Identification of ROBO1 as a Novel Hepatocellular Carcinoma Antigen and a Potential Therapeutic and Diagnostic Target

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Abstract

Purpose: Hepatocellular carcinoma is the most common primary malignancy of the liver and accounts for as many as one million deaths annually worldwide. The present study was done to identify new transmembrane molecules for antibody therapy in hepatocellular carcinoma.

Experimental Design: Gene expression profiles of pooled total RNA from three tissues each of moderately differentiated and poorly differentiated hepatocellular carcinoma were compared with those of normal liver, noncancerous liver tissue in hepatocellular carcinoma patients, 30 normal tissue samples, and five fetal tissue samples. Target genes up-regulated specifically in hepatocellular carcinoma were validated by immunohistochemical analysis and complement-dependent cytotoxicity assay using monoclonal antibodies generated against target molecules.

Results: The human homologue of the Drosophila Roundabout gene, axon guidance receptor homologue 1, ROBO1/DUTT1, a member of the immunoglobulin superfamily, was highly expressed in hepatocellular carcinoma, whereas it showed only a limited distribution in normal tissues. On immunohistochemical analysis using a newly generated anti-ROBO1 monoclonal antibody, positive signals were observed in 83 of 98 cases of hepatocellular carcinoma (84.7%). The mAb B2318C induced complement-dependent cytotoxicity in ROBO1-expressing cell lines and in the liver cancer cell line PLC/PRF/5. Strikingly, the ectodomain of ROBO1 was detected not only in the culture medium of liver cancer cell lines (PLC/PRF/5, HepG2, etc.) but also in sera from hepatocellular carcinoma patients (6 of 11).

Conclusions: This is the first report that ROBO1 is overexpressed in hepatocellular carcinoma and shed into serum in humans. These observations suggest that ROBO1 is a potential new serologic marker for hepatocellular carcinoma and may represent a new therapeutic target.

Hepatocellular carcinoma is one of the most prevalent types of cancer worldwide, and its incidence is still increasing (1). Roughly 80% of people with hepatocellular carcinoma have liver cirrhosis. In addition, chronic infection with either hepatitis B or C virus, with which >170 million people are infected worldwide, also significantly increases the risk of developing hepatocellular carcinoma. Aflatoxins, produced by a mold that is a contaminant of nuts, beans, and grains, have also been suggested to be major risk factors for the development of this type of cancer. Hepatocellular carcinoma is curable by surgery; yet, this option is generally only applicable in 10% to 15% of presentations. In spite of the high mortality rate associated with hepatocellular carcinoma, there has been little progress in its diagnosis and treatment. The identification of new targets for the early detection of hepatocellular carcinoma, whereas the tumor is still small, would likely have a significant positive effect on the prognosis of this disease. Targeted therapies are dramatically changing the treatment modalities for breast, lung, and colon cancers. In breast cancer therapy, trastuzumab (Herceptin), a humanized recombinant monoclonal antibody (mAb) that recognizes the extracellular domain of HER2 transmembrane protein, is among the first immunologic target-specific drugs that have been licensed for clinical use, and its development represents a model for the
integration of new agents with classic treatment strategies (2, 3). Therefore, the identification of tumor-associated cell surface antigens is critical in the development of tumor-targeted antibody therapy (3).

The ideal expression pattern of a cancer-specific target antigen for antibody therapy is that is should be abundant and homogeneous on the surface of tumor cells yet absent from normal tissues and adjacent noncancerous tissue (3). To identify potential targets for hepatocellular carcinoma, we screened for genes up-regulated in hepatocellular carcinoma by DNA microarray analysis (4). Microarray analysis has been applied to various aspects of cancer research, such as classification of cancer, elucidation of mechanisms of carcinogenesis, discovery of therapeutic targets, and the development of tumor markers (5–8). Recently, microarray studies on hepatocellular carcinoma presented gene lists, including a number of overexpressed (5–8). Recently, microarray analysis has been applied for genes up-regulated in hepatocellular carcinoma by DNA microarray analysis (4). Microarray analysis was done essentially as described previously (17, 18).

Quantitative real-time PCR. Tissues were lyzed directly in IsoGen (Nippon Gene, Osaka, Japan) and total RNA was extracted according to the manufacturer’s instructions. Aliquots of 5 μg of total RNA were reverse transcribed into cDNA, using SuperScriptII (Invitrogen, San Diego, CA) with oligo-(dt) primers. Quantitative real-time PCR was done using SYBR Green I nucleic acid gel stain (BMA, Rockland, ME) with an iCycler iQ Detection System (Bio-Rad, Hercules, CA). Primers were as follows: 5′-GCAATCGCGTTGAAATCTGTA-3′ and 5′-TTGAGACGTGGAACCAAGACCCAGTG-3′ for ROBO1; 5′-GAAAGGAGATCTACCTGCCTGCGACC-3′ and 5′-CCCTGGCTGCTATCACCACATGCTG-3′ for β-actin. PCR conditions were as follows: one cycle of 94°C for 3 minutes followed by 40 cycles of 94°C for 15 seconds, 63°C for 15 seconds and 72°C for 30 seconds. The expression level of ROBO1 was first calculated as the relative ratio of ROBO1 to β-actin in each sample.

Generation of anti-ROBO1 antibodies. We reported that foreign proteins can be displayed on the surface of Autographa californica multiple nuclear polyhedrosis virus (AcMNPV), and that mAbs can be produced by introducing the recombinant virus into mice in the absence of a protein purification step (19–21). Anti-ROBO1 antibodies were generated against various ROBO1 partial protein sequences using this system. The first immunoglobulin domain (Ig1; amino acids 22-115) and the third fibronectin III domain (Fn3; amino acids 738-855) of ROBO1 were amplified by PCR with the following primers: 5′-GGTACCCTCTCGAGAAGAATTTTCCAC-3′ and 5′-GGTACCCCTCTCGAGAAGAATTTTCCAC-3′ and 5′-GGTACCGAGAACACCTGCTGACCA-3′ and 5′-GGTACCGAGAACACCTGCTGACCA-3′ for Ig1; 5′-GGTACCGAGAACACCTGCTGACCA-3′ and 5′-GGTACCGAGAACACCTGCTGACCA-3′ and 5′-GGTACCGAGAACACCTGCTGACCA-3′ and 5′-GGTACCGAGAACACCTGCTGACCA-3′ for Fn3 and ligated into the pmg64 gene. Culture media of 5×109 cells infected with recombinant baculoviruses encoding Ig1-BV and Fn3-BV were harvested and used as immunogens. Mouse mAbs (A2741A and B2318C) were then generated by the conventional method.

Immunoblotting analysis. The full-length ROBO1 cDNA was subcloned into the pcDNA3.1/V5-His TOPO TA vector (Invitrogen) by a PCR-based method using the ROBO1 primers 5′-ACCATGATTCGAGGAGGCCGCTC-3′ and 5′-GCTTCTTCGGTCCCTGCAAAAGATTTTCCAC-3′ and 5′-GCTTCTTCGGTCCCTGCAAAAGATTTTCCAC-3′ and 5′-GCTTCTTCGGTCCCTGCAAAAGATTTTCCAC-3′ and 5′-GCTTCTTCGGTCCCTGCAAAAGATTTTCCAC-3′ for Ig1; 5′-GGTACCGAGAACACCTGCTGACCA-3′ and 5′-GGTACCGAGAACACCTGCTGACCA-3′ and 5′-GGTACCGAGAACACCTGCTGACCA-3′ and 5′-GGTACCGAGAACACCTGCTGACCA-3′ for Fn3 and ligated into the pmg64 gene. Culture media of 5×109 cells infected with recombinant baculoviruses encoding Ig1-BV and Fn3-BV were harvested and used as immunogens. Mouse mAbs (A2741A and B2318C) were then generated by the conventional method.

Materials and Methods

Tissue and serum samples. Eight hepatocellular carcinoma samples, including five cases of moderately differentiated hepatocellular carcinoma and three cases of poorly differentiated hepatocellular carcinoma, and adjacent noncancerous liver tissues were obtained with informed consent from patients who underwent hepatectomy for hepatocellular carcinoma at the Tokyo University Hospital, Tokyo, Japan and the Saitama Cancer Center, Saitama, Japan. All samples were frozen immediately after resection and stored at −80°C until further analysis. Sera from three healthy adults and six patients with hepatocellular carcinoma were collected at the Tokyo University Hospital. In addition, sera from five patients with chronic hepatitis, from five patients with liver cirrhosis, and five patients with hepatocellular carcinoma were collected at the Yokohama City University School of Medicine.

Cell lines. COS7, HEK293, and the hepatoblastoma cell line HepG2 were obtained from the American Type Culture Collection (Manassas, VA). The hepatocellular carcinoma cell lines HLE, HuH7, and PLC/PRF/5 and the hepatoblastoma cell line HuH6 were purchased from the Health Science Research Resource Bank (Osaka, Japan). An additional 14 tumor cell lines derived from different tumor types (nasal, lung, colon, brain, uterine cervix, and breast) were described previously (16).

RNA extraction and microarray analysis. Tissues or cells were lysed directly in IsoGen reagent (Nippon Gene, Osaka, Japan) and homogenized. Total RNA was extracted according to manufacturer’s instructions. Purchased RNA representing 30 different adult normal tissues, five different fetal tissues, and pooled total RNA from three samples each of moderately differentiated hepatocellular carcinoma, poorly differentiated hepatocellular carcinoma, and liver cirrhosis, along with total RNA from 19 cancer cell lines were analyzed using the HG-U133A array (Affymetrix, Santa Clara, CA) containing probes for 22,000 human genes. Further information on the source of RNA can be provided on request or is available at http://www.lsbm.org/db/index.html. Microarray analysis was done essentially as described previously (16).
separated on 8% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Hybond P; Amersham Biosciences, Uppsala, Sweden). After blocking the membrane with 2% nonfat milk in TBS containing 0.05% Tween 20 for 1 hour, the membranes were incubated with A7241A (1.0 μg/mL), anti-V5 antibody (1:5,000; Invitrogen), or anti-β-actin antibody (1:2,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) as a primary antibody. Horseradish peroxidase–conjugated anti-mouse IgG antibody (1:50,000; Jackson ImmunoResearch Laboratories, West Grove, PA) was used as a secondary antibody, and Enhanced Chemiluminescence PLUS Detection System (Amersham Biosciences) was used for chemiluminescent detection.

### Immunohistochemistry

A total of 98 hepatocellular carcinoma specimens, including 15 well-differentiated, 69 moderately differentiated, and 14 poorly differentiated hepatocellular carcinomas, were prepared from the archives at the Tokyo University Hospital. Using a tissue microarrayer (Beecher Instruments, Silver Spring, MD), the area of interest in the donor paraffin block was cored twice with a needle 2 mm in diameter and transferred to the recipient paraffin block. Sections 4-μm thick were cut from the tissue microarray block and deparaffinized in xylene, washed in ethanol, and rehydrated in TBS. ROBO1 was stained with anti-ROBO1 mAb A7241A (20 μg/mL). Antigen retrieval was done in 10 mmol/L citrate buffer solution (pH 6) at 121°C for 10 minutes, and primary antibodies were applied for 1 hour followed by secondary staining with DAKO Envision Reagent (DAKO Ltd., Cambridge, United Kingdom) for A7241A and DAKO LSAB2 Reagent (DAKO) for the others. All sections were counterstained with Meyer's hematoxylin.

ROBO1 staining was scored according to the Clinical Trial Assay recommendations (0-3+) and was evaluated independently by two pathologists (I.S. and N.Y.). The results were compared, and any discrepancies were resolved by consensus at a meeting after further histopathologic reviews. The data were analyzed by the Fisher’s exact test, and P < 0.01 was considered significant.

### Establishment of ROBO1-expressing HEK293 cells

To establish ROBO1-V5 overexpressing HEK293 cell clones (ROBO1.HEK293), HEK293 cells were transfected with 1 μg of ROBO1-V5/pCDA3.1 plasmid using FuGENE 6 (Roche Diagnostics, Indianapolis, IN). Cells were selected in the presence of 500 μg/mL Geneticin (Life Technologies, Rockville, MD) in DMEM supplemented with 10% fetal bovine serum. Baby rabbit complement (Cedarlane, Hornby, Ontario, Canada) was added at a final concentration of 25% and incubated at 37°C for 90 minutes. After incubation, 100 μL of supernatant was collected, and calcine concentration was measured by fluorometry with excitation and emission at 494 and 517 nm, respectively. Calcine concentrations in the supernatants of the cells incubated without complement and antibodies were considered spontaneous calcine release, and those of cells incubated in 1% NP40 solution were considered maximum calcine release. Cytotoxicity (%) was determined from the formula \( \frac{A - C}{B - C} \times 100 \), where A, B, and C represent calcine release in each experiment, maximum calcine release, and spontaneous calcine release, respectively. All experiments were done in triplicate, and the figures show the means ± SD.

### Results

**ROBO1 mRNA is specifically up-regulated in hepatocellular carcinoma.** To identify targets for antibody therapy in hepatocellular carcinoma, gene expression profiles of pooled total RNA from three tissues each of moderately differentiated and poorly differentiated hepatocellular carcinomas were compared with those from normal liver, noncancerous liver tissues in hepatocellular carcinoma patients, 30 normal tissue samples, and five fetal tissue samples. The goal was to identify genes that are up-regulated in hepatocellular carcinoma and that encode proteins localized to the cell surface for antibody accessibility. These genes should also exhibit little or no expression in vital organs to minimize target-driven undesirable side effects associated with antibody therapy. Genes with the desired expression profile were screened by extensive bioinformatics analysis to determine their structural and functional classifications along with their potential for cell surface localization.

The ROBO1 gene expression pattern fulfilled all the desired characteristics. The ROBO1 gene was overexpressed in hepatocellular carcinoma specifically compared with other normal tissues and noncancerous (adjacent noncancerous liver tissues) except fetal tissues (Fig. 1A). In addition, ROBO1 is a member of the neural cell adhesion molecule family of receptors and is a type I transmembrane molecule.

To verify overexpression of ROBO1 in hepatocellular carcinoma, we analyzed ROBO1 expression in eight pairs of hepatocellular carcinoma tissues and adjacent noncancerous areas by quantitative real-time PCR. More than a 2-fold up-regulation of ROBO1 expression was observed in all eight pairs of hepatocellular carcinoma tissues (Fig. 1B).

**Protein expression of ROBO1 in hepatocellular carcinoma.** To characterize ROBO1 protein expression in hepatocellular carcinoma, we generated anti-ROBO1 mAbs using gp64-fused ROBO1 BV antigens. The mAb A7241A was generated by immunization with the Ig1-BV antigen and was used subsequently to detect ROBO1 protein by immunoblotting analysis and immunohistochemical analysis. When we analyzed the lysates of COS7 cells transfected with full-length cDNA of ROBO1 (ROBO1-V5/pCDA3.1) with the mAb A7241A, we obtained the same results as with an anti-V5-tag antibody, which showed a band of ~260 kDa specific to transfected cells (Fig. 2A), suggesting that the mAb A7241A could specifically detect recombinant ROBO1 protein. Next, to confirm whether the mAb A7241A could detect endogenous ROBO1, cell lysates from 19 cancer cell lines (brain, lung, colon, stomach, liver,
breast, and uterine cervix) were screened by immunoblotting analysis. The mAb A7241A detected ROBO1 protein only in cancer cell lines that showed high levels of ROBO1 mRNA expression by GeneChip analysis (Fig. 2B and C), suggesting that there is a correlation between ROBO1 mRNA and protein levels in cancer cell lines.

Overexpression of ROBO1 in hepatocellular carcinoma specimens was confirmed by immunohistochemical analysis using the mAb A7241A, whereas ROBO1 staining was not detected in any normal tissues except the brain (Supplementary Fig. S1). Staining with the mAb A7241 was observed specifically in the cancerous area but not in the adjacent noncancerous areas, such as those showing liver cirrhosis and hepatitis (Fig. 3). The results of immunohistochemical analysis of ROBO1 status were correlated with the GeneChip ROBO1 expression data (Fig. 2A). The cancer cell membrane was stained strongly in poorly differentiated hepatocellular carcinoma by the mAb A7241A, confirming membrane localization of ROBO1. ROBO1 expression was observed in 11 of 15 cases of well-differentiated hepatocellular carcinoma (73.3%), in 59 of 69 cases of moderately differentiated hepatocellular carcinoma (85.5%), and in 13 of 14 cases of poorly differentiated hepatocellular carcinoma (92.9%; Table 1). ROBO1 staining was especially strong in poorly differentiated hepatocellular carcinoma compared with well-differentiated and moderately differentiated hepatocellular carcinoma (Fisher’s exact test, \( P = 0.005 \)).

Detection of the ectodomain of ROBO1 in culture media and sera of patients with hepatocellular carcinoma. In the ROBO1 immunoblotting pattern, the 120-kDa band was detected specifically in ROBO1-V5/COS7 (Fig. 2A) and in liver cancer cell lines (Fig. 2C), suggesting that the outer domain of ROBO1 may be shed into the culture media of liver cancer cell lines.

Culture media from ROBO1-V5/COS7, ROBO1-V5/HEK293, PRC/PREF/5, HuH6, HuH7, and HepG2 cells cultured under serum-free conditions (CHO-S-SFMII; Life Technologies) were analyzed by immunoblotting analysis with the mAb A7241A.
The 120-kDa band, thought to be the shed ectodomain of ROBO1, was detected in all lanes. This 120-kDa band was also detected by immunoblotting analysis with other ROBO1 mAbs. Next, soluble ROBO1 was detected by the same method using sera from patients with hepatocellular carcinoma. Sera from 11 patients with hepatocellular carcinoma, three normal healthy individuals, five patients with chronic hepatitis, and five patients with liver cirrhosis were screened (Fig. 4B). Culture media of PLC/PRF/5 cells, ROBO1-V5/COS7, and mock (pcDNA3.1) transfected COS7 cells were used as controls. Soluble ROBO1 was detected strongly in 6 of the 11 serum samples with an apparent molecular size similar to the shed ROBO1 protein found in the culture media of PLC/PRF/5 cells and ROBO1/COS7. In contrast, there were no significant bands in sera from normal adults, patients with chronic hepatitis, or patients with liver cirrhosis.

**Cytotoxicity of anti-ROBO1 mAb.** We examined whether the anti-ROBO1 mAbs have complement-dependent cytotoxicity activity against cells expressing ROBO1. First, we screened for mAbs that gave positive results on flow cytometric analysis. The mAb B2318C, which was generated by immunization with the Fn3-BV antigen, reacted strongly to the liver cancer cell lines PRF/PLC/5, HepG2, HuH6, HuH7, and ROBO1_HEK293 (Fig. 5A) but not to mock-transfected HEK293 cells.
Next, complement-dependent cytotoxicity activity of the mAb B2318C was examined against ROBO1_HEK293 cells and the liver cancer cell line PLC/PRF/5. The mAb B2318C showed cytotoxicity against ROBO1_HEK293 and PLC/PRF/5 in a dose-dependent manner but not against control HEK293 cells (Fig. 5B-D), suggesting that ROBO1 may be a suitable target for antibody therapy of hepatocellular carcinoma.

### Discussion

In this study, we showed that ROBO1 mRNA is highly expressed in hepatocellular carcinoma using GeneChip analysis (Affymetrix) and quantitative real-time PCR (Fig. 1). ROBO1 is highly conserved in mice and humans; thus, it was difficult to generate mAbs due to immunologic tolerance. Therefore, we used a baculovirus display system to produce immunized antigen, which we have used previously to generate mAbs against human nuclear receptors (19–21). Using this procedure, we successfully generated the mAbs A7241A and B2318C against ROBO1 displayed budded baculovirus immunogens (Ig1-BV and Fn3-BV), with which we showed that the expression level of ROBO1 protein showed a strong correlation with its level of transcription in hepatocellular carcinoma. Moreover, the cell membranes of hepatocellular carcinoma, especially in poorly differentiated hepatocellular carcinoma, were stained strongly with anti-ROBO1 mAb on immunohistochemical analysis (Fig. 3), indicating that ROBO1 is localized on the surface of hepatocellular carcinoma cells. However, using conventional glutathione S-transferase fusion proteins as antigens, we failed to obtain high-affinity anti-ROBO1 mAbs suitable for immunohistochemical analysis and cell cytotoxicity assay (data not shown).

We showed that the mAb B2318C can kill both the liver cancer cell line PLC/PRF/5 and ROBO1-overexpressing HEK293 cells by complement-dependent cytotoxicity activity. We also confirmed that the mAb B2318C has antibody-dependent cell cytotoxicity against cells overexpressing ROBO1 (Supplementary Fig. S2). These observations suggest that ROBO1 is a promising target for antibody therapy in hepatocellular carcinoma.

The ligands of ROBO1 have been reported to be human homologues of Slit 1 to Slit 3, which play a vital role in axon guidance by signaling through Robo receptors (13, 14). Slit 2 is also a candidate tumor suppressor gene, which is silenced epigenetically in lung, breast, and colon cancers, and conditioned medium containing recombinant Slit 2 was shown to reduce growth of breast and colorectal cancer cells and to induce apoptosis in a colorectal cancer cell line (22, 23). ROBO1 is also a candidate tumor suppressor gene (15, 24). Therefore, the Slit-ROBO1 signaling pathway may be involved in the growth suppression signal in cancer cells. The expression of Slit 2 and also Slit 1 and Slit 3 in hepatocellular carcinoma is very low in poorly differentiated hepatocellular carcinoma (Supplementary Fig. S3); meanwhile, ROBO1 is overexpressed in hepatocellular carcinoma. Interestingly, mutations in the ROBO1 gene were not identified in liver cancer cell lines (data not shown), suggesting that ROBO1 may be overexpressed by negative feedback in hepatocellular carcinoma. Further studies to characterize the precise physiologic function of ROBO1 in hepatocellular carcinoma are currently under way in our laboratory.

In addition, we show that the ectodomain portion of ROBO1 is shed from the cell surface, and that this fragment of ROBO1 is present in the sera of patients with hepatocellular carcinoma but not in normal healthy adults or in patients with liver cirrhosis or chronic hepatitis. It is well established that the ectodomain of certain transmembrane molecules can be cleaved off by proteolysis, with the resulting shed fragment exerting important biological functions. Clearly, large-scale studies are needed to evaluate the potential of ROBO1 as a serologic marker for the early detection of hepatocellular carcinoma. We are currently pursuing the large-scale testing of an ELISA system capable of detecting ROBO1 in patient serum.

From a diagnostic perspective, shed ROBO1 may represent an excellent diagnostic marker for hepatocellular carcinoma.

### Table 1. Correlation between expression of ROBO1 and the differentiation of hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Level</th>
<th>WD</th>
<th>PD</th>
<th>MD</th>
<th>Total</th>
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<td>+</td>
<td>4</td>
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<td>2+</td>
<td>7</td>
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<td>3+</td>
<td>3</td>
<td>4</td>
<td>36</td>
<td>43</td>
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<tr>
<td>Positive rate</td>
<td>11/15 (73.3%)</td>
<td>13/14 (92.9%)</td>
<td>5969 (85.5%)</td>
<td>83/98 (84.7%)</td>
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NOTE: A total of 98 hepatocellular carcinoma specimens, including 15 well-differentiated, 69 moderately differentiated, and 14 poorly differentiated cases, were stained with anti-ROBO1 mAb A7241A. The staining was scored according to the Clinical Trial Assay recommendations (0–3+) evaluated independently by two pathologists (J.S. and N.Y.). 0+, no staining; 1+, weak staining; 2+, moderate staining; 3+, strong staining.

Abbreviations: WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated.
Fig. 5. Complement-dependent cytotoxicity activity against ROBO1_HEK293 cells and liver cancer cell PRC/PRF/5 with anti-ROBO1 mAb (B2318C). A, flow cytometric analysis of recognition of ROBO1 HEK293 cells and liver cancer cell lines (PLC/PRF/5, HepG2, HuH6, and HuH7) with the mAb B2318C. y-axis, relative cell number; x-axis, fluorescent staining intensity on a logarithmic scale. B, calcein-labeled ROBO1 HEK293 cells and HEK293 cells (Mock) were incubated with 25% baby rabbit complement (Cedarlane) in the presence of 0 or 10 μg/mL mAb B2318C. C, calcein-labeled PRC/PRF/5 cells were incubated with 25% baby rabbit complement (Cedarlane) in the presence of 0 or 10 μg/mL mAb B2318C. D, calcein-labeled ROBO1/HEK293 cells were incubated with 25% baby rabbit complement (Cedarlane) and various concentrations of the B2318C mAb.
Yet, from a therapeutic perspective, it presents potential efficacy limitations to the use of ROBO1 as an antibody-based therapeutic to target tumor cells. It is of course possible that serum ROBO1 may accelerate the clearance of anti-ROBO1 therapeutic antibodies from the patient or impair the targeting of those antibodies to hepatocellular carcinoma tissues. Although, HER2, a well-known target for antibody therapy in breast cancer, is also a shed antigen, there is no significant correlation between shed antigen concentration and response status of patients treated with anti-HER2 antibody (25). Further experiments are needed to clarify the therapeutic potential of ROBO1 as an antibody-based therapy for the treatment of hepatocellular carcinoma.

This study is the first report showing that ROBO1 is expressed specifically at high levels in hepatocellular carcinoma, and that ROBO1 is shed from the membrane at a level detectable in the serum of patients with hepatocellular carcinoma. These results indicate that ROBO1 is a potential new serologic marker of hepatocellular carcinoma and may represent a new therapeutic target.

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References

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