SHORT COMMUNICATION

DLG1: Chromosome Location of the Closest Human Homologue of the *Drosophila Discs Large* Tumor Suppressor Gene

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The Drosophila discs large tumor suppressor protein, Dlg, is the prototype of a newly discovered family of proteins termed MAGUKs (membrane-associated guanylate kinase homologues). MAGUKs are localized at the membrane–cytoskeleton interface, usually at cell–cell junctions, where they appear to have both structural and signaling roles. They contain several distinct domains, including a modified guanylate kinase domain, an SH3 motif, and one or three copies of the DHR (GLGF/PDZ) domain. Recessive lethal mutations in the *discs large* tumor suppressor gene interfere with the formation of septate junctions (thought to be the arthropod equivalent of tight junctions) between epithelial cells, and they cause neoplastic overgrowth of imaginal discs, suggesting a role for cell junctions in proliferation control. A homologue of the Dlg protein, named Hdlg, has been isolated from human B lymphocytes. It shows 65–79% identity to Dlg in the different domains, and it binds to the cytoskeletal protein 4.1. Here, we report that the gene for lymphocyte Hdlg, named DLG1, is located at chromosome band 3q29. This finding identifies a novel site for a candidate tumor suppressor on chromosome 3.

Genetic work in *Drosophila* has led to the identification of over 50 genes in which mutations lead to loss of cell proliferation control and which are therefore considered tumor suppressor genes (TSGs) (6, 28). At least 22 of these genes have been cloned and the coding region sequenced. Most of them have clear mammalian homologues, and in many cases the sequence similarity is high enough to suggest the possibility that the mammalian homologue may also function as a tumor suppressor.

Mutations in *Drosophila* TSGs are generally recessive lethals that cause overgrowth of specific tissues at certain developmental stages. The mutant phenotypes include overgrowth of the central nervous system of the embryo or larva, of the imaginal discs, of the hematopoietic system, and of the gonads (28). Here, we are concerned with the human homologue of the *lethal(1)-discs large* (*dlg*) tumor suppressor gene. Mutations in *dlg* cause the imaginal discs to overgrow, lose their epithelial structure, and lose their ability to differentiate even after transplantation into a wildtype larval host. The *dlg* gene encodes a 960-amino-acid protein (Dlg) that is expressed in most epithelial tissues, including imaginal discs, as well as in other tissues throughout development (30, 31). The carboxyl-terminal 179 amino acids of Dlg show strong homology (35.5% identity) to yeast guanylate kinase (GUK) (1), an enzyme that transfers a phosphate group from ATP to GMP, converting it to GDP. Dlg also contains the 59-amino-acid SH3 domain (22), which is found in many membrane-associated signal transduction proteins and which mediates binding to other proteins, including GTPase-activating protein (25). The N-terminal half of the molecule contains three copies of a newly identified 80- to 90-amino-acid motif called DHR/GLGF/PDZ (4), which has now been identified in a diverse group of proteins (24). The DHR domain appears to mediate the binding of these proteins to the plasma membrane (2, 10, 13, 27).

In epithelial cells, Dlg is localized in an apical belt of the lateral cell membrane at the position of a specialized structure, called the septate junction, that connects cell neighbors (31). The role of these junctions is unknown, although they have often been considered the invertebrate equivalent of vertebrate tight junctions (23). Dlg is required for septate junction structure, as shown by the absence or reduction of septate junctions in epithelial cells of *dlg* mutant larvae (D. F. Woods and P. J. Bryant, unpublished). The analysis of this gene suggests that cell interactions important for growth control occur at septate junctions, and the presence of a guanylate kinase-homologous domain suggests that the interactions may involve guanine nucleo-

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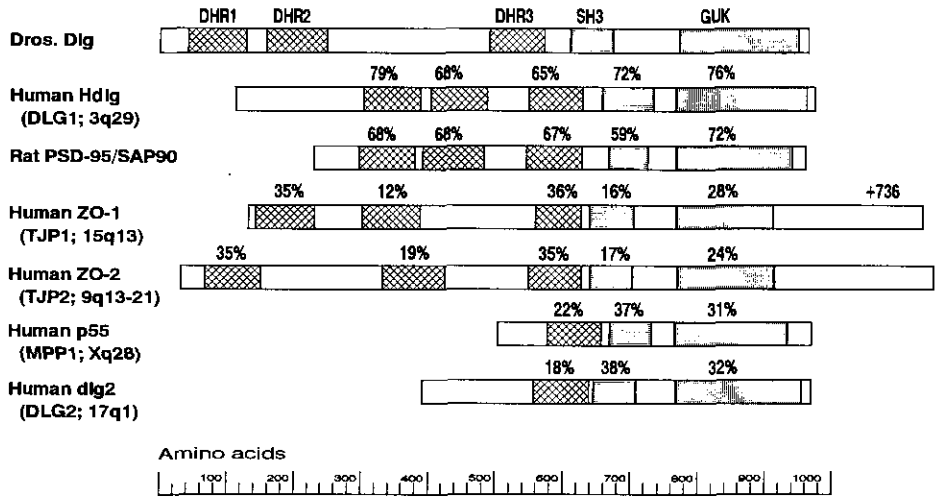

FIG. 1. Domain structure and amino acid sequence similarities between members of the MAGUK family. Drosophila DlgA (31) is compared with human Hdlg (13); rat PSD-95/SAP90 (4, 11); human ZO-1 (29), which extends 736 amino acids to the right; human ZO-2 (9); human p55 (26); and human dlg2 (16). The chromosome positions are indicated below the gene names. DHR1, DHR2, DHR3, discs-large-homologous regions (24, also designated GLGF or PDZ); SH3, src-homology region 3 (22); GUK, guanylate kinase-homologous region. Numbers outside the boxes indicate percentage of amino acid identity compared with the corresponding domain of the *Drosophila* sequence.

tides that could act as messenger molecules within the cell.

Six genes with similar domain structure and significant sequence similarity to *dlg* have been found in mammals (Fig. 1). These are the genes encoding the membrane-associated human lymphocyte protein Hdlg (13), homologous to rat SAP97 (19), PSD-95/SAP90, a protein component of synaptic junctions (4, 11); ZO-1 and ZO-2, major protein components of tight junctions (5, 8, 9, 18, 29); p55, which is the major palmitoylated protein of the erythrocyte membrane cytoskeleton (26); and dlg2, a protein similar to p55 but of unknown function (16). Four of the human membrane-associated guanylate kinase homologues (MAGUKs) (Hdlg, PSD-95/SAP90, ZO-1, and ZO-2) have a domain structure similar to Dlg, including three DHRs, an SH3, and a GUK domain. In these proteins as well as in Dlg, the putative ATP-binding site of the GUK domain has a 3-amino-acid deficiency (32), suggesting that the catalytic function has been modified during evolution. The other two family members (p55 and dlg2) include GUK and SH3 domains but contain only one DHR domain and do not have a deficiency in the ATP-binding site of the GUK domain. Based on domain organization as well as detailed sequence comparison, the closest mammalian homologue to Dlg is Hdlg/SAP97 (13, 19) (Fig. 1). The human homologue Hdlg is expressed in a variety of cell types including epithelia, where it is localized at the cell membrane at regions of cell–cell contact (13). Its similar structure and subcellular localization are consistent with a function similar to that of *Drosophila* Dlg. The rat protein, SAP97, is also expressed in a variety of tissues, including brain, heart, muscle, liver, lung, intestine, and testis. It is found in synaptic junctions as well as along the basolateral surfaces of cultured epithelial cells in the intestine and choroid plexus (19). Like the previously identified human MAGUK p55, Hdlg binds directly to the membrane cytoskeletal protein 4.1 (13). The presence of Hdlg isoforms with and without the protein 4.1 binding domain suggests that the tissue-specific cytoskeletal interactions of Hdlg may be regulated by alternative splicing of its transcripts (13).

In this paper, we report the chromosome position of the human gene encoding Hdlg, named DLG1. The chromosomal assignment was determined by hybridization of a somatic cell hybrid panel containing DNA from defined human chromosomes. The somatic cell hybrid panel was obtained from BIOS Inc. The mapping panel consists of DNA isolated from 24 human/rodent somatic cell hybrids retaining one or two human chromosomes. All but two of the hybrids retain a single intact human chromosome. The blot was hybridized with a 1.0-kb cDNA probe specific for human lymphocyte Hdlg (13). The cDNA probe, which corresponds to the three DHR domains in Hdlg (13), hybridized with DNA fragments produced from the hybrid containing human chromosome 3. The hybridizing bands were of a mobility similar to those detected from the *Pst*I digestion of the human genomic DNA (data reviewed but not shown). These results indicate that the DLG1 gene is located on human chromosome 3. The subchromosomal localization of the DLG1 gene was determined by fluorescence *in situ* hybridization. Using PCR primers specific for the DLG1 gene (P7 sense primer: 5′-CAACAGAAGCTGTTCTTC; P8 antisense primer: 5′-CTGTGGTATGGTGGGTAG), two P1 clones were isolated. The identity of the P1 clone 3131 was established by Southern blot analysis (Fig. 2A). The DNA from an 80-kb P1 clone 3131 was purified, biotinylated, and used to detect the Hdlg gene. Ten metaphase cells with single or double chromatid hybridizations were examined.

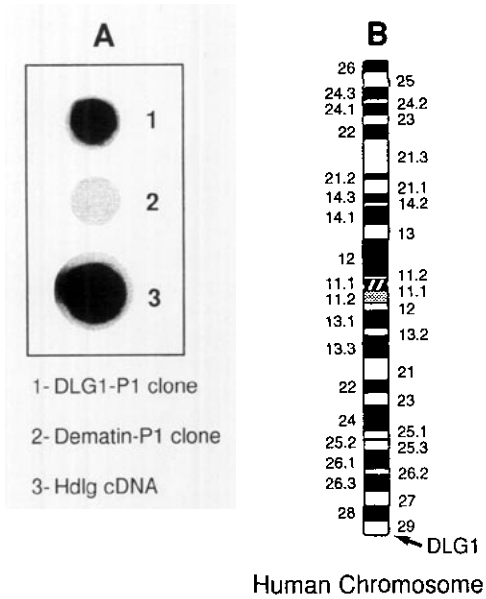

FIG. 2. The subchromosomal localization of the DLG1 gene to 3q29. (**A**) Identity of the DLG1 genomic P1 clone (3131) was confirmed by Southern blot analysis. All three DNA spots were hybridized with a full-length cDNA probe specific for human lymphocyte Hdlg (13). (**B**) Idiogram of human chromosome 3 showing the location of the P1 genomic clone 3131 at 3q29.

All revealed hybridization on the long arm of chromosome 3 (Fig. 2B). By simultaneous viewing of the hybridization signals with DAPI-banded chromosomes, P1 clone 3131 mapped to 3q29 (data reviewed but not shown).

A search of the literature indicated that no tumor suppressor genes have been localized to chromosome band 3q29 (14). The location of the DLG1 gene on the 3q29 region, therefore, identifies a novel site for a candidate tumor suppressor gene. In a subset of patients with Philadelphia-positive chronic myeloid leukemia, chromosomes 3 and 21 undergo reciprocal translocation (3q26.2 and 21q22.2) (3, 7). Although the transferrin receptor, which is located on 3q29, is translocated in these patients, no structural alterations in the primary structure of the transferrin receptor were observed as a consequence of translocation (12). The identification of the DLG1 locus on 3q29 suggests that the structural integrity of the DLG1 gene should be examined in these patients. Similarly, a significant loss of heterozygosity on chromosome arm 3q has been observed previously in several cancers, including cervical carcinoma (17), male germ tumors (21), osteosarcomas (33), multiple endocrine neoplasia type 2 (20), and thyroid tumors (15). Whether the DLG1 gene plays any role in the progression of these cancers remains to be tested.

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