Chromosomal Localization of the Human *ECT2* Proto-oncogene to 3q26.1 → q26.2 by Somatic Cell Analysis and Fluorescence *in Situ* Hybridization

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The mouse *ect2* oncogene was identified in a search of mitogenic signal transducers using a novel expression cloning strategy. In this system, the introduction of an appropriate expression-cDNA library prepared from the mRNA of one cell type into another can result in the morphological transformation of the recipient cells (2, 3). The predicted Ect2 protein has sequence similarity within a central core of 255 amino acids to the products of the breakpoint-cluster gene, *bcr*, the yeast cell cycle gene, *CDC24*, the *dbl* and *vav* oncogenes, and the *RasGRF* gene (3). Baculovirus-expressed Ect2 protein shows specific binding to Rho and Rac proteins, indicating that Ect2 is a new member of transforming proteins that can interact with Rho-like proteins of the Ras superfamily. The *ect2* foci exhibit an unusual stellate morphology and are capable of forming tumors when subcutaneously inoculated in nude mice (3). These results indicate that the *ect2* gene acts as an oncogene (3). To investigate the involvement of the *ect2* in human tumors, we isolated the human homolog, *ECT2*, of mouse *ect2* and determined its chromosomal localization.

To localize the *ECT2* proto-oncogene regionally to a human chromosome, fluorescence *in situ* hybridization (FISH) was performed on metaphase chromosomes using a biotinylated human *ECT2* cDNA as a probe. Since the signals obtained by the probe were scattered throughout all chromosomes, we

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reduced the background by competition with human genomic sequences (added in hybridization solution). Among the 114 signals in a total of 40 (pro)metaphase cells photographed, 53 signals were located on chromosomal region 3q26.1 → q26.2. Thirteen cells had symmetrical double spots in this area on one homolog of chromosome 3 (Fig. 1). The size of these 53 signals was found to be the largest in each cell. The other 61 signals were scattered without any coincidence.

We also analyzed the segregation of the human *ECT2* sequence in panels of human–hamster somatic cell hybrids by PCR analysis. We designed four forward and four reverse primers from various regions of the human *ECT2* cDNA to amplify segments of 171–356 bp. Of eight combinations of the primers tested, two pairs amplified different-size fragments from human and hamster genomic DNA. One of the pairs, JL6 and JL30, was used to amplify segments of the *ECT2* gene from two subsets of the human–hamster somatic cell hybrid DNAs (BIOS, New Haven, CT). The results unambiguously localized *ECT2* to human chromosome 3 (data not shown), which is consistent with the FISH analysis. Therefore, we concluded that the human *ECT2* gene is located within the human chromosomal region 3q26.1 → q26.2.

The mouse *ect2* gene product can be activated by the N-terminal truncation, and NIH/3T3 cells transfected by activated *ect2* expression vectors are tumorigenic in nude mice (2). Since the human *ECT2* gene has a similar structure to mouse *ect2* and the truncated, but not full-length, cDNA clones possess transforming activity (Miki *et al.*, unpublished observations), it is conceivable that amino-terminal truncation of the product by chromosomal rearrangement could cause *ect2* activation and lead to tumor formation. However, the regulatory domain of the Ect2 protein is relatively small, and therefore the occurrence of such rearrangement is probably rare. Common chromosomal rearrangements in the region 3q26, where *ECT2* has been mapped, are associated with acute myeloid leukemia and myelodysplastic syndromes (5, 9). It is possible that activation of *ECT2* or a closely located unknown oncogene is responsible for these diseases. Recently, the t(3;21)(q26;q22) translocation, which is one of the consistent chromosomal abnormalities found in blastic crisis of chronic myelocytic leukemia (CML), was shown to generate the *AML1–EVI1* fusion gene (4). Thus, the human homolog of the mouse *evi-1* gene, which was originally isolated as a common site of viral integration in murine myeloid leukemias (6), is closely located to *ECT2* and rearranged in CMLs. One of the proviral integration sites in mouse myeloblastic leukemias induced by Friend murine leukemia virus has been identified and designated *Fim3* (1, 10). The human homolog, *FIM3*, of the mouse *Fim3* locus has been mapped to 3q27 (8). Although *FIM3* is separated from *ECT2*, rearrangements of the *FIM3* locus may affect *ECT2* expression.

The *ECT2* locus has been mapped to 3q26.1 → q26.2 in this study. The *ECT2* gene represents a new member of a class of oncogenes whose products are related to regulators of the Ras-related Rho subfamily of small GTP-binding proteins. The present study may provide a clue to help determine the relationship between chromosomal abnormalities and functional alterations of such oncogenes. Further studies of chromosomal alterations at this locus may clarify the role of the *ECT2* oncogene in human malignancies.

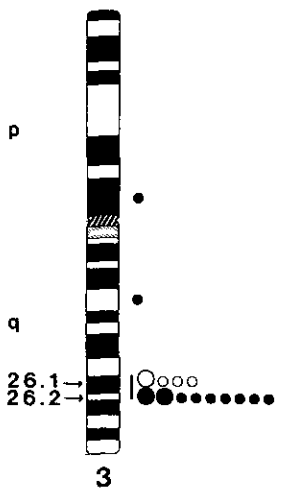

FIG. 1. Idiogram of human chromosome 3 showing the distribution of signals after fluorescence *in situ* hybridization with the biotinylated human *ECT2* cDNA probe. Open circles, symmetrical double spots; solid circles, single spot. Large circles, 10 counts; small circles, one count. Signals were counted in 40 (pro)metaphases observed. R-banded chromosomes were prepared by standard methods (13) with some modifications (11). A human full-length *ECT2* cDNA, clone 1M, was isolated from a cDNA library in λpCEV27 prepared from a normal human epithelial cell line, B5/589 (Chedid *et al.*, in preparation). The cDNA insert was isolated as two *Sal*I fragments (sizes 2.8 and 1.7 kb), and an equimolar mixture of the fragments was labeled with biotin-16–dUTP. Hybridization was carried out as reported (11, 12). The signal amplification procedure was performed by published methods (7). The slides were stained with propidium iodide (1.0 μg/ml, Sigma). They were observed under a Nikon OPTIPHOT-2-EFD2 microscope (B-2A filter). Kodak Ektachrome film (ASA 100) was used for microphotography of chromosomes.

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