Isolation and Characterization of a Novel Human Lung-specific Gene Homologous to Lysosomal Membrane Glycoproteins 1 and 2: Significantly Increased Expression in Cancers of Various Tissues

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Abstract

We have isolated and characterized a novel human lung-specific gene and observed its increased expression in cancers arising from various tissues. The cDNA, designated TSC403, contained an open reading frame of 1248 nucleotides encoding 416 amino acids; the deduced amino acid sequence showed significant similarities to lysosomal membrane glycoproteins (lamps) 1 and 2. We localized the gene to chromosomal band 3q27, a genomic region that is often amplified in human cancers of several tissue types. We detected a high level of the TSC403 transcript in primary cancers of the esophagus, colon, fallopian tube, ovary, breast, and liver, although expression of this gene was barely detectable in corresponding normal tissues. These findings indicated that up-regulation of the TSC403 transcript may be related to the development and/or progression of cancer in humans.

Introduction

Cancer is one of the most common causes of death worldwide. Development of carcinoma is a multistage process involving stepwise accumulation of somatic alterations that usually include activation of oncogenes and/or inactivation of tumor suppressor genes. A major goal in cancer research is to identify genes whose alterations lead to tumorigenesis and to exploit that information for establishing useful diagnostic tools and effective therapies. Lamp-1 and lamp-2 are lysosomal membrane glycoproteins whose "sialyl-Lex" (sialosilated Lewis" antigen) termini, specific to granulocytes and monocytes (3, 4), serve as ligands for the protein-tyrosine kinase, p160ROCK, which is often amplified in several types of human breast carcinomas. We have observed that lamp-1 and -2 may, therefore, promote or facilitate the metastatic process.

Here, we report isolation of a novel human lung-specific gene, TSC403, which encodes a protein significantly similar to lamps 1 and 2, and document significantly increased expression of this gene in human cancers of several types.

Materials and Methods

Differential Display. The differential display procedure was performed essentially as described by Liang and Pardee (14). Subcloning of amplified cDNA fragments was described previously (15). Nucleotide sequences were determined by means of an ABI 377 auto-sequencer (Applied Biosystems).

Northern Blot Analysis. Human Multiple-tissue Northern blots I and II (Clontech Laboratories, Inc.) were probed with a cDNA fragment (TSC403) obtained from the differential display, which had been labeled with α-[32P]dCTP by the random labeling kit (Boehringer Mannheim). The membranes were prehybridized and then hybridized according to the manufacturer's protocol. Washed membranes were autoradiographed for 16 h at -80°C.

Screening of cDNA. A human normal lung cDNA library was constructed using oligo(dT)-primed normal human lung cDNA and Uni-ZAP[TM]XR. In total, 1 X 10⁶ clones were screened with the α-[32P]dCTP-labeled cDNA fragment (TSC403). Positive clones were selected and their insert DNAs were excised in vivo in pBlueScript II SK(-), according to the supplier's recommendation.

Radiation-hybrid Mapping. Using PCR primers generated from the 3'-UTR of the TSC403 cDNA, we tested the amplified product against the Genbridge-4 whole-genome radiation hybrid panel (Research Genetics, Huntsville, AL; Ref. 16). The primer sequences were as follows: forward, TCTGCACTTCATATCC; reverse, AGACTAAAGTCAGGACCTTG. The amplification results were submitted to the Radiation Hybrid Mapper server at the Whitehead Institute/Massachusetts Institute of Technology Center for Genome Research(http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl).

Mapping by FISH.2 FISH for chromosomal assignment was performed as described elsewhere (17), using as the probe 0.5 μg of cosmid DNA. FISH image was captured with a CCD camera system (CytoVision; Applied Imaging).

Northern Blot Analysis of TSC403 mRNA in Cancer Tissues. Human breast cancer and lung cancer tissue samples were purchased from In vitroGen. The probe consisted of the coding region of TSC403 cDNA labeled with α-[32P]dCTP (Boehringer Mannheim). The membranes were prehybridized and then hybridized according to the manufacturer's protocol. Washed membranes were autoradiographed for 24 h at -80°C.

Human Tissue Samples. Fresh surgical specimens of 10 esophagus and 3
Fig. 1. Expression of TSC403 in normal human tissues, using as a probe the lung-specific cDNA fragment isolated by differential display. β-actin served as a control. PBL, peripheral blood lymphocytes.

breast cancers, and their adjacent normal mucosae, were obtained from patients undergoing surgery. The materials, obtained immediately after the surgical procedure, were frozen in liquid nitrogen and stored at -80°C.

RT-PCR Analysis of TSC403 mRNA in Esophageal Carcinomas and Breast Cancer. Total RNA was isolated from resected specimens by TRIZOL (Life Technologies, Inc.). cDNA was prepared from 2 μg of total RNA by dT<sub>15</sub> priming and synthesized the same as in the differential display experiments. The levels of TSC403 mRNA expression were analyzed by RT-PCR in normal tissues and primary cancers. Glyceraldehyde-3-phosphate dehydrogenase levels were measured as a control.

### Table 1: Amino Acid Sequence Alignment of TSC403, Lamp-1, and Lamp-2

<table>
<thead>
<tr>
<th>TSC403</th>
<th>Lamp-1</th>
<th>Lamp-2</th>
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<tbody>
<tr>
<td><strong>Alignment</strong></td>
<td><strong>Alignment</strong></td>
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<tr>
<td>AARFDDGHIGFQTAANIKPTTTPATKNTATT97</td>
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<td>98</td>
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<td>88</td>
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Fig. 2. Alignment of amino acid sequences of TSC403 and lamps 1 and 2. Identities are indicated by gray background.
Results

Isolation of Full-length TSC403 cDNA and Characterization of Predicted Protein. We compared differential display patterns using mRNAs isolated from human brain, lung, liver, pancreas, stomach, spleen, breast, prostate, heart, skeletal muscle, kidney, and visceral fat, and identified a PCR product (TSC403) that was expressed specifically in the lung. This PCR product was subcloned and sequenced. The TSC403 fragment showed no significant homology to any sequence registered in the GenBank or European Molecular Biology Laboratory databases (releases 104.0 and 52.0, respectively). To confirm the expression pattern revealed in the differential display experiment, we performed Northern blot analysis using the TSC403 fragment as a probe. As shown in Fig. 1, a transcript of approximately 3.3 kb corresponding to TSC403 was observed specifically in the lung, although very weak signals were detected in the testis and spleen after 48 h of exposure (data not shown). To isolate the full-length cDNA, we screened a lung cDNA library using the fragment isolated through the differential display experiment as a probe, and isolated a clone containing an insert of approximately 3.0 kb. The full cDNA consisted of 3126 nucleotides, including 1248 nucleotides of open reading frame (GenBank accession No. AB013924), which would encode a protein of 416 amino acids with a calculated molecular weight of Mr 44,315,000. The predicted TSC403 protein contained multiple sites for N-glycosylation, a possible hydrophobic signal sequence near the NH2 terminus, and one possible transmembrane domain near the COOH terminus. A comparison with known proteins in the public databases using the FASTA program identified significant similarities to lamp-1 (30% identity) and lamp-2 (28% identity; Fig. 2; Ref. 18). Hence, this gene product may be a new member of the molecular family of lysosomal membrane glycoproteins.

Chromosomal Localization of the TSC403 Gene. To determine the chromosomal location of the novel gene, a FISH experiment was carried out using as the probe a cosmid clone containing the TSC403 cDNA sequence. Among 100 typical R-banded (pro) metaphase spreads examined, the signals were localized specifically to band q27 of chromosome 3 (Fig. 3). Radiation-hybrid mapping also localized TSC403 to chromosome 3, near marker WI-6365 (lod score >3), a site consistent with the location defined by FISH.

Up-regulation of TSC403 mRNA in Human Cancers. From its structural similarity to lamps and its chromosomal localization, we considered that TSC403 might be involved in carcinogenesis. To examine whether TSC403 expression is altered in human cancers, we performed Northern blot analysis using RNAs isolated from cancers that had arisen in several different tissues. Fig. 4 shows that expression of TSC403 mRNA was significantly up-regulated in carcinomas of the esophagus, colon, rectum, urater, stomach, breast, fallopian tube, thyroid, and parotid tissues. Expression of TSC403 mRNA also was up-regulated in three of four additional colon cancers, in two of four liver cancers, and in two of four ovarian cancers examined (Fig. 4). In addition, RT-PCR experiments detected TSC403 transcript in 6 of 10 primary esophageal carcinomas and 2 of 3 primary breast cancers, although expression was barely detectable in corresponding normal tissues from the same patients.

Discussion

A cascade of cellular, biochemical, and genetic events occur during the development and progression of solid tumors, leading to malignancy and, ultimately, to metastasis. Discoveries of genetic alterations in numerous oncogenes and tumor suppressor genes have stimulated the search for additional genes that may promote or suppress cell growth, the spread of cancer cells, and/or metastasis.

We have described here the isolation and characterization of a novel lung-specific gene that bears significant similarity to lysosomal membrane glycoproteins (lamps). Lamp-1 and lamp-2, found in a variety of cell types both normal and malignant, have been implicated in the regulation of cell growth, in cell adhesion, and in metastasis. Although lamps are located primarily in lysosomes, they are also constitutively expressed at the surfaces of platelets, T and B lymphocytes, monocytes, and several types of cancer cells, especially those with highly metastatic potential (2, 19). An increase of lamp-1 on cell surfaces significantly increases adhesion of colon cancer cells to endothelial cells, but this adhesion can be inhibited by soluble lamp-1 (13). Being ligands for E-selectin, lamps may promote metastasis by helping cancer cells bind to endothelial cells. Structural similarities suggest that TSC403 plays similar roles (i.e., cell-to-cell adhesion, regulation of cell growth, and cancer metastasis).

In some of the primary cancer samples we examined, TSC403 expression was significantly up-regulated, and our results support the view that increased expression of TSC403 may have a critical role in the development and/or progression of several types of cancer. Considering its structural similarity to lamps, TSC403 protein at the surface of cancer cells may mediate their binding to endothelial cells at the sites of metastasis. It will be interesting to determine whether increased expression of TSC403 protein at the cell surface does, in fact, lead to a higher incidence of metastasis. In view of our assignment of TSC403 to chromosomal band 3q27, a region that is often amplified in cancers of various tissue origins (20–22), TSC403 may be a target gene whose expression is up-regulated by those amplification events. Further studies are needed to substantiate correlations between aberrant TSC403 expression and various stages of carcinogenesis.

The biological function of the novel gene product reported here is unknown. We observed its expression specifically in the lung among the normal human tissues examined, but we found elevated expression of TSC403 in a large proportion of cancerous tissues whose normal counterparts expressed hardly detectable amounts of the product. Because no RNA materials from lung cancers were available to us, we were unable to compare the expression of TSC403 in normal versus cancerous lung tissue. Its tissue specificity indicates that this protein is likely to play an important role in the development and/or maintenance of normal structure and function of the lung. This type of activity may imply some advantage for progression of cancer cells when the gene product appears on cell surfaces in other tissues.
Fig. 4. A, expression of TSC403 in human cancers and corresponding normal tissues. The coding region of TSC403 cDNA served as the probe for Northern blotting. Arrows, TSC403 transcript. B, expression of TSC403 mRNA in 10 primary esophageal carcinomas and 3 primary breast cancers (C), and in corresponding normal tissues (N). Glyceraldehyde-3-phosphate dehydrogenase levels were measured as a control.
Acknowledgments

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References


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