Cytogenet Cell Genet 75:222–223 (1996)

Cytogenetics and Cell Genetics

Assignment of the gene for a ubiquitin-conjugating enzyme (UBE2I) to human chromosome band 16p13.3 by in situ hybridization

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Rationale and significance

We have recently cloned a cDNA encoding a protein which interacts with a transcription factor, MITF (Microphthalmia-associated transcription factor), using the yeast two-hybrid system (Iwata et al., in preparation). The predicted protein called UBE2I (GenBank: U45328) is ~60% homologous to a ubiquitin-conjugating enzyme (E2) of *Saccharomyces cerevisiae* UBC9 (Seufert et al., 1994) and that of *Schizosaccharomyces pombe* Hus5 (Al-Kohdairy et al., 1994). One of the functions of a ubiquitin-conjugating system is to degrade transcription factors such as MATα2 (Hochstrasser and Varhavsky, 1990) and Gcn4 (Kornitzer et al., 1994). MITF is a transcription factor which is involved in melanocyte differentiation (Tachibana et al., 1994, 1996). Mutations of the MITF gene appear to cause Waardenburg syndrome type 2 (WS2; MIM193510) in patients from some families (Tassabehiji et al., 1995; Nobukuni et al., 1996). Given the association between UBE2I and MITF gene products, we speculate that mutations of the UBE2I gene may cause WS2 or similar diseases. We here localized the UBE2I gene on chromosome 16 band p13.3. Among several diseases mapped to 16p13.3, the autosomal dominant congenital cataract with microphthalmia (Yokoyama et al., 1992; MIM156850) is the one most likely to involve a mutation of the UBE2I gene, since microphthalmia can be caused by mutations of the MITF homolog in mice (Steingrimsson et al., 1994). We are now analyzing the possible mutation of the UBE2I gene in an individual showing congenital cataract with microphthalmia (Yokoyama et al., 1992).

Materials and methods

Human metaphase cells were prepared from phytohemugglutinin-stimulated lymphocytes. Purified DNA from a P1 clone containing the UBE2I gene was labeled with digoxigenin-dUTP by nick translation and used as a probe. In one-color FISH, digoxigenin-labeled probe was combined with sheared human DNA, and hybridized to metaphase chromosomes in a solution containing 50% formamide, 10% dextran sulfate and 2 × SSC, and detected with fluorescein-conjugated antidigoxigenin antibodies as described previously (Stokke et al., 1995). Chromosomes were then counter-stained with propidium iodide. For two-color FISH a biotin labeled probe specific for the heterochromatic region of chromosome 16 was employed. Chromosome 16 specificity was confirmed by the cohybridization of marker D16S422 (Dr. Valentine, unpublished data). The probe was co-hybridized with UBE2I probe and detected by avidin-Texas red. Chromosomes were then counterstained by 4',6-diamidino-2-phenylindole.

Probe name: UBE2I-P1/9032
Probe type: genomic DNA
Insert size: ~85 kb
Vector: P1 phage
Proof of authenticity: DNA sequencing
Gene reference: Watanabe et al. (1996), Iwata et al. (in preparation) and this report

Results

One-color FISH resulted in specific labeling of the distal short arm of a group E chromosome. Two-color experiments resulted in the specific labeling of the short arm of chromosomes which are co-labeled with the probe specific to chromo-

Received 7 February 1996; revision accepted 29 July 1996.

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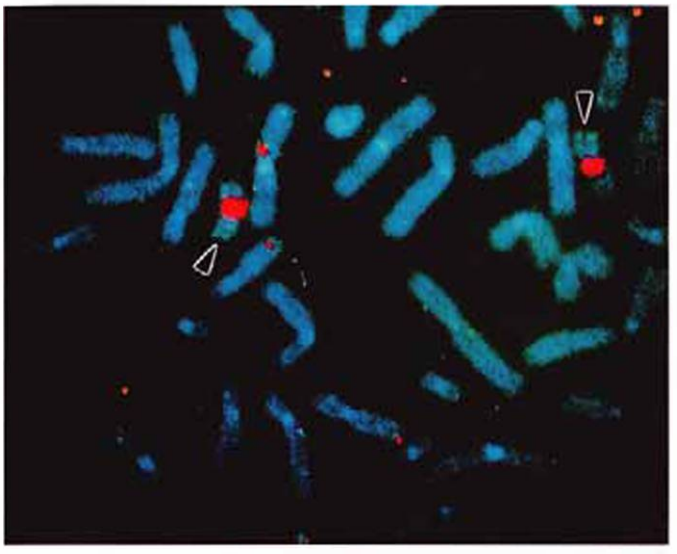

Fig. 1. In situ hybridization of digoxigenin-labeled UBE2I-P1/9032 probe to human metaphase cells resulted in specific labeling at the p13.3 region of chromosome 16 (green signals; arrow heads). Note that the chromosome is labeled with a probe specific for the heterochromatic region of chromosome 16 (pink signals).

some 16 (Fig. 1). Measurements of 10 specifically hybridized chromosome 16's demonstrated that the UBE2I gene is located at a position which is 98% of the distance from the centromere to the telomere of chromosome arm 16p, an area that corresponds to band 16p13.3.

Location: 16p13.3
No. of cells examined: 80
No. of cells with specific signals: 68
Most precise assignment: 16p13.3
Location of background signals (sites with > 2 signals): none observed

Note: During the review of the manuscript, a cDNA for UBE2I was cloned from human fetal-brain library and the gene was mapped (Watanabe et al., 1996).

Acknowledgements

We thank Dr. M. Valentine for his technical assistance and for his gift of a probe specific for the heterochromatic region of chromosome 16.

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