BRIEF REPORTS

Assignment of a Human Melanoma Associated Gene MG50 (D2S448) to Chromosome 2p25.3 by Fluorescence *in Situ* Hybridization

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The incidence of malignant melanoma is increasing faster than that of any other cancer in the United States and worldwide, with the possible exception of lung cancer in women (1). The primary cause of this malignancy is believed to be exposure to ultraviolet radiation, although approximately 10% of cutaneous malignant melanomas (CMM) arise in familial settings suggesting heritable components (2). Extensive genetic studies, including linkage analysis, cytogenetics, and molecular analysis, provide strong evidence for involvement of at least five loci on chromosomes 1, 6, 7, and 9 (3). In a recent report, a locus involved in development of familial melanoma (MLM) was assigned to chromosome 9p13–p22 by linkage analysis (4). In addition, chromosomes 2, 3, 10, and 11 are altered in approximately 30% of all melanomas (2, 3). Clearly, many other loci also contribute to this highly complex malignancy, and identification of the specific genes involved will contribute to the understanding of melanocyte transformation and disease progression.

Human metastatic melanoma is generally resistant to chemotherapy, but is sometimes responsive to immunotherapy (5). These observations suggest that melanoma cells express genes encoding proteins recognized by cytotoxic and/or helper T-lymphocytes (T-cells). Examples of genes known to induce such a response include the "MAGE," melanoma antigen genes described by Van Der Bruggen *et al.* (6), and the melanoma markers ICAM-1 (7), GPIIb/IIIa (8, 9), and Muc-18 (10).

We have undertaken a study aimed at the identification of specific genes involved in the development of melanoma, particularly those encoding proteins or polypeptides that elicit a humoral or cellular anti-tumor immune response. We have previously reported the isolation, by molecular subtraction, of several novel gene sequences expressed in human melanoma (11). Here we report the chromosomal localization of one of these novel gene sequences, clone 50 (MG50, for melanoma associated gene 50, locus name D2S448) to 2p25.3 by fluorescence *in situ* hybridization (FISH).

D2S448 represents an interesting gene for several reasons. First, it shows a restricted pattern of expression. The 8.5-kb mRNA corresponding to this gene was detected in three of four melanoma cell lines tested, in addition to a fibroblast, a glioblastoma (U138MG), and a breast carcinoma line (734B). Expression of D2S448 was not observed in colon carcinoma lines (HT-29 and Hutu-80), a myeloid leukemia line (K562), nor a Burkitt's lymphoma cell line (Daudi) (11). Second, the nucleotide sequence of a 1.5-kb partial D2S448 cDNA clone shows no significant sequence homology to any gene currently in Gene Bank. Last, as evidenced in this report, D2S448 localizes to a chromosomal region frequently involved in CMM (2) as well as in uveal melanoma (12).

To identify the chromosomal locus encoding this gene, 1 μg of a human genomic clone G23MG50, which is 17.25-kb and contains at least 2 exons corresponding to D2S448, was labeled with biotin and hybridized to human metaphase chro-

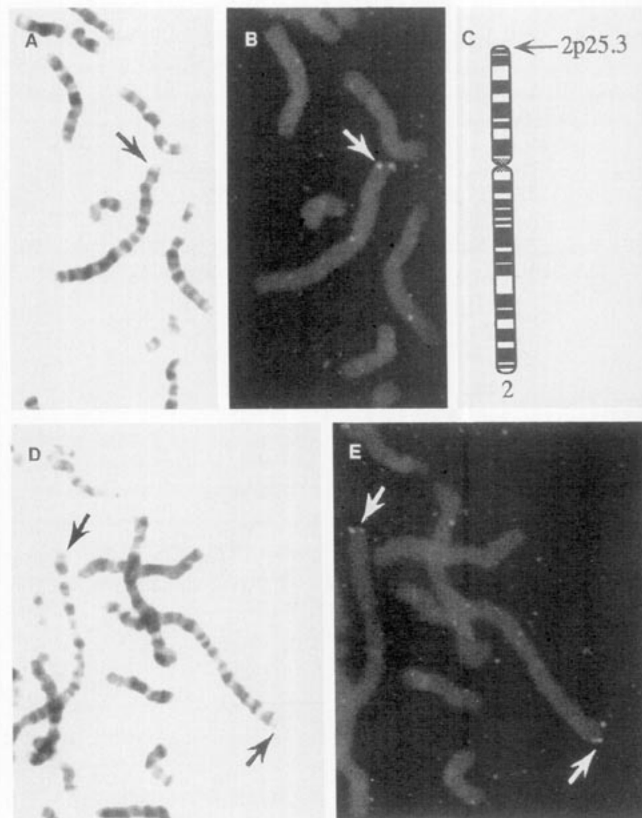

FIG. 1. Localization by FISH of the human melanoma associated gene D2S448 to 2p25.3. (**A,D**) G-banded partial metaphase chromosomes (arrows indicate chromosomes 2). (**B,E**) Identical partial metaphase chromosomes after FISH with the biotin-labeled D2S448 probe documenting the localization of the fluorescent signal to 2p25.3 (arrows). (**C**) Idiogram of chromosome 2.

The locus, probe, and primers described in this paper have been entered in the Genome Data Bank under the following Accession Nos.: locus D2S448 (G00-252-144), probe G23MG50 (G00-252-150), primers D39/D40 (G00-252-147).

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mosomes as previously described (13). A total of 44 metaphase cells were examined, and 42 of these had "double" fluorescent signals, one on each chromatid over the distal short arm of chromosome 2. All 42 cells had double signals observed on both chromosome 2 homologs. G banding of the same cells (using Wright stain) allowed direct comparison with the results obtained by FISH and confirmed that the hybridization signal was localized to 2p25.3 (Fig. 1).

Other genes associated with disorders mapping to this region are ACTH deficiency, acid phosphatase (ACP-1), thyroid iodine peroxidase deficiency, and carbamoylphosphate synthase-1 deficiency (14). However, none of these genes has been associated with the development of malignancy. At this time, the biological role of D2S448 is unknown, and there are currently no data demonstrating involvement of D2S448 in melanoma progression. However, alterations in chromosome 2 (region 2pter−q11) are common in melanoma and are considered to be late events in tumor progression as determined on the basis of cytogenetic analysis of early stage lesions, such as benign and dysplastic nevi, and on the basis of multiple tumors of the same individual consistently having the same chromosomal aberrations (2). The restricted expression pattern of D2S448 and its localization to this region of chromosome 2 suggest an involvement in melanoma, either directly in tumor progression or as a marker for late events in progression of this disease. We are currently undertaking a molecular characterization of D2S448 to determine its role in the biology and genetics of melanoma. If this gene proves to encode a melanoma associated antigen, it may lead to valuable tools for active specific immunotherapy.

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