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Assignment of the Human *TIM* Proto-Oncogene to 7q33→q35

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ABSTRACT: *The human transforming gene TIM has been mapped to human chromosome 7 region q33→q35 by fluorescence in situ hybridization with R-banded chromosomes. Rearrangements within this region have been reported to occur in acute myeloid leukemia cells.*

INTRODUCTION

The *TIM* oncogene was isolated by an expression cloning strategy [1] as a cDNA clone with transforming activity in NIH/3T3 fibroblasts [2]. The 2.3-kb *TIM* cDNA encodes a predicted protein of 60-kD containing a Dbl-Homology (DH) domain [2]. The DH motif is shared by several signal transducing molecules including Bcr, Cdc24, Dbl, Vav, Ras-GRF, Ect2, Lbc, and Tiam-1 [2–6]. Because Dbl can activate Cdc42, a small GTP-binding protein of the Rho family, by guanine nucleotide exchange [7] and Dbl, and Ect2 can associate with subsets of Rho family proteins [4], these molecules are implicated as regulators of small GTP-binding proteins. Rho family proteins are known to regulate cytoskeletal organization [8]. Cdc24 and Tiam-1 are involved in yeast budding control and invasiveness of T cells, respectively [9, 6], suggesting that these molecules control cytoskeletal organization. Therefore, the *TIM* oncogene may be also involved in the control of cytoskeletal organization through regulation of small GTP-binding proteins.

Our initial studies on the characterization of *TIM* indicated that this novel gene resides on human chromosome 7 by using human–hamster somatic cell hybrids [2]. To shed further light on the potential involvement of the *TIM* gene in cancer, the sublocalization of the gene on chromosome 7 was determined by fluorescence in situ by hybridization (FISH) in the present study.

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Received July 18, 1994; accepted January 17, 1995.

MATERIALS AND METHODS

R-banded chromosomes were prepared by standard methods [10] with some modifications [11]. Peripheral blood lymphocytes from a healthy male were stimulated with phytohemagglutinin (Welcome, HA 15) and cultured in TC 199 medium. At 48 hours, thymidine (300 μg/mL, Sigma) was added to the culture. After 15.5 hours, the cells were washed and treated with bromodeoxyuridine (25 μg/mL, Sigma) for 6.5 hours. Chromosome preparation was carried out by standard methods. Slides were stained with Hoechst 33258 (1 μg/mL, Sigma), heated for 3 minutes, and exposed at 75°C for 6 minutes under a 20-W black light (Toshiba FL20SBLB). The exposed slides were rinsed in distilled water and stored at −80°C until use.

A human *TIM* cDNA (3.6 kb) was isolated from a B5/589 human epithelial cell cDNA library in λ pCEV27 by using the 2.3-kb *TIM* cDNA as a probe, and used for fluorescence in situ hybridization (FISH). The probe was labeled by nick translation with biotin-16-dUTP (Boehringer). Hybridization was carried out as reported [11, 12]. The signal amplification procedure was carried out with fluorescein avidin DCS (Vector) and biotinylated anti-avidin goat antibody (Vector) by published methods [13, 14]. The slides were stained with propidium iodide (0.5 μg/mL, Sigma). They were observed using a Nikon OPTIPHOT-2-EFD2 microscope (B-2A filter). Kodak Ektachrome film (ASA 100) was used for microphotography of chromosomes.

RESULTS

To determine the regional localization of the *TIM* proto-oncogene, fluorescence in situ hybridization was carried out on (pro)metaphase human chromosomes using a biotinylated human *TIM* cDNA as a probe. Among 50 (pro)metaphases observed, 43 cells showed symmetrical double (or more) spots on at least one homologue of chromosome 7. The region of the signals was localized to 7q33→q35 (Fig. 1). No other chromosomes showed double spot signals. Therefore, it was con-

Cancer Genet Cytogenet 83:87–89 (1995)

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0165-4608/95/$9.50
SSDI 0165-4608(95)00017-J

Figure 1 The localization of the human *TIM* proto-oncogene to human chromosome 7 region q33→q35 by fluorescence in situ hybridization (FISH) with R-banded chromosomes. Arrows indicate spots of the signals after FISH using biotinylated *TIM* cDNA as a probe. The signal might be very dim after publication.

cluded that the human *TIM* gene is located within the human chromosome 7 region q33→q35. This result was consistent with our previous localization of the gene on chromosome 7 by analyzing a panel of human-hamster somatic cell hybrids [2].

DISCUSSION

The proto-oncogene *TIM* has been mapped to human chromosome 7 region q33→q35 in this study using FISH methods. Within this chromosomal region, a chromosome anomaly, del(7) (q11–34→q22–36), has been reported in over 10 cases of acute myeloid leukemia (AML) [15]. Other genes in this region that play a role in human diseases include the human *MET* proto-oncogene (7q31 [16]), the gene for the cystic fibrosis transmembrane conductance regulator (CFTR), (7q31.2 [16]), and the *BRAF* proto-oncogene, (7q34 [17, 18]) (Takai and Yuasa, unpublished data, 7q32→q34 by FISH). Whether oncogenic activation of *TIM* or one of these other genes contributes to the development of AML remains to be determined.

We are grateful to Dr. Steven Tronick for continued support and critical reading of the manuscript.

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