Mapping of a Newly Discovered Human Gene Homologous to the Apoptosis Associated-Murine Mammary Protein, MFG-E8, to Chromosome 15q25

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We report here the identification and mapping of a human PAC clone containing the human homolog of the murine MFG-E8 gene (7). The murine MFG-E8 cDNA sequence encodes a 463-amino-acid polypeptide with two epidermal growth factor-like repeat domains linked to two 142-residue tandem repeated C-terminal domains. These latter domains share 42% overall amino acid identity with the C1 and C2 domains of human coagulation factors VIII and V (7) and contain a 22-amino-acid sequence with over 80% homology to a phosphatidylserine binding region in the human coagulation factors (3). Buse *et al.* (in preparation) have shown that MFG-E8 expression undergoes a 10- to 20-fold induction in mouse mammary epithelial cells concordant with the induction of apoptosis in these same cells in the mammary gland at the end of lactation. This induced apoptosis is critical for the normal process of involution, the developmental phase of breast tissue remodeling wherein epithelial cells are recognized and ingested by infiltrating macrophages and the mammary gland becomes repopulated with adipocytes and other stromal cells as it returns to the pre-pregnant state. Buse *et al.* also show that MFG-E8 has phosphatidylserine binding specificity and, at the onset of involution, is exposed as a peripheral membrane protein on the outer surface of epithelial cells destined to be ingested by macrophages. Since movement of phosphatidylserine from the inner to the outer leaflet of plasma membrane has been implicated as a signal for macrophage recognition of lymphocytes induced to enter apoptosis (2), MFG-E8 has been postulated to be an inducible marker that allows macrophage recognition of apoptotic epithelial cells.

The human homolog of murine MFG-E8 was identified in a human infant brain cDNA library (1) by a database search. This human cDNA clone has been sequenced (Wu and Parry, unpublished data) and contains coding sequence for a 387-amino-acid polypeptide of which 263 (68%) are identical or conserved matches with the mouse protein. The sequence extends 20 bases into a 3′ untranslated region. We call this cDNA huMFG-E8. Larocca *et al.* (4) have reported a partial cDNA sequence, obtained from a human breast tumor, that identically matches with hMFG-E8 from nucleotide 514 (huMFG-E8 numbering) to 1190 and then extends an additional 600 bases into a 3′ untranslated region. Larocca *et al.* (4) have also observed a mRNA corresponding to this cDNA to be significantly higher than in normal breast tissue in Northern blots of two human breast tissue carcinoma lines and a human ovary carcinoma cell line.

A single PAC clone was isolated by screening a human PAC library arrayed on high-density filters (Genome Systems). Prehybridization and hybridization were performed according to the Genome Systems protocol. A 425-bp hybridization probe was generated by amplification from huMFG-E8 using the PCR in glass capillary tubes (Idaho Technologies). PCR was performed in 10-μl volumes containing 10 m*M* Tris–HCl, pH 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 250 μg/ml BSA (Gibco-BRL), 100 μM for each dNTP, 10 pmol of each primer, and 0.5U AmpliTaq. An amplification product from the huMFG-E8 cDNA template was observed using 35 PCR cycles (94°C for 0 s; 58°C for 3 s; 72°C for 15 s). The primers used are huMFG-E8F1(5′-ggtgcatgtcaacctgtttgagac-3′) and huMFG-E8B1(5′-cagtccagttcgcacgtcattac-3′) located in the C-terminal region of the cDNA template (nucleotides 576–1002). Labeling was performed using a Rediprime random primer labeling kit (Amersham) and unincorporated $\alpha[^{32}P]$-dCTP was removed by using ProbeQuant G-50 microcolumns (Pharmacia Biotech). Hybridization was performed for 24 h followed by a posthybridization wash performed according to the Genome Systems protocol; autoradiography was performed for 3 days. The single positive clone was ordered from Genome Systems and confirmed by Southern blot hybridization with the same probe (data not shown).

The huMFG-E8 PAC clone was localized on human target chromosomes by fluorescence *in situ* hybridization (FISH) as described elsewhere (6). Briefly, DNA was extracted from an overnight culture using a modified alkaline lysis method (5). The DNA was then labeled with digoxigenin–dUTP (Boehringer-Mannheim) by nick-translation using the DNase-polymerase mix from the BioNick Labeling System (BRL Life Technology). Labeled probe was then hybridized to slides containing human metaphase chromosomes. Hybridized signal was detected with FITC–anti-digoxigenin antibody (Boehringer-Mannheim). The location of the hybridization signal on chromosome 15 band q25 was determined by DAPI banding and the fractional length measurement Flpter 0.86 was determined by digital imaging microscopy (8). The mapping data for the huMFG-E8 clone may be viewed at the Lawrence Berkeley National Laboratory, Resource for Molecular Cytogenetics home page at http://www-rmc.lbl.gov.

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Mapping of the Ras-GRF2 Gene (GRF2) to Mouse Chromosome 13C3-D1 and Human Chromosome 5q13, near the Ras-GAP Gene

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The ras proto-oncogenes encode guanine nucleotide binding proteins that are key transducers of signals essential for cell growth, differentiation, and survival (1, 16). Mutations that lead to chronic Ras activation are prevalent in a wide range of human tumors (2). Extracellular signals influence Ras activity through two classes of regulators: guanine nucleotide exchange factors (GEFs), which activate Ras by catalyzing the exchange of GDP for GTP, and GTPase activating proteins (GAPs), which stimulate Ras' intrinsic GTPase activity to return Ras to its inactive GDP-bound state (1). We recently reported the cloning of mouse cDNAs encoding Ras-GRF2, a widely expressed protein that functions as a specific GEF for Ras proteins (7, 9). Ras-GRF2 contains a catalytic domain similar to the Ras-specific exchange factor encoded by the budding yeast gene *CDC25* (4), and it also has an IQ motif, two pleckstrin homology (PH) domains, and a single Dbl homology (DH) domain (9). The primary structure of Ras-GRF2 is most similar to Ras-GRF1 (6, 20, 21), which was originally called Cdc25Mm (17) and which has been mapped to mouse chromosome 9 (11) and human chromosome 15q24 (19). Ras-GRF2 mediates Ras-dependent activation of the mitogen-activated protein kinase ERK1 in response to calcium ionophore treatment of cells and is associated with the calcium sensor calmodulin (9). Overexpression of Ras-GRF2 in human kidney epithelial cells causes morphological transformation characterized by decreased intercellular contacts suggesting that Ras-GRF2 may be encoded by a proto-oncogene and involved in human cancers (9). Here we report the mapping of the Ras-GRF2 gene locus in humans and mice by fluorescence *in situ* hybridization (FISH).

Using a mouse Ras-GRF2 cDNA (9) as a probe, mouse genomic Ras-GRF2 clones were isolated from a Lambda DASH library. To determine the Ras-GRF2 chromosomal localization in mice, a 9-kb genomic Ras-GRF2 clone was labeled with biotin-11–dATP using the BioNick labeling kit (GIBCO-BRL). The FISH procedure was performed on mouse lymphocyte chromosomes prepared as described in Fang *et al.* (10). Briefly, chromosome slides were baked at 55°C for 1 h. After RNase I treatment, slides were denatured in 70% formamide and 10% dextran sulfate and mouse cot I DNA and prehybridized for 15 min at 37°C. The probe was then loaded on the denatured slides and hybridized overnight. Slides were subsequently washed and detected as well as amplified as described (12). FISH signals (Fig. 1c) and the DAPI banding pattern (Fig. 1d) were photographed separately, and assignment of the Ras-GRF2 gene locus was achieved by superimposing FISH signals with DAPI banded chromosomes (13).

Ninety-one of 100 murine metaphase cells examined (91%) showed signals on both chromatids of one or both chromosome 13 homologues, which were identified by the DAPI banding pattern. Ten mitotic cells were photographed and all of the signals scored within bands 13C3 to 13D1, as shown in Figs. 1c and 1d.

To determine the human Ras-GRF2 locus, a murine Ras-GRF2 cDNA was used as a probe to isolate human genomic Ras-GRF2 clones from a P1 artificial chromosome (PAC) library. Human FISH mapping with normal human lymphocyte chromosomes counterstained with propidium iodide and DAPI was performed essentially as described above, except that the slides were not baked and the probe, not the slides, was preannealed with cot-1 DNA following denaturation in 70% formamide at 75°C. Biotinylated probe was detected with avidin–fluorescein isothiocyanate (FITC). Images of meta-

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