The Tumor Suppressor Protein TSLC1 Is Involved in Cell-Cell Adhesion*

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**| The abbreviations used are: NCAM, neural cell adhesion molecule; BS3, bis(sulfosuccinimidyl) suberate; CEA, carcinoembryonic antigen; DIC, differential interference contrast; GFP, green fluorescent protein; HA, hemagglutinin; HBSS, Hanks' balanced salt solution; HEK, human embryonic kidney; Ig, immunoglobulin; IgCAMs, immunoglobulin superfamily cell adhesion molecules; MDCK, Madin-Darby canine kidney; PBS, phosphate-buffered saline; FCS, fetal calf serum.

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TSLC1 is a tumor suppressor gene encoding a member of the immunoglobulin (Ig) superfamily. The significant homology of its extracellular domain with those of other Ig superfamily cell adhesion molecules (IgCAMs) has raised the possibility that TSLC1 participates in cell-cell interactions. In this study, the physiological properties of TSLC1 were investigated in Madin-Darby canine kidney (MDCK) cells expressing TSLC1 tagged with green fluorescent protein (GFP) as well as in the cells that express endogenous TSLC1. Biochemical analysis has revealed that TSLC1 is an N-linked glycoprotein with a molecular mass of 75 kDa and that it forms homodimers through cis interaction within the plane of the cell membranes. Confocal laser scanning microscopy of the cells expressing TSLC1 showed the localization patterns characteristic to adhesion molecules. At the beginning of cell attachment, TSLC1 accumulated in interdigitated structures at cell-cell boundaries, but, when cells reached a confluence, TSLC1 was distributed all along the cell membranes. In polarized cells, TSLC1 was recruited to the lateral membrane, implying trans interaction of TSLC1 between neighboring cells. In support of this notion, MDCK cells expressing TSLC1-GFP showed a significant level of cell aggregation in the absence or presence of Ca\(^{2+}\) and Mg\(^{2+}\). Taken together, these results indicate that TSLC1 mediates intracellular adhesion through homophilic interactions in a Ca\(^{2+}/\)Mg\(^{2+}\)-independent manner.

We have recently identified a tumor suppressor gene TSLC1 on chromosome 11q23.2 by functional complementation of a human lung cancer cell line, A549, through suppression of tumorigenicity in nude mice (1). Furthermore, we have demonstrated the two-hit inactivation of TSLC1 in primary non-small cell lung cancer, hepatocellular carcinoma, and pancreatic cancer, implying its involvement in various human cancers (1). TSLC1 encodes a member of the immunoglobulin superfamily proteins comprising three Ig-like C2-type domains, a single hydrophobic membrane-spanning a-helix, and a cytoplasmic domain containing a putative signaling motif (1). From the significant homology of its extracellular domain with those of NCAM1 and NCAM2, we have inferred that TSLC1 is capable of mediating cell-cell interaction.

Cell adhesion molecules generally fall into four major classes: the cadherins, the integrins, the selectins, and the Ig superfamily. Among them, Ig superfamily cell adhesion molecules (IgCAMs) are the largest, numbering well over 100 members in vertebrates (2). These well-characterized molecules include NCAMs (3), L1 family CAMs (4), and nectins (5–8). Whereas cadherins and integrins require divalent cations such as Ca\(^{2+}\) or Mg\(^{2+}\) for their adhesive activities (9), IgCAMs are usually Ca\(^{2+}\)- or Mg\(^{2+}\)-independent (10). Moreover, most IgCAMs have preferences for homophilic and/or heterophilic interactions (10). In combination with these interactions, IgCAMs promote a variety of cell-cell associations through cis interaction within the plane of the membranes and/or trans interaction across the membranes (2, 11). For instance, the heterophilic cis interaction between L1 and NCAM appears to enhance the homophilic trans-binding activity of L1 (12, 13). Nectins form cis-heterodimers that undergo homophilic and heterophilic trans interactions with each other to mediate cell-cell adhesion (6, 11).

In this study, the biochemical properties and subcellular localization of TSLC1 were investigated in the cells expressing TSLC1 tagged with GFP or endogenous TSLC1. We report the physiological properties of TSLC1 along with several lines of evidence that TSLC1 is a single transmembrane glycoprotein involved in cell-cell aggregation through homophilic trans interaction.

EXPERIMENTAL PROCEDURES

Cells—Human embryonic kidney (HEK) 293 (JCRB9068) and MDCK cells (JCRB9029) were obtained from the Human Research Resources Bank (Osaka, Japan). HEK293 cells were maintained in Dulbecco’s modified Eagle’s medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal calf serum (FCS), whereas MDCK cells were grown in Eagle’s minimum essential medium (Sigma Chemical) supplemented with 0.1 mM nonessential amino acids (In Vitrogen, Carlsbad, CA), 1.0 mM sodium pyruvate (In Vitrogen), and 10% FCS. Human colorectal carcinoma Caco-2 (HTB-37) cells were purchased from the American Type Culture Collection (Rockville, MD). The cells were maintained in Dulbecco’s modified Eagle’s medium (Sigma Chemical Co., St. Louis, MO) supplemented with 1% fetal calf serum (FCS), whereas MDCK cells were grown in Eagle’s minimum essential medium (Sigma Chemical) supplemented with 0.1 mM nonessential amino acids (In Vitrogen, Carlsbad, CA), 1.0 mM sodium pyruvate (In Vitrogen), and 10% FCS.

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from ATCC (Rockville, MD) and maintained in Eagle’s minimum essential medium supplemented with 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate, and 20% FCS. All media used in this study contained 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen).

Antibodies—For immunoprecipitation and immunoblot analysis of green fluorescent protein (GFP), monoclonal and polyclonal antibodies against GFP were purchased from MBL (Nagoya, Japan). Rabbit polyclonal antibodies against HA (sc-7392) and ZO-1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and Zymed Laboratories Inc. (South San Francisco, CA), respectively. A rabbit polyclonal antibody against TSLC1 (C-2) was raised against 18 synthetic polypeptides of the C terminus of TSLC1 coupled with keyhole limpet hemocyanin and purified with an affinity column (Asahi Technoglass, Japan). Secondary antibodies for immunoblot analysis were from Amersham Biosciences (Buckinghamshire, England). Fluorescein isothiocyanate- or rhodamine-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratory, West Grove, PA) was used as a secondary antibody for immunofluorescence staining.

Construction of Expression Vectors—Construction of pTSLC1-GFP was described previously (1). For the construction of pTSLC1-HA, an HA epitope tag (YPYDVPDYA) was introduced at the C terminus of TSLC1, which was cloned into the expression vector pcDNA3 (pTSLC1) in our previous study (1).

To prepare MDCK cells stably expressing TSLC1-GFP (MDCK/TSLC1-GFP), MDCK cells were transfected with pTSLC1-GFP using LipofectAMINE Plus reagent (Invitrogen) according to the manufacturer’s protocol. Stable clones were selected in the media containing 500 μg/ml Geneticin (G418) (Invitrogen). Stably transfected cells were maintained in the presence of 300 μg/ml G418. HEK293 cells were transiently transfected with pTSLC1-GFP and/or pTSLC1-HA as described above.

Protein Analysis—Cell lysates were prepared using a lysis buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 20 mM EDTA, and a protease inhibitor mixture (Calbiochem, San Diego, CA). The cell extract was centrifuged at 12,000 rpm for 1 min at 4°C to remove insoluble material, and the supernatant was collected. Protein concentration of each sample was determined by the method of Bradford using a protein assay dye reagent (Bio-Rad, Hercules, CA). For immunoprecipitation, cell lysates were incubated with an appropriate primary antibody overnight at 4°C and then added with protein A-Sepharose (Amersham Biosciences). Immunoprecipitates were rinsed four times with the lysis buffer, suspended in a sample buffer (Invitrogen) containing 50 mM dithiothreitol, and incubated at 70°C for 4 min. Rabbits polyclonal antibodies against HA (sc-7392) and ZO-1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and Zymed Laboratories Inc. (South San Francisco, CA), respectively. A rabbit polyclonal antibody against TSLC1 (C-2) was raised against 18 synthetic polypeptides of the C terminus of TSLC1 coupled with keyhole limpet hemocyanin and purified with an affinity column (Asahi Technoglass, Japan). Secondary antibodies for immunoblot analysis were from Amersham Biosciences (Buckinghamshire, England). Fluorescein isothiocyanate- or rhodamine-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratory, West Grove, PA) was used as a secondary antibody for immunofluorescence staining.

RESULTS

N-Linked Glycosylation of TSLC1—In an attempt to elucidate the physiological function of TSLC1, an expression vector for TSLC1 tagged with GFP at its C terminus (pTSLC1-GFP) was introduced into MDCK cells, and stable transfectants (MDCK/TSLC1-GFP) cells were obtained. 200 μg of protein samples prepared from the cells was immunoprecipitated with anti-GFP polyclonal antibody followed by immunoblot analysis with anti-GFP monoclonal antibody, generating a single band of N-linked glycosylated form is 50 kDa. By subtracting the molecular mass of GFP, 27 kDa, the size of TSLC1 was estimated at ~75 kDa. The sequence analysis of TSLC1 predicted that its extracellular domain bears six NXS(S/T) motifs for N-linked glycosylation (Fig. 1A). We, therefore, enzymatically digested the TSLC1-GFP protein with N-glycosidase F to release putative N-linked oligosaccharides. Approximately one half of the 100-kDa species was replaced with an 80-kDa species, confirming that TSLC1 was indeed glycosylated (Fig. 1B, lane 3) even though a 100-kDa species remained detectable even after the treatment, presumably due to incomplete digestion with N-glycosidase F. Consistent with this result, treating MDCK/TSLC1-GFP cells with tunicamycin for 48 h, an antibiotic that inhibits N-linked glycosylation, also gave rise to a band at 80 kDa (Fig. 1B, lanes 4 and 5). These results indicate that the size of TSLC1 in a deglycosylated form is ~50 kDa. Dimerization of TSLC1 on Plasma Membrane—Previous studies have shown that the majority of IgCAMs function through homophilic and/or heterophilic interaction (2, 10). We therefore investigated pre-existing forms of TSLC1 on the cell membrane by treating the monolayer of nonpolarized MDCK/
TSLC1-GFP cells with BS³, a noncleavable membrane-impermeable cross-linker. Protein samples were prepared in the presence of iodoacetamide to prevent the formation of nonspecific disulfide linkages. Immunoprecipitation of the cross-linker-treated samples followed by immunoblot analysis yielded an additional signal with a molecular mass of ~200 kDa (Fig. 2A, upper band), which seemed to represent the dimerized form of TSLC1-GFP present on the cell surface. We thus examined whether TSLC1 forms homodimers by transiently coexpressing TSLC1-GFP and TSLC1-HA in human embryonic kidney 293 cells. The protein samples prepared from these cells were immunoprecipitated with anti-GFP polyclonal antibody followed by incubation in the presence (lanes 1, 2, and 4) or absence (lanes 3 and 5) of N-glycosidase F at 37 °C overnight and then subjected to 4–12% gradient SDS-PAGE. Immunoreactive signals were detected with anti-GFP monoclonal antibody. Solid and open arrowheads indicate signals for glycosylated and unglycosylated forms, respectively. Positions of the molecular size markers are shown on the left.

Without a chemical cross-linker, a single band was detected at 75 kDa (Fig. 2C), which was compatible with the molecular size of TSLC1 estimated from the size of TSLC1-GFP (~100 kDa) described above. By contrast, with 3 mM BS³, an immunoreactive signal was detected predominantly at 150 kDa on the blot (Fig. 2C), indicating that TSLC1 molecules were dimerized through homophilic interaction. These results led us to analyze the pre-existing forms of endogenous TSLC1 on the cell membrane. Several cell lines were tested for their ability to express TSLC1 on the cell surface. A monolayer of TSLC1-GFP/MDCK cells was treated with 3 mM BS³ prior to protein sample preparation in a lysis buffer containing 10 mM iodoacetamide. 200 µg of protein samples was immunoprecipitated with a rabbit anti-GFP polyclonal antibody and subjected to immunoblot analysis with an anti-GFP monoclonal antibody. The arrow indicates putative homodimers of TSLC1-GFP. B, coimmunoprecipitation of TSLC1-GFP and TSLC1-HA. HEK293 cells were transfected with αTSLC1-GFP, αTSLC1-HA, or both. Protein samples were prepared from transfected HEK293 cells. 200 µg of protein samples was immunoprecipitated with a rabbit anti-GFP polyclonal antibody and subjected to immunoblot analysis with an anti-GFP monoclonal antibody (left panel) or an anti-HA monoclonal antibody (right panel). The signals corresponding with the TSLC1-GFP and TSLC1-HA molecules are marked with open and closed arrowheads, respectively. C, homophilic cis dimerization of endogenous TSLC1 in HEK293 cells. A single-cell suspension of the HEK293 cell was incubated in the presence (+) or absence (−) of 3 mM BS³. 5 µg of protein samples was subjected to 4–12% SDS-PAGE followed by Western blot analysis using anti-TSLC1 polyclonal antibody (CC2). The positions of the molecular size markers are shown on the left of each panel.

FIG. 2. Homophilic cis dimerization of TSLC1. A, cross-linking of TSLC1-GFP on the cell surface. A monolayer of TSLC1-GFP/MDCK cells was treated with 3 mM BS³ prior to protein sample preparation in a lysis buffer containing 10 mM iodoacetamide. 200 µg of protein samples was immunoprecipitated with a rabbit anti-GFP polyclonal antibody and subjected to immunoblot analysis with an anti-GFP monoclonal antibody. The arrow indicates putative homodimers of TSLC1-GFP. B, coimmunoprecipitation of TSLC1-GFP and TSLC1-HA. HEK293 cells were transfected with αTSLC1-GFP, αTSLC1-HA, or both. Protein samples were prepared from transfected HEK293 cells. 200 µg of protein samples was immunoprecipitated with a rabbit anti-GFP polyclonal antibody and subjected to immunoblot analysis with an anti-GFP monoclonal antibody (left panel) or an anti-HA monoclonal antibody (right panel). The signals corresponding with the TSLC1-GFP and TSLC1-HA molecules are marked with open and closed arrowheads, respectively. C, homophilic cis dimerization of endogenous TSLC1 in HEK293 cells.
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TSLC1-GFP

DIC

A1

A2

B1

B2

C1

C2

FIG. 3. Subcellular localization of TSLC-GFP in MDCK cells. MDCK/TSLC1-GFP cells were seeded on culture slides at various cell densities. A few days later, the cells were fixed and observed under a laser scanning confocal microscope with a filter set suitable for GFP detection (panels A1, B1, and C1) and differential interference contrast (DIC) (panels A2, B2, and C2). Bars represent 10 μm. Note: TSLC1-GFP was seen at the tip of filopodia that were about to attach to the neighboring cells as well as in the cytosol (panel A1). An interlocked finger-like distribution of TSLC1-GFP was observed at the cell-cell contact site of the opposing cells in panel B1. In a confluent monolayer, TSLC1-GFP was localized all along the cell-cell border (panel C1).

GFP cells at a range of cell densities by confocal laser scanning microscopy. When cells were well spread, TSLC1 molecules were distributed to punctate structures in the cytoplasm and at the tip of cell surface protrusions that were about to make contact with neighboring cell surfaces (Fig. 3, A1–A2). It is intriguing that, at the initial stage of cell-cell attachment, TSLC1 was concentrated to zipper-like structures at the cell-cell boundaries, where filopodia are thought to penetrate and embed into opposing cells (Fig. 3, B1–B2). Once cells became confluent, TSLC1 protein was localized to all along cell membranes (Fig. 3, C1–C2). We next viewed subcellular localization of endogenous TSLC1 in HEK 293 cells. Immunostaining for endogenous TSLC1 with a CC2 antibody exhibited similar patterns to those of TSLC1-GFP exogenously introduced into MDCK cells at either low (Fig. 4, A1–A3) or high (Fig. 4, B1–B3) cell density.

The distribution of TSLC1 was further examined in polarized MDCK/TSLC1-GFP cells by confocal laser scanning microscopy. The cells were also stained for ZO-1, a protein restricted to tight junction (15), to compare its localization with that of TSLC1-GFP. In the X–Y confocal sections, TSLC1-GFP was distributed to honeycomb-like structures at cell-cell boundaries as well as ZO-1 (Fig. 5A, upper panels). In the X–Z vertical cross-section, ZO-1 staining revealed intense dots along the apical cell surface, where the apical and lateral cell membranes meet (Fig. 5A, lower panel in red). In contrast, TSLC1-GFP was detected predominantly on the lateral surface of the cells (Fig. 5A, lower panel in green) showing distinctive localization from that of ZO-1. Distribution of endogenous TSLC1 was also examined in polarized Caco-2 cells found to express endogenous TSLC1 (data not shown). Immunostaining for endogenous TSLC1 and ZO-1 in the polarized Caco-2 cells (Fig. 5B) exhibited very similar patterns to those of TSLC1-GFP in MDCK cells showing the lateral-specific localization (Fig. 5A). These results suggest that TSLC1 molecules achieve lateral localization through its trans interaction across the membranes as often seen in other adhesion molecules. It is also noteworthy that the subcellular localization seen in TSLC1-GFP/MDCK cells was compatible with that of endogenous TSLC1.

TSLC1 Mediates Ca2+/Mg2+-independent Cell-Cell Aggregation in MDCK Cells—In the last set of experiments, we employed a well-established cell aggregation assay (14) to further assess trans interaction of TSLC1. MDCK/TSLC1-GFP or the parental MDCK cells were dissociated, resuspended in HBSS, and incubated at 37 °C for various periods of time. The requirement for divalent cations such as Ca2+ and Mg2+ in TSLC1-mediated adhesion activity was also tested in Ca2+/ Mg2+-free HBSS in the presence or absence of 10 mM EDTA. Although parental MDCK cells showed little aggregation in the time frame of these experiments, MDCK cells expressing TSLC1-GFP significantly aggregated in a time-dependent manner (Fig. 6A). The degree of aggregation of MDCK/TSLC1-GFP was comparable in either the presence or absence of Ca2+ and Mg2+ (Fig. 6A), indicating that TSLC1-mediated cell aggregation does not require either of the divalent cations. When incubated in HBSS with the divalent cations, the parental MDCK cells exhibited weak cell-aggregation activity (Fig. 6A), presumably caused by Ca2+-dependent cadherin endogenously expressed in MDCK cells. Taken together, these results suggest that TSLC1 mediates intercellular adhesion through homophilic trans interaction in a Ca2+/Mg2+-independent manner.

DISCUSSION

TSLC1 is a unique tumor suppressor gene, because it was identified by its direct activity of tumor suppression in nude mice when introduced in cancer cells. When TSLC1 was introduced into A549 cells lacking endogenous TSLC1, cell growth in vivo was significantly suppressed, whereas the growth rate of the cells in vitro was not affected (1). Based on this observation, we have assumed that suppression of a malignant phenotype of cancers by TSLC1 is not due to direct arrest of the cell cycle but to some other mechanism, such as the regulation of cell growth through cell-to-cell or cell-to-substrate interaction. In support of this notion, the amino acid sequence analysis of TSLC1 revealed significant homology of the extracellular domain of TSLC1 with those of NCAM1 and NCAM2. We, therefore, have assessed our hypothesis by characterizing TSLC1 and demonstrated that TSLC1 is a member of IgCAMs. Biochemical analysis has shown that TSLC1 is an N-linked glycosylated protein. The use of a chemical cross-linker in a single cell suspension has demonstrated that TSLC1 molecules predominantly exist as cis-homodimers on the cell membranes of the dispersed single cells. It remains to be clarified if the homophilic cis interaction plays a role in the adhesive function. To facilitate further investigation, studies are in progress developing antibodies against the extracellular domain of TSLC1 as well as TSLC1 mutants able to impede the dimerization. It is also intriguing to see if removal of the N-linked glycosylation on TSLC1 affects the homophilic interaction, because the modification of NCAM with polysialic acid, another post-transcrip-
tional modification, has been reported to modulate its function by inhibiting its homophilic interaction with the bulky sugar residues (10).

Analysis of the subcellular localization of TSLC1 with confocal microscopy revealed the characteristic pattern to adhesion molecules. In nonpolarized cells, TSLC1 molecules were accumulated at the cell-cell contact sites. Similar patterns of subcellular localization were reported in other adhesion molecules, including E-cadherin and nectins (5–7). In polarized cells, TSLC1 was recruited to the lateral membrane but not to the apical or basal membranes, suggesting the trans interaction of TSLC1 between apposing cells. In favor of this idea, the aggregation assay has demonstrated that TSLC1 mediates cell aggregation through homophilic trans interaction. In addition, we have found that this adhesive activity is independent of diva-

Fig. 4. Subcellular localization of endogenous TSLC1 in HEK293 cells. HEK293 cells were seeded on culture slides at various cell densities. A few days later, the cells were permeabilized and stained for TSLC1 with an anti-TSLC1 polyclonal antibody (CC2) as described under “Experimental Procedures.” Laser scanning confocal microscopy was performed with a filter set suitable for rhodamine detection (panels A1 and B1) and differential interference contrast (DIC) (panels A2 and B2). Bars represent 10 μm. Note: At low cell density (panels A1–A3), staining for TSLC1 showed either “zipper-like” structures or completely sealed stable lines at cell-cell boundaries (A1). When confluent, a honeycomb-like distribution of TSLC1 was seen (B1).

Fig. 5. Lateral localization of TSLC1 in the polarized cells. The cells grown on the Transwell filter unit were fixed, permeabilized, and immunostained with indicated primary antibodies as described under “Experimental Procedures.” Laser scanning confocal microscopy was performed with a filter set suitable for fluorescein and rhodamine detection. The representative sets of X–Y and X–Z sections are shown here. A, MDCK/TSLC1-GFP was stained for tight junction protein ZO-1. Green corresponds to TSLC1-GFP molecules, and red corresponds to the immunodetected ZO-1. Note that a distinct distribution of TSLC1 from that of ZO-1 was observed in the merged panel. B, Caco-2 cells were stained for either TSLC1 (green) or ZO-1 (red). Dual staining for TSLC1 and ZO-1 was not possible, because polyclonal antibodies against them are produced in the same species. Scale bars indicate 10 μm.

Fig. 6. Cell aggregation activity of TSLC1 through homophilic trans interaction. A, Ca2+ - and Mg2+-independent aggregation of MDCK/TSLC1-GFP. The parental MDCK (dotted lines) or MDCK/TSLC1-GFP (solid lines) cells were treated with trypsin in the presence of EDTA and then dispersed by pipetting to obtain a single-cell suspension. Each single-cell suspension was rotated in HBSS containing Ca2+ and Mg2+ (circles), Ca2+- and Mg2+-free HBSS (squares), or Ca2+ - and Mg2+-free HBSS containing 5 mM EDTA (triangles) for 20, 40, and 60 min. The degree of aggregation of cells was represented by the ratio of the total particle number at time t of incubation (Nt) to the initial particle number (No). Data shown here indicate the average Nt/No in duplicate experiments. B, cell aggregation of MDCK/TSLC1-GFP cells (left panel) and control MDCK cells (right panel) after incubating for 60 min in Ca2+- and Mg2+-free HBSS containing 10 mM EDTA.

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heterophilic on the blot by longer exposure (data not shown), suggesting showed several additional bands with higher molecular weight in a monolayer of nonpolarized MDCK/TSLC1-GFP cells characteristics of IgCAMs.

Lent cations, confirming that TSLC1 retains one of the characteristics of IgCAMs.

It should be noted that chemical cross-linking of TSLC1-GFP in a monolayer of nonpolarized MDCK/TSLC1-GFP cells showed several additional bands with higher molecular weight on the blot by longer exposure (data not shown), suggesting heterophilic trans/cis interaction of TSLC1 with other proteins that are endogenously expressed in the cells. Alternatively, these may represent a small amount of higher order homooligomers of TSLC1-GFP. Growing evidence indicates that most adhesion molecules transduce signaling by associating with other adhesion molecules and/or receptors for a soluble ligand (4, 16, 17). Hence, identification of hetero-interacting partners for TSLC1 may lead to finding novel signaling pathways underlying its biological functions.

Accumulating reports indicate that Ig superfamily proteins are multifunctional and participate in the regulation of various cellular activities. In some instances, they play a role in regulating cellular response in the immune system (18, 19). These examples include sialic acid binding Ig-like lectins (siglecs) expressed in hematopoietic cells, which are reported to downregulate cellular activation of immune system to prevent inappropriate auto-reactivity (18, 19). Furthermore, many of the Ig superfamily proteins are associated with malignancy. Whereas TSLC1 has been identified as a tumor suppressor, glycosphatidylinositol-anchored carcinoembryonic antigen, another homophilic Ca\(^{2+}\)-independent adhesion IgCAM, promotes de-differentiation and is one of the widely used tumor markers in clinical medicine (20, 21). In this study, we have demonstrated that TSLC1 is involved in cell-cell adhesion. Recent extensive studies on adhesion molecules have revealed that adhesion has profound effects on cells that go far beyond merely gluing them together (2). They play important and diverse roles in many aspects of cell behavior during the normal development, growth, or maintenance of tissues (22). Altered adhesion properties have been recognized as a feature of cancer cells, and this is true for E-cadherin, another tumor suppressor (23). Notably, its alterations are often observed in the advanced stages of epithelial cancers (24). Loss of E-cadherin function, besides disrupting cell-cell adhesion, was suggested to convey signals that actively induce tumor invasion and/or metastasis (2, 23). It therefore is probable that aberrant TSLC1 contributes to invasion or metastasis to surrounding or distal tissues in a similar manner. Elucidating the molecular mechanisms through which TSLC1 regulates cell-cell interactions may provide appropriate molecular targets for future cancer therapy.

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