Leukocyte cell-derived chemotaxin 2 (LECT2) has been shown to act as a tumor suppressor in hepatocellular carcinoma (HCC). However, the underlying mechanism has not yet been completely defined. Here, we employ a LECT2-affinity column plus liquid chromatography coupled with tandem mass spectrometry to identify LECT2-binding proteins and found that MET receptor strongly interacted with LECT2 protein. Despite the presence of hepatocyte growth factor, the LECT2 binding causes an antagonistic effect to MET receptor activation through recruitment of protein tyrosine phosphatase 1B. The antagonistic effect of LECT2 on MET activation also mainly contributes to the blockage of vascular invasion and metastasis of HCC. Furthermore, serial deletions and mutations of LECT2 showed that the HxGxD motif is primarily responsible for MET receptor binding and its antagonistic effects. **Conclusion:** These findings reveal a novel, specific inhibitory function of LECT2 in HCC by the direct binding and inactivation of MET, opening a potential avenue for treating MET-related liver cancer. (Hepatology 2014;59:974-985)

Hepatocellular carcinoma (HCC) is the most common form of liver cancer worldwide. Liver transplantation, surgical resection, and local-regional therapy, such as transarterial chemoembolization, have resulted in great progress, but, overall, prognosis is poor. The available treatment options are...
largely unsuccessful as a result of a high frequency of tumor recurrence and metastasis. Studies of the molecular pathophysiology of HCC revealed that growth factors and their corresponding receptors are commonly overexpressed and/or dysregulated in HCC. Such receptor axes include hepatocyte growth factor (HGF)/MET, insulin growth factor (IGF)/IGF-1 receptor (IGF-1R), vascular endothelial growth factor (VEGF)/VEGFR, and ErbB family receptor tyrosine kinases. Activation of these receptors and their corresponding downstream signaling cascades can lead to angiogenesis, cell proliferation, and metastasis in HCC. Hence, identifying novel therapeutic targets and effective treatments, particularly against receptor tyrosine kinase pathways, is urgent for this fatal disease.

Leukocyte cell-derived chemotaxin 2 (LECT2) was originally identified as a chemotactic factor for neutrophils and stimulates the growth of chondrocytes and osteoblasts. The subsequent isolation of LECT2-coding complementary DNA suggested that it is predominantly expressed in the liver. In a previous study, LECT2 was identified as a direct target gene of β-catenin in the liver. β-catenin-induced LECT2 expression suppresses tumor progression through its inflammation-suppressive effects. This balancing force of LECT2 to nuclear β-catenin was proposed to affect tumorigenesis of HCC. However, the LECT2 expression did not correlate with the status of hepatitis B and C virus infection or cirrhosis, suggesting the tumor-suppressive effects of LECT2 may be beyond inflammation. Furthermore, whether LECT2 is a clinically downstream molecule of β-catenin remains illusive, because LECT2 expression did not correlate with CTTNB mutations. Recently, LECT2 expression was also found to suppress HCC cell invasion in vitro and negatively correlated with vascular invasion in HCC patients. Although accumulating evidence supports LECT2 as an important tumor suppressor in HCC, the membrane-binding receptor as well as downstream-acting mechanisms of LECT2 on HCC progressions remain largely unclear.

In this study, we have identified LECT2 as a novel endogenous MET antagonist that reduces phosphorylation by direct interaction with the MET receptor and recruitment of protein tyrosine phosphatase 1B (PTP-1B).

**Materials and Methods**

**Cell Culture, Transfection, and Established Stable Clone.** Hepatoma cell lines (i.e., SK-Hep1, HepG2, and Huh7) were grown in Dulbecco’s modified Eagle’s medium medium containing 10% fetal bovine serum (Life Technologies, Inc., Grand Island, NY) at 37°C in a humidified atmosphere of 5% CO2/95% air. SK-Hep1, HepG2, and 293T cells were from American Type Culture Collection (Manassas, VA). Huh7 cells were from Joint Conference of Restoration Branches. For establishment of stable cell lines, both empty and pSecTag2A-LECT2-hygroycin B or pLKO-shLECT2-puromycin and shLuciferase (purchased from the National RNAi Core Facility) vectors were transfected into hepatoma cells using Lipofectin reagent (Invitrogen Life Technology, Carlsbad, CA) or infected with lentivirus. Target sequences for shLECT2 were CTATTGCCCTTGCAGAAAGTT (shLECT2-1) and GCATAACATCGCATGTGC-ACA (shLECT2-2). Stable cell populations were selected, and single clones were confirmed to have prominent LECT2 expression by western blotting analysis.

**Western Blotting and Immunoprecipitation.** Western blotting and immunoprecipitation (IP) analysis were performed, as previously described, by indicating antibodies (Abs; listed in the Supporting Materials). For western blotting, the indicated cells were harvested and homogenized in a lysis buffer. For establishment of stable cell lines, both empty and pSecTag2A-LECT2-hygroycin B or pLKO-shLECT2-puromycin and shLuciferase (purchased from the National RNAi Core Facility) vectors were transfected into hepatoma cells using Lipofectin reagent (Invitrogen Life Technology, Carlsbad, CA) or infected with lentivirus. Target sequences for shLECT2 were CTATTGCCCTTGCAGAAAGTT (shLECT2-1) and GCATAACATCGCATGTGC-ACA (shLECT2-2). Stable cell populations were selected, and single clones were confirmed to have prominent LECT2 expression by western blotting analysis.

**Additional Supporting Information** may be found in the online version of this article.
developed with enhanced chemiluminescence western blotting reagents (Merck Millipore). For IP, cells were lysed on ice with IP lysis buffer (50 mM of Tris [pH 7.4], 150 mM of NaCl, 1% Triton X-100, and 1% Nonidet P-40) and immunoprecipitated with the indicated Abs combined with protein A agarose beads (Sigma-Aldrich). The collected protein complex was washed four times with IP buffer and eluted by boiling with protein sample buffer under reducing conditions. Then, proteins were resolved on SDS-PAGE and analyzed by western blotting.

Full additional materials and methods are described in the Supporting Information.

Results

MET Is a Direct Binding Target of LECT2. To identify receptor(s) that are targeted by the LECT2 protein in regulating HCC progression, we utilized a LECT2-Fc immobilized affinity column to isolate candidate receptors from the SK-Hep1 cell membrane proteins and analyzed by nanoLC-MS/MS (liquid chromatography coupled with tandem mass spectrometry). Multiple proteins were detected and assumed to be bound by LECT2, including receptor tyrosine kinases (RTKs) and their associated factors (Supporting Table 1). RTKs play an important role in regulating cell motility and invasation of multiple cancer cells.10,11 Next, we used a human phospho-RTK array to detect alterations in phosphorylated RTKs after LECT2 treatment. Interestingly, we found that the phosphorylation of hepatocyte growth factor receptor (HGFR/MET), but not epidermal growth factor receptor, was strongly inhibited after treatment with LECT2 recombinant protein (Supporting Fig. 1). MS analysis also revealed that MET was a LECT2-associated protein (Fig. 1A). To further examine the interaction between LECT2 and MET proteins, we performed coimmunoprecipitation (Co-IP) experiments and confirmed that interactions between exogenous expressed LECT2 and MET in 293T cells and endogenous expressed LECT2 and MET in Huh7 cells (Fig. 1B,C). An in vitro binding assay also revealed that the rLECT2-Fc protein directly binds to the extracellular domain (1-932 amino acids) of recombinant MET protein (Fig. 1D). In addition, confocal microscopy analysis demonstrated that endogenous LECT2 is highly expressed and located in the cytoplasm or colocalized with the MET receptor at the Huh7 cell membrane (Fig. 1E, lower panel). We also observed the same colocalization of MET and recombinant LECT2 protein in SK-Hep1 cells (Fig. 1E, upper panel). Together, these results demonstrated that LECT2 directly binds to the MET receptor at the cell membrane.

LECT2 Inhibits MET RTL Activity Without Competing With HGF Binding. We next examined whether the invasion-suppressive activity of LECT2 is mediated through MET binding. We first observed that MET phosphorylation (Tyr 1234/1235) levels were significantly decreased in the LECT2-overexpressing SK-Hep1 cells and elevated in the LECT2-knockdown Huh7 cells (Supporting Fig. 2A). Next, we tested whether LECT2 could antagonize HGF, the native MET ligand-induced12 MET phosphorylation. Interestingly, recombinant Fc-tagged LECT2, but not Fc, demonstrated a concentration-dependent inhibition in the HGF-induced MET phosphorylation (Supporting Fig. 2B) and invasion of SK-Hep1 cells (Supporting Fig. 2C). SU11274, a MET kinase inhibitor, also suppressed MET phosphorylation in LECT2-knockdown Huh7 cells and returned the invasive ability to the control level (Supporting Fig. 2D). Furthermore, overexpression of the wild-type (WT) MET dogmatically increased phosphorylation and restored the invasiveness of SK-Hep1/LECT2 cells, whereas overexpression of the kinase-dead MET (K1110A) mutant did not (Supporting Fig. 2E).

To evaluate whether LECT2 competed with HGF binding to MET, a competition enzyme-linked immunosorbent assay (ELISA) was used. The results demonstrated that the binding of HGF (0-100 nM) with MET was not affected with the addition of excess LECT2 (100 nM) and vice versa (Supporting Fig. 3A,B). Furthermore, flow cytometry (FCM) also revealed that HGF binding with cell-surface MET was not altered by coinubcation with 0.5-10.0 nM of LECT2 protein (Supporting Fig. 3C). Similarly, LECT2 binding with cell-surface MET also remained consistent in the presence of up to 5 nM of HGF (Supporting Fig. 3D), confirming that LECT2 and HGF do not interfere with the binding of each other with the MET receptor. Furthermore, HGF protein can only be coimmunoprecipitated with LECT2 Ab in the presence of MET receptor (Supporting Fig. 3E).

LECT2 Functionally Binds on α-chain of MET Extracellular Domain. HGF has previously been shown to bind to the MET terminal Ig3-412 and, with lower affinity, the semaphorin domain.13 To identify the LECT2-binding site on the MET receptor, a series of expression constructs containing different portions of the MET receptor extracellular domain were generated (Fig. 2A). LECT2 had strong interaction with the α-chain and relatively weak interaction with the SEMA
Fig. 1. Identification of MET as a LECT2-binding protein. (A) Tandem MS (MS-MS) data confirm the detection of MET peptides in the LECT2-precipitated complex. The representative spectra of two peptides (SEGSPLVLVLPYMK and AFFMLDGILSK) are shown. (B) 293T cells were transfected with control vector, LECT2, and MET as indicated. Cell lysates were immunoprecipitated with anti-LECT2 or anti-MET and immunoblotted with anti-LECT2 and anti-MET Ab. (C) Endogenous interactions between LECT2 and MET were evaluated in high endogenous LECT2-expressing cell lines. Huh7 cell lysates were immunoprecipitated with anti-MET, anti-LECT2, or normal human IgG Abs and immunoblotted with anti-LECT2 and anti-MET Ab. (D) An in vitro binding assay was used to demonstrate direct binding between LECT2 and MET. The Fc-tagged recombinant LECT2 protein and the His-tagged MET extracellular domain (1-932 amino acids) were incubated and purified using a protein A affinity column. Washed precipitates were subjected to SDS-PAGE. The Fc recombinant protein and poly-His peptide were used as the control. (E) Colocalization of LECT2 and MET. SK-Hep1 cells treated with recombinant Fc-tagged LECT2 or Fc for 30 minutes and Huh7 cells were fixed and stained for LECT2 (green) and MET (red). Nuclei were counterstained with DAPI (blue). Scale bars: 20 μm. (B-E) Each experiment was repeated three times. IgG, immunoglobulin G; DAPI, 4′,6-diamidino-2-phenylindole.
and IPT repeat domains (Fig. 2A). We subsequently dissected the α-chain into four fragments to identify the minimal MET ectodomain (Fig. 2B). Co-IP results revealed that LECT2 had strong interaction with α-chain fragment 2 (residues 92-175) and a weaker interaction with α-chain fragment 3 (residues 159-242; Fig. 2B). An α-chain fragment with residues 159 to 175 deletion (α-D159-175) lacked most of the binding capacity to LECT2 (Fig. 2B, right panel). Invasion assays also demonstrated that α-D159-175 competed out the LECT2 inhibitory effect on invasion ability and MET receptor phosphorylation in SK-Hep1 cells (Fig. 2C).

The HxGxD Motif Is Critical for LECT2’s Inhibitory Activity. To map the MET-interacting LECT2 region, we divided LECT2 into three fragments and performed co-IPs (Fig. 3A). Co-IP results demonstrated that fragments D1 (residues 19-77) and D2...
(residues 53-117) sufficiently bound MET and significantly inhibited its phosphorylation (Fig. 3A). Conditional medium of fragments D1 and D2 also retained invasion-suppressive abilities to SK-Hep1 cells, such as LECT2 (Fig. 3B). Taken together, these results indicated that the 53-77 amino acid (aa) region of LECT2 binds to the 159-175 aa region of the MET α-chain and mediates the inhibition of MET phosphorylation.

To structurally characterize the LECT2 protein, we compared its amino acid sequence with known protein domains. We identified a M23 peptidase domain located between aa 51 and 147. The M23 peptidase domain contained a highly conserved HxGxD motif at aa 53-57 (Supporting Fig. 4A,B), a putative enzyme-active site previously also known to be involved in protein-protein interactions.14 To further evaluate the
function of the HxGxD motif, MET mutants harboring substitutions in this domain were generated to determine the importance of this domain to its activity (Supporting Fig. 4C). Significantly, we found that mutations at aa 53-57 (i.e., mLECT2-a and -b), but no other LECT2 aa (i.e., mLECT2-c and -d), disrupted MET-binding capacity (Fig. 3C) and significantly decreased LECT2-inhibitory effects on MET phosphorylation and invasion ability (Fig. 3D).

Furthermore, SK-Hep1 cells expressing WT LECT2 or LECT2 containing mutations outside of the presumed MET-binding site (mLECT2-d) grew small tumors, compared with HxGxD motif-mutated LECT2 (mLECT2-b) cells. Control and mLECT2-b
cells had higher frequency of intrahepatic metastasis, as confirmed by histopathology (Fig. 4A). In addition, HxGxD mutant cells (mLECT2-b) exhibited strong extravascularization and lung metastasis activities, as determined by survival colonies from mouse inferior vena cava (IVC) blood (Fig. 4B), minced lung tissue (Fig. 4C), and histopathological analysis (Fig. 4D). On the contrary, orthotopically xenografted models revealed that LECT2-knockdown Huh7 cells developed larger tumor masses, more circulating cancer cells, more intrahepatic metastases, and more lung metastasis than control Huh7 cells (Supporting Fig. 5A-D). Based on these data, we suggest that the HxGxD motif is important for the LECT2 function in vascular invasion and metastasis.

**LECT2 Inhibits MET Activation by Recruiting PTP1B.** Phosphorylation of MET can be regulated by phosphotyrosine phosphatases (PTPs), such as density-enhanced protein-tyrosine phosphatase-1 (DEP-1), PTP-1B, and T-cell PTP (TC-PTP).15-17 Because LECT2 reduced phosphorylation of MET without affecting HGF binding or MET protein levels, we examined whether LECT2 could affect interaction between MET and PTPs. We found an increased association between PTP-1B, but not DEP-1 or TC-PTP, and MET receptor in LECT2-overexpressing SK-Hep1 cells (Fig. 5A). In contrast, the association between PTP-1B and MET was decreased in LECT2-knockdown cells (Fig. 5A). Furthermore, the association between MET and PTP-1B was elevated in response to treatment with 2.5 nM of recombinant LECT2, even in the presence of 40 ng/mL of HGF (Fig. 5B).

After depletion of PTP-1B, but not TC-PTP, by specific small interfering RNA (siRNA), the recombinant LECT2 protein had no effect on HGF-induced MET phosphorylation (Fig. 5C). When the HxGxD motif was mutated, PTP-1B failed to be recruited to MET, accompanied with the retaining of MET phosphorylation and cell invasion abilities (Fig. 3D). The associations of MET downstream adaptor proteins, such as growth factor receptor-bound protein 2 (Grb2), Src, GRB2-associated binding protein 1 (Gab1), and p85, were impaired by LECT2 protein with or without HGF administration (Fig. 5D). Downstream signaling of the HGF/MET axis, as with Raf-1 and extracellular signal-regulated kinase (Erk) phosphorylation levels, were significantly decreased in LECT2-overexpressing SK-Hep1 cells and increased in LECT2-knockdown Huh7 cells (Fig. 5E). Despite a slight decrease in recruitment of p85 to MET, phosphorylation of protein kinase B (Akt) was not affected in response to LECT2 treatment (Fig. 5E). These results support the role of LECT2 as a negative regulator of MET signaling in HCC.

**LECT2 Was Negatively Correlated With the Phospho-MET Level in Patients With HCC.** MET expression is an important prognostic factor for vascular invasion and HCC progression.18,19 To determine the clinical significance of our findings, we analyzed LECT2 and phospho-MET levels in primary tumors from HCC patients (n = 73). As expected, there was a significant inverse correlation between LECT2 and MET phosphorylation level in these specimens (P = 0.0004; Fig. 6A,B). Furthermore, LECT2 and MET were coimmunoprecipitated with each other in all four nonvascular invasive tumor specimens tested. MET phosphorylation levels were almost undetectable in these nonvascular invasive specimens. In contrast, MET was precipitated without LECT2 and had high phosphorylation levels in the vascular-invasive specimens (Fig. 6C). We also observed that patients with high LECT2 expression and low p-MET/MET level had less vascular-invasive tumors with longer survival time, whereas patients with low LECT2 expression and high p-MET/MET level had more vascular-invasive tumors and shorter survival time (Fig. 6D,E).

**Discussion**

Through cell functional assays, animal studies, and biochemical and molecular dissections, we demonstrated that LECT2 has unique functions in HCC tumor suppression through a mechanism involving the interaction with the MET receptor and subsequent inhibition of its activation. Of note, our data demonstrate that LECT2 associates with MET without affecting HGF binding. LECT2 was able to suppress HGF-induced MET activation, even in the presence of excess levels of HGF. The absence of LECT2 strongly promoted HCC progression, presumably as a result of increased HGF/MET activation. Furthermore, our results also demonstrate that LECT2 directly binds to the z-chain of MET, leading to recruitment of PTP1B. The recruitment of PTP-1B leads to the
MET dephosphorylation and dissociation of adaptor proteins, including Gab1, Grb2, p85, and Src (Fig. 7).

Despite clinical observations, the functional roles and molecular mechanisms of LECT2 in HCC have not been fully investigated. LECT2 was identified as a direct target gene of β-catenin in mouse liver. Recently, β-catenin-activated LECT2 has been shown to antagonize the pro-inflammatory effects of nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 and β-catenin. Furthermore, β-catenin-activated transgenic mice also developed highly malignant HCC with lung metastasis in a LECT2-depleted background (LECT2+/−). The HGF/MET-mediated activation of β-catenin has been well documented, and it is possible that LECT2 expression may also be regulated by MET-activated β-catenin. However, given our current data showing an inverse correlation between LECT2 and MET, it is unlikely that LECT2 is

**Fig. 5.** LECT2 inhibits MET activation and downstream signaling by recruiting PTP-1B. (A) SK-Hep1 LECT2-overexpressing and Huh7 LECT2-knockdown cells were immunoprecipitated with a MET Ab. The association of PTPs was examined with the indicated Abs. (B) SK-Hep1 cells were treated with or without recombinant LECT2 protein (2.5 nM) in the presence or absence of HGF (40 ng/mL), followed by IP with a MET Ab. The association of MET with different PTPs was examined by western blotting. (C) SK-Hep1 cells were transfected with siScramble, siPTP1B (siRNA ID: s11507; Ambion, Cambridge, MA), siTC-PTP (siRNA ID: s11510; Ambion), and control siLuciferase alone or they were treated with 2.5 nM of Fc-tagged recombinant LECT2 protein in the presence of HGF (40 ng/mL). Cells were then subjected to western blotting and Co-IP to evaluate MET phosphorylation and PTP associations. (D) SK-Hep1 cells were treated with or without Fc-tagged recombinant LECT2 protein (2.5 nM) in the presence of HGF (40 ng/mL), followed by IP with a MET Ab. MET phosphorylation and adapter protein associations were detected. (E) LECT2 gene expression status interferes with MET activation and downstream signaling. MET phosphorylation status and its downstream effectors, including Raf-1, Akt, Erk, p38, or glycogen synthase kinase 3 beta were measured by western blotting. (A–E) Each experiment was repeated three times.
activated by the MET-activated β-catenin pathway. Although a previous report suggested that LECT2 expression correlates with a deregulated Wnt-signaling pathway in hepatocytes and hepatoblastoma cells, LECT2 was not up-regulated in all HCC specimens harboring β-catenin-activated mutations, suggesting additional regulation machinery for LECT2 expression in HCC. Nevertheless, the molecular interplay between MET, β-catenin, and LECT2 in HCC malignancy requires further investigation.

The HGF/MET axis plays a pleotropic role in cell proliferation, migration, invasion, angiogenesis, and survival. The central role of MET activity in cancer progression and disparities in quiescent HGF/MET signaling in normal tissue and overexpression in tumors may provide a degree of tumor selectivity for therapeutic intervention, making HGF or MET inhibition an attractive strategy in oncology. A variety of HGF/MET axis inhibitors have been developed, including small-molecule compounds targeting MET kinase activity, neutralizing p-ERK expression levels of LECT2, MET, and phosphorylated MET were analyzed by immunoblotting in 73 HCC specimens. Representative blottings of high- and low-LECT2-expression specimens are shown. β-actin was used as an internal control, and sample C from one specimen was used as an external control between blottings. (B) A protein expression scatter diagram of LECT2/β-actin versus p-MET/MET is shown. Blue dots represent the expression level in individual specimens in the sample cohort, and a regression line is also shown on the plot. (C) Phosphorylated MET and LECT2-MET interactions were examined in clinical specimens from 4 patients with nonvascular invasive HCC and 4 with vascular invasive HCC. (D) Correlation between LECT2, phospho-MET protein levels, and vascular invasion status. A total of 73 HCC specimens were analyzed by immunoblotting. After normalization with MET level (i.e., phospho-MET/MET), the average expression level was recognized in arbitrary units (AU). Low phospho-MET/MET ratio (AU < 0.1); high phospho-MET/MET ratio (AU > 0.1). L2High+pMLow means high LECT2 and low phospho-MET/MET ratio; L2Low+pMHigh means low LECT2 and high phospho-MET/MET ratio; L2High+pMHigh=L2Low+pMLow means combination of low LECT2/low phospho-MET/MET ratio and high LECT2/high phospho-MET/MET ratio. Nonvascular invasion is depicted by blue columns, whereas vascular invasion is depicted by red columns. (E) Kaplan-Meier’s analysis of the overall survival of 73 patients with HCC stratified by LECT2 protein level and phospho-MET/MET ratio.
anti-MET\textsuperscript{25} and anti-HGF Abs,\textsuperscript{26} decoy receptors,\textsuperscript{27} and HGF-derived factors, such as NK4.\textsuperscript{28} Remarkably, most of these inhibitors act as competitive antagonists to the HGF/MET axis, with the exception of RTK inhibitors. In our study, LECT2 predominately binds to aa 159-175 of the MET \(\alpha\)-chain and does not interrupt HGF binding, as demonstrated by an ELISA competition assay and FCM analysis. Unlike known MET antagonists, LECT2 acts as a noncompetitive MET antagonist. Such endogenous allosteric regulation is frequently observed for metabolic enzymes, but is rarely found for RTKs. Allosteric antagonists may prevent ligand-induced conformational changes required for receptor activation. Furthermore, the association of PTP-1B and the dissociation of adaptor proteins, such as Gab1, Grb2, and Src, which are induced by LECT2 binding, may also result from conformational stabilization or changes in MET. Future structural analyses, such as X-ray crystallography or nuclear magnetic resonance spectroscopy, may provide a better understanding of how LECT2 alters MET activation. Taken together, our results suggest that LECT2 is a novel MET antagonist, and the epitope at aa 159-175 in the MET \(\alpha\)-chain may be a potential target for developing MET inhibitors.

In conclusion, we have revealed a significant correlation between LECT2 expression and MET activation, which is extensively recognized as a key regulator to HCC aggressiveness. Our findings also uncover a novel mechanism of MET regulation by the LECT2 protein and display the potential for developing LECT2 as an HCC therapeutic agent.

Acknowledgment: The authors thank Yu-Ling Wu for technical help, Dr. Morag for providing the WT MET expression construct, Dr. Ireton for providing the kinase-dead mutant (K1110A) MET construct, Hey-Chi Hsu for research suggestions, Ming Gao for cell culture and experimental suggestions, Nei-Li Chan for protein structure analysis, and Andrew H.J. Wang for proteomic MS analyses with support from the Core Facilities for Protein Structural Analysis located at the Institute of Biological Chemistry, Academia Sinica, who is supported by a National Science Council grant (NSC100-2325-B-001-029) and the Academia Sinica.

References

1. Huynh H, Ong RW, Li PY, Lee SS, Yang S, Chong LW, et al. Targeting receptor tyrosine kinase pathways in hepatocellular carcinoma. Anticancer Agents Med Chem 2011;11:560-575.
2. Hiraki Y, Inoue H, Kondo J, Kamiyama A, Yoshitake Y, Shukunami C, Suzuki F. A novel growth-promoting factor derived from fetal bovine cartilage, chondromodulin II. Purification and amino acid sequence. J Biol Chem 1996;271:22657-22662.
3. Yamagoe S, Yamakawa Y, Matsuo Y, Minowada J, Mizuno S, Suzuki K. Purification and primary amino acid sequence of a novel neutrophil chemotactic factor LECT2. Immunol Lett 1996;52:9-13.
4. Yamagoe S, Mizuno S, Suzuki K. Molecular cloning of human and bovine LECT2 having a neutrophil chemotactic activity and its specific expression in the liver. Biochem Biophys Acta 1998;1396:105-113.
5. Ovejero C, Cavard C, Perianin A, Haviroo T, Vermeulen J, Godard C, et al. Identification of the leukocyte cell-derived chemotaxin 2 as a direct target gene of beta-catenin in the liver. Hepatology 2004;40:167-176.
6. Anson M, Crain-Donoyelle AM, Baud V, Chereau F, Gougelet A, Terris B, et al. Oncogenic beta-catenin triggers an inflammatory response that determines the aggressiveness of hepatocellular carcinoma in mice. J Clin Invest 2012;122:586-599.
7. Ong HT, Tan PK, Wang SM, Hian LL, Hui KM. The tumor suppressor function of LECT2 in human hepatocellular carcinoma makes it a potential therapeutic target. Cancer Gene Ther 2011;18:399-406.
8. Zucman-Rossi J, Benhamouche S, Godard C, Boyault S, Grimer G, Balbaud C, et al. Differential effects of inactivated Axin1 and activated beta-catenin mutations in human hepatocellular carcinomas. Oncogene 2007;26:774-780.
9. Hua KT, Tan CT, Johansson G, Lee JM, Yang PW, Lu HY, et al. N-alpha-acetyltransferase 10 protein suppresses cancer cell metastasis by binding PIX proteins and inhibiting Cdc6/2/Rac1 activity. Cancer Cell 2011;19:218-231.
10. Brunelleschi S, Penengo L, Santoro MM, Gaudino G. Receptor tyrosine kinases as target for anti-cancer therapy. Curr Pharm Des 2002;8:1959-1972.
11. Summy JM, Gallick GE. Src family kinases in tumor progression and metastasis. Cancer Metastasis Rev 2003;22:337-358.
12. Basilio C, Arnesano A, Galluzzo M, Comoglio PM, Michieli P. A high affinity hepatocyte growth factor-binding site in the immunoglobulin-like region of Met. J Biol Chem 2008;283:21267-21277.
13. Stamos J, Lazarus RA, Yao X, Kirchoffer D, Wiesmann C. Crystal structure of the HGF beta-chain in complex with the Sema domain of the Met receptor. EMBO J 2004;23:2325-2335.
14. Bamford CV, Francescutti T, Cameron CE, Jenkinson HF, Dymock D. Characterization of a novel family of fibronectin-binding proteins with M23 peptidase domains from Treponema denticola. Mol Oral Microbiol 2010;25:369-383.
15. Sangwan V, Abella J, Lai A, Bertos N, Stubble M, Tremblay ML, Park M. Protein-tyrosine phosphatase 1B modulates early endosome fusion and trafficking of Met and epidermal growth factor receptors. J Biol Chem 2011;286:45000-45013.
16. Wang Z, Wang M, Carr BL. Involvement of receptor tyrosine phosphatase DEP-1 mediated PI3K-collin signaling pathway in sorafenib-induced cytoskeletal rearrangement in hepatoma cells. J Cell Physiol 2010;224:559-565.
17. Sangwan V, Pailleux GN, Abella JV, Dube N, Monast A, Tremblay ML, Park M. Regulation of the Met receptor-tyrosine kinase by the protein-tyrosine phosphatase 1B and T-cell phosphatase. J Biol Chem 2008;283:34374-34383.
18. Kaposi-Novak P, Lee JS, Gomez-Quiroz L, Coulaud C, Factor VM, Thorgersson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. J Clin Invest 2006;116:1582-1595.
19. Ke AW, Shi GM, Zhou J, Wu FZ, Ding ZB, Hu MY, et al. Role of overexpression of CD151 and/or c-Met in predicting prognosis of hepatocellular carcinoma. Hepatology 2009;49:494-503.
20. Herynk MH, Tian R, Radinsky R, Gallick GE. Activation of c-Met in colorectal carcinoma cells leads to constitutive association of tyrosine-phosphorylated beta-catenin. Clin Exp Metastasis 2003;20:291-300.
21. Nakopoulou L, Gkioupoulou H, Keramopoulos A, Giannopoulos I, Athanasiadou P, Mavrommatis J, Davaris PS. c-met tyrosine kinase
receptor expression is associated with abnormal beta-catenin expression and favourable prognostic factors in invasive breast carcinoma. Histopathology 2000;56:313-325.

22. Purcell R, Childs M, Maibach R, Miles C, Turner C, Zimmermann A, Sullivan M. HGF/c-Met related activation of beta-catenin in hepatoblastoma. J Exp Clin Cancer Res 2011;30:96.

23. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol 2003;4:915-925.

24. Trusolino L, Comoglio PM. Scatter-factor and semaphorin receptors: cell signalling for invasive growth. Nat Rev Cancer 2002;2:289-300.

25. Martens T, Schmidt NO, Eckerich C, Fillbrandt R, Merchant M, Schwall R, et al. A novel one-armed anti-c-Met antibody inhibits glioblastoma growth in vivo. Clin Cancer Res 2006;12:6144-6152.

26. Petrelli A, Circosta P, Granziero L, Mazzone M, Pisacane A, Fenoglio S, et al. Ab-induced ectodomain shedding mediates hepatocyte growth factor receptor down-regulation and hampers biological activity. Proc Natl Acad Sci U S A 2006;103:5090-5095.

27. Michieli P, Mazzone M, Basilico C, Cavassa S, Sottile A, Naldini L, Comoglio PM. Targeting the tumor and its microenvironment by a dual-function decoy Met receptor. Cancer Cell 2004;6:61-73.

28. Matsumoto K, Nakamura T. NK4 (HGF-antagonist/angiogenesis inhibitor) in cancer biology and therapeutics. Cancer Sci 2003;94:321-327.