations of PJ syndrome (Jenne et al. 1998). Adenoma malignum (AM) is one of the malignant tumors commonly associated with PJ syndrome. A loss of heterozygosity (LOH) study of a sporadic case of AM revealed that a putative tumor suppressor gene for AM was located at D19S216 on chromosome 19p13.3, a distinct region for PJ syndrome, and that the gene(s) played an important role in AM tumorigenesis (Lee et al. 1998) (candidate 1).

Structural rearrangements of chromosome 19 are also observed in pancreatic carcinomas, resulting in a high frequency of 19p13.3 losses, suggesting that the gene(s) of importance in the development of pancreatic carcinomas are located in the region (Hoglund et al. 1998) (candidate

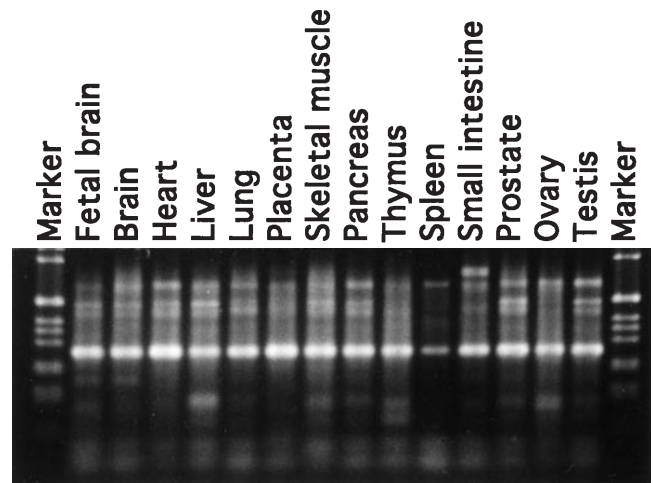


Fig. 2 Reverse transcriptase-PCR in various human tissues was performed with the same primer set as that used for the analysis shown above, and for thermal cycle profile was described for the chromosome mapping shown in *Fig. 1*. Templates of the human tissue poly(A)⁺ RNAs were purchased from Clontech (Palo Alto, CA, USA)

2). The most common nonrandom translocation of childhood pre-B acute lymphoblastic leukemia is t(1;19)(23;p13), which frequently involves the *E2A* gene at 19p13.3 (Nourse et al. 1990). A novel pre-B cell line, TS-2, has been identified with t(1;19) translocation not involving the *E2A* gene (Yoshinari et al. 1998). These findings suggest that TS-2 may have a genetic abnormality involving uncharacterized gene(s) at 19p13.3 distinct from the *E2A* gene (Yoshinari et al. 1998) (candidate 3).

Considering its ubiquitous expression in a wide variety of human tissues (Fig. 2), ZIP kinase seems to be involved in a basic housekeeping function in cells. Our precise chromosomal positioning data for the gene could contribute toward on-going positional candidate approaches for the above-mentioned disease genes linked to this genomic locus.

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