Supplementary Material and Methods

Recombinant L1 fusion protein

Recombinant L1 fragments produced in insect cells were described (1). Production and purification of human L1-Fc constructs were described earlier (2). The CHL1-Fc protein comprising the complete ectodomain was constructed and expressed in a similar way. GST-fusion proteins encoding the cytoplasmic part of human L1 or CHL1 were produced by standard procedures.

Cell Adhesion Assays

Cell adhesion assay was performed as described earlier (2). In brief, purified L1-Fc (10μg/ml) was coated to LABTEK glass chamber slides (Nunc, Wiesbaden, Germany) for 16 h at 4 °C. Following coating, the wells were blocked with 3% bovine serum albumin in PBS for 1 h at room temperature and washed with Hanks' balanced salt solution containing 10 mM HEPES. For binding, cells (1 x 10^6/200μl) were preincubated with purified antibody (20 μg/ml) for 10 min at 37°C and transferred to the chamber slides. The binding assay was performed for 30 min at 37°C, following fixation in 4% glutaraldehyde in PBS after briefly dipping into PBS.

Quantitative real-time PCR

This assay was described before (3). Primers for qPCR were designed with the DNA Star Program and were produced by MWG (Ebersberg, Germany). β-actin was used as an internal standard.

Surface Plasmon resonance (SPR) equilibrium analysis

The binding analysis was performed using a BIAcore 3000 equipped with a CM5 sensor chip. Briefly, a BIAcore CM5 chip was activated with EDC/NHS and various levels of L1-Fc were captured onto the activated surface. The remaining active sites were blocked by ethanolamine/HCl. L1-mAb were bound to the L1-Fc surface and allowed to dissociate over time. The association and dissociation phases for each injection over each density surface were subjected to kinetic analysis.

Probe labeling and Illumina Sentrix BeadChip array hybridization

Tumor samples from mice were treated in a tissue homogenizer and total RNA was extracted

by using RNeasy® mini kit (Qiagen Inc., Valencia, CA, USA) according to the manufacture's instructions. The concentration and quality of total RNA was verified by electrophoresis using the total RNA Nano chip assay on an Agilent 2100 Bioanalyzer (Agilent Technologies GmbH, Berlin, Germany). RNA concentrations were determined using the NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). All of the RNA samples were stored at −80 °C until used for microarray analysis.

Biotin-labeled cRNA samples for hybridization on Illumina human Sentrix-6 BeadChip arrays (Illumina Inc., San Diego, CA, USA) were prepared according to Illumina's recommended sample labeling procedure based on a previously published protocol (4). Data extraction was done for all beads individually, and outliers are removed by taking only beads with an expression value > 20 before using the 2.5 MADs rule. All remaining data points are used for the calculation of the mean average signal for a given probe, and standard deviation for each probe was calculated. Normalization and statistical analysis using all hybridisations where carried out through Chipster (Version 1.3, Finnish IT Center for Science CSC). Chipster performs DNA microarray data analysis with R/Bioconductor. Quantile normalizing was choosen to make the expression value follow the same distribution on all chips. Raw data evaluated according to MIAME standards are deposited at GEO at NCBI (Acc-Nr. GSE19171). Differentially regulated genes are filtered according to their standard deviation from the gene mean. Deviation is expressed as a percentage of data to be filtered out (eg. 3 SDs = 99.7%). Regulated genes were functionally clustered using DAVID functional annotation tool (5). DAVID uses an algorithm to measure the significance of the association between the data set and the canonical pathway by a ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway. The gene enrichments are measured by EASE Score, a modified Fisher Exact p-value. P-value smaller than 0.05 are considered strongly enriched in the annotation categories. The treshold was set to highest classification stringency.

References

1. Gouveia RM, Morais VA, Peixoto C, et al. Production and purification of functional truncated soluble forms of human recombinant L1 cell adhesion glycoprotein from Spodoptera frugiperda Sf9 cells. Protein Expr Purif 2007; 52: 182-93.

2. Oleszewski M, Beer S, Katich S, et al. Integrin and neurocan binding to L1 involves distinct Ig domains. J Biol Chem 1999; 274: 24602-10.
3. Gast D, Riedle S, Issa Y, et al. The cytoplasmic part of L1-CAM controls growth and gene expression in human tumors that is reversed by therapeutic antibodies. Oncogene 2008; 27: 1281-9.
4. Van Gelder RN, von Zastrow ME, Yool A, Dement WC, Barchas JD, Eberwine JH. Amplified RNA synthesized from limited quantities of heterogeneous cDNA. Proc Natl Acad Sci U S A 1990; 87: 1663-7.
5. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4: 44-57.