Hepatocyte growth factor activates Wnt pathway by transcriptional activation of LEF1 to facilitate tumor invasion

Fang-I Huang, Yu-Ling Chen, Chih-Ning Chang, Ray-Hwang Yuan and Yung-Ming Jeng

1Graduate Institute of Pathology, National Taiwan University, 2Department of Pathology and 3Department of Surgery, National Taiwan University Hospital, Taipei 100, Taiwan

*To whom correspondence should be addressed. Tel: +886 2 23123456 ext. 65455; Fax: +886 223934172; Email: mrna0912@yahoo.com.tw

Hepatocyte growth factor (HGF) is a secretory protein that plays important roles in cancer growth and metastasis. Lympohoid-enhancing factor 1 (LEF1) is a transcription factor mediating Wnt/β-catenin signaling. Using microarray analysis, we found HGF induced expression of LEF1 in liver and breast cancer cell lines. HGF induced expression of LEF1 through phosphatidylinositol 3-kinase/Akt and nuclear factor-kappa B (NF-κB) signaling. Multiple NF-κB-binding sites were mapped within 3 kb upstream of LEF1 transcription initiation site. NF-κB binding to a site 2 kb upstream of LEF1 transcription initiation site was confirmed by chromatin immunoprecipitation assay. Knockdown of LEF1 inhibited the expression of Slug and zinc finger E-box-binding homeobox 2 (ZEB2) and markedly attenuated HGF-induced tumor migration and invasion. Using immunohistochemical staining, we found LEF1 was frequently expressed in multiple types of carcinoma but not in the non-tumorous epithelial cells. Our finding suggest that transcriptional activation of LEF1 is a mechanism of cross talk between HGF/c-Met and Wnt/β-catenin pathways and is essential for HGF-induced tumor invasion.

Introduction

Hepatocyte growth factor (HGF), also known as scatter factor, is pleotrophic cytokine capable of inducing cell proliferation, migration and invasion, angiogenesis and survival in cancer cells (1,2). HGF is mainly secreted from fibroblasts in tumour stroma, whereas its receptor, c-Met receptor tyrosine kinase, is present in epithelial cancer cells (2). Hence, HGF and c-Met form a simple paracrine signaling loop that mediates development and progression of cancers. High level of HGF or overexpression of c-Met are frequently observed in many types of cancer and often associated with poor prognosis (3).

Wnt/β-catenin pathway is another important pathway for tumor growth and invasion (4). In the absence of Wnt signaling, the protein level of β-catenin is tightly controlled by a multiprotein complex that contains adenomatous polyposis coli (APC), axin and glycogen synthetase kinase-3β, which phosphorylates the N-terminal of β-catenin and targets it for degradation by the proteasome system (5). Upon activation by Wnt signaling, β-catenin accumulates in cytoplasm and translocates to nuclei, where it associates with members of the T-cell factor/lymphoid-enhancing factor (TCF/LEF) family of transcription factor to turn on Wnt target genes (6). Mutations of APC or β-catenin are frequent events in several types of cancer, including colorectal cancer, endometrial cancer, and hepatocellular carcinoma (7–9). Besides genetic mutation and Wnt proteins, other growth proteins, such as epidermal growth factor and HGF also can enhance β-catenin/LEF-dependent transcription by increasing cytoplasmic β-catenin (10,11).

Another possible mechanism for modulating Wnt/β-catenin signaling is by the variable use of the nuclear binding partners of β-catenin, namely TCF4 and LEF1. They belong to the high mobility group family that regulates gene expression by inducing structural alteration in the DNA helix (12). TCF4 is the main binding partner of β-catenin in mammary and gut epithelium and is essential for maintenance of the crypt stem cells of gut (13). TCF4 knockout mice showed depletion of stem cell components of small intestine and replaced by differentiated non-dividing stem cells (14). In contrast, the expression of LEF1 is restricted to pre-T and B lymphocytes (15). LEF1 has been reported to be expressed in colorectal cancer and breast cancer (16,17). When overexpressed, LEF1 enhances tumor invasion by inducing epithelial to mesenchymal transition (EMT) (18). Therefore, overexpression of LEF1 in cancer may result in a shift of binding partner of β-catenin from TCF4 to LEF1 that enables EMT and tumor invasion. The mechanism of overexpression of LEF1 in cancer is still unknown. LEF1 has been reported to be transcriptional activated by β-catenin/TCF4 complex and it is thought to be an amplifier of Wnt/β-catenin signaling (19). However, until now, the association of activated β-catenin signaling and LEF1 expression in cancer samples has not been confirmed.

In this paper, we show LEF1 is overexpressed in multiple types of cancer. Its expression is induced by HGF through the Akt/NF-κB pathway. Overexpression of LEF1 is essential for HGF-induced tumor invasion. These results indicate transcriptional activation of LEF1 by HGF is a mechanism of cross talk between HGF/c-Met and Wnt/β-catenin in coordinating tumor migration and invasion.

Materials and methods

Cell culture

Liver cancer cell line HepG2, breast cancer cell line MDA-MB-231 and viral package cell line 293T were grown in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. All cells were grown at 37°C in a humidified atmosphere composed of 95% air and 5% CO2. The cells were passaged with trypsin/EDTA at 70–90% confluence.

Microarray analysis

Total RNA was isolated from HepG2 cell line treated with or without HGF (10 ng/ml) using TRIzol (Life Technologies, Invitrogen, Carlsbad, CA) and then extracted using an RNeasy mini kit (Qiagen, Valencia, CA), according to the manufacturer’s instructions. The complementary DNA was labeled with Cy3-ICTP or Cy5-ICTP and then applied to a microarray chip. The microarray experiment and data analysis were done by Welsegen Biotech (Taipei, Taiwan) using the Agilent Oligo Chip (Agilent Human Whole Genome Oligo 4 × 44 K Microarray). Microarrays were scanned by laser scanner and the microarray signal intensities were measured to identify gene expression differences and ratios of gene expression.

RNA isolation and real-time reverse transcription–PCR

Total RNA was isolated from cell lines using RNeasyTM C&T (PROtech, Taipei, Taiwan). Real-time PCR was performed with the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) using the SYBR Green method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping gene, served as a control for RNA quantity. The primers for LEF1 were LEF1-F (5′-CGAGCCCTAATATGTTAAAGC-3′) and LEF1-R (5′-GGTTGAGATAGCTTCCCTTATG-3′). The primers used for GAPDH were GAPDH-F (5′-AGCCTGACGATCCAGAT-3′) and GAPDH-R (5′-TGTGGTCATGAGTCCTTCAGAT-3′).

Boyden chamber assay

For invasion assays, we used modified Boyden chambers with filter inserts (pore size, 8 µm) coated with Matrigel (40 µg; Collaborative Biomedical, Becton Dickinson Labware, San Jose, CA) in 24-well dishes (Nucleopore, Pleasanton, CA). Cells (2 × 10⁵) in 100 µl of serum-free Dulbecco’s modified Eagle’s medium were placed in the upper chamber and 500 µl of Dulbecco’s

Abbreviations:

EMT, epithelial to mesenchymal transition; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HGF, hepatocyte growth factor; NF-κB, nuclear factor-kappa B; LEF1, lymphoid-enhancing factor 1; TCF, T-cell factor; siRNA, small hairpin RNA.

© The Author 2012. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com
Transcriptional activation of LEF1

modified Eagle’s medium with 2% fetal bovine serum and 40 ng/ml HGF was placed in the lower chamber. After 18 h in culture, cells were fixed in 4% paraformaldehyde for 10 min and then stained with 0.05% crystal violet and 4',6-diamidino-2-phenylindole in phosphate buffered saline with 10% Tween 20 for 15 min. Cells on the upper side of the filters were removed with cotton-tipped swabs, and the filters were washed with PBS. Cells on the underside of the filters were viewed and counted under a fluorescent microscope. Each group was plated in triplicate in each experiment, and each experiment was repeated three times.

Western blot

Protein samples (10–50 μg each) were separated via 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then electrotransferred onto nitrocellulose membranes (Amersham, Buckinghamshire, UK). The membranes were allowed to react with primary and secondary antibodies at the optimum dilutions and the immunoreactive signals were detected using an enhanced chemiluminescence kit (Millipore, Bedford, MA). The antibodies used included LEF1 (1:1000; GeneTex, San Antonio, TX), β-catenin (1:8000; BD Transduction Laboratories, Lexington, KY), Snail (1:3000; AbCam, Cambridge, MA), Slug (1:500, rabbit polyclonal; Abgent, San Diego, CA), zinc finger E-box-binding homeobox 2 (ZEB2; 1:8000; BD Transduction Laboratories, Lexington, KY), Snail (1:3000; Sigma, St Louis, MO) and β-actin (1:4000; Sigma). The band densities were quantified using AlphaEaseFC software (AlphaInnotech, San Leandro, CA).

RNA interference

For the knockdown of the endogenous LEF1, the following target sequences were constructed in the small hairpin RNA (shRNA) vector pLKO.1: shLEF1-1: 5'-GCACGTATCAACCGAGTCTTT-3'; shLEF1-2: 5'-CCGGTACATAATGATGCCAAA-3'; shLEF1-3: 5'-CCACACTGAGCTGACCAAAT-3'; shLEF1-4: 5'-GACCTAAT-3'; shLEF1-5: 5'-GCACGGAAAGAAAGACAGCTA-3'. An shRNA vector against lacZ (pLKO.1–shLacZ) was used as a negative control. These lentiviral vectors were constructed by The RNAi Consortium and were distributed by the RNAi core laboratory of Academia Sinica (Taipei, Taiwan). For production of lentiviral particles, 293T cells were transfected with 4 μg pLKO.1 lentiviral vectors along with 0.4 μg of envelope plasmid pMD.G and 3.6 μg of packaging plasmid pCMVΔR8.91. Viral particles were collected 24 and 48 h after transfection. To prepare LEF1 knockdown cells, MBA-MD-231 cells were infected with lentiviruses for 24 h. Fresh medium containing 2.5 μg/ml puromycin was replaced every 3 days for 2 weeks for drug-resistant cell selection.

Wounding assay

MDA-MB-231 cells were grown to confluence in six-well culture plates and pretreated with 40 ng/ml HGF for 16 h. Cell layers were scraped with a sterile pipet tip and reincubated at 37°C. Photographs were taken at indicated time points. Migration from the edge of the injured monolayer was quantified by measurement of the distance between the wound edges. Experiments were done in triplicate.

Chromatin immunoprecipitation

MDA-MB-231 cells were serum starved for 24 h and pretreated with or without BAY-117082 (20–40 μM) for 2 h and followed by 40 ng/ml of HGF treatment for 6 h. Formaldehyde (final concentration: 1%) was added for crosslinking. DNA was sheared by sonication. The sheared chromatin fragments were immunoprecipitated with antibody specific for p65 subunit NF-kB (Santa Cruz Biotechnology, Santa Cruz, CA) or control immunoglobulin G (Santa Cruz Biotechnology) at 4°C for 16 h. After dissociating the DNAs from the immunoprecipitated chromatin, the DNAs underwent PCR amplification with four pairs of specific primers from each putative NF-kB-binding site.

Pathway analysis

Small molecules used in pathway analysis included SU11274 (0.5 μM; Sigma), lithium chloride (LiCl) (40 μM), XAV939 (1 μM; Sigma), PD08093 (20 μM; Sigma), Wortmannin (200 nM; Sigma) and BAY-117082 (20–40 μM; Calbiochem, San Diego, CA).

Tumor samples

Tumor samples and corresponding non-tumorous tissue from patients were retrieved from the database of Department of Pathology, National Taiwan University Hospital. The study was conducted according to the regulation of Ethics Committee, and the specimens were anonymous and analyzed in a blinded manner.

Immunohistochemical analysis

Tissue sections (5 μm) were dewaxed and rehydrated. Antigen retrieval was done by autoclaving the slides in Trisodium Trifyl Phosphate (Cell Marque, Hot Springs, AR) at 121°C for 10 min. After blocking with 3% H2O2 and 5% fetal bovine serum, the slides were allowed to react with a rabbit monoclonal antibody against LEF1 (1:100, C12A5; Cell Signaling) at 4°C overnight. The slides were then incubated with polymer-HRP reagent (Dako Cytomation, Glostrup, Denmark). The peroxidase activity was visualized with diamino-benzidine tetrahydroxychloride solution (Dako). The sections were counterstained with hematoxylin. Dark brown nuclear staining was defined as positive and no staining was defined as negative. For negative control, we replaced the primary antibody with 5% fetal bovine serum. Lymphocytes in slides served as internal positive control.

Results

HGF enhances expression of LEF1

To test whether HGF is essential for fibroblast-induced tumor invasion, we isolated cancer-associated fibroblasts from an ovarian cancer. The primary fibroblasts were grown to confluency in the lower chamber of Boyden chamber. As shown in Figure 1A and B, the invasiveness of liver cancer cell line HepG2 and breast cancer cell line MDA-MB-231 induced by fibroblast was markedly inhibited by c-Met inhibitor SU11274, indicating that HGF/c-Met signaling plays important roles in tumor invasion induced by fibroblasts. After confirming the functional role of HGF in tumor invasion, we treated HepG2 cells with HGF (10 ng/ml) for 8 h and identified the
genes induced by HGF by microarray analysis. The microarray data were deposited in the GEO database (GEO accession number: GSE35994). Of the most significantly upregulated genes, we found previously reported HGF targets, including amphiregulin (AREG) (20), metalloproteinase 3 (MMP3) (21), interleukin-8 (IL-8) (22) and early growth response 1 (Egr1) (23). We confirmed the induction of these known targets by reverse transcription–PCR (Supplementary Figure S1, available at Carcinogenesis online).

Of the novel genes identified, we were particularly interested in LEF1 because of its known functional role in Wnt/b-catenin signal pathway. After treating with HGF, the mRNA and protein levels of LEF1 were significantly elevated in both HepG2 and MDA-MB-231 cells (Figure 1C and D).

**Induction of LEF1 by HGF is not due to accumulation and nuclear translocation of b-catenin**

HGF has been reported to activate canonical Wnt signaling by inducing accumulation and nuclear translocation of β-catenin in several cell line and animal systems (24–26). LEF1 was reported to be directly activated by Wnt/β-catenin signaling by binding of β-catenin/TCF4 complex to its promoter (19). Therefore, the induction of LEF1 by HGF may be caused by the activation of β-catenin signaling. So we decided to test whether HGF activates β-catenin pathway in our cell line systems. After treated with HGF for 6 h, we did not find accumulation or increased nuclear translocation of β-catenin in both MBA-MB-231 and HepG2 cells (Figure 2A and B). Furthermore, we found that treatment of both MBA-MB-231 and HepG2 cells with the Wnt/β-catenin activator lithium chloride (LiCl) results in the accumulation of β-catenin but the expression levels of LEF1 was not affected (Figure 2C). Treating MBA-MB-231 cells with β-catenin inhibitor VAX939 reduced the protein level of β-catenin but the expression level of LEF1 was not affected (Figure 2D). To be noteworthy, one of the cell lines we used, HepG2, is a hepatoblastoma cell line with constitutive activation of β-catenin pathway due to deletion of exon 3 (27). The finding that expression of LEF1 is inducible by HGF in HepG2 indicates this induction is independent of β-catenin signaling.

**LEF1 is induced by HGF through the Akt/NF-κB pathway**

We next examined whether the ERK or phosphatidylinositol 3-kinase/Akt signaling pathway is involved in HGF-mediated expression of LEF1 in MDA-MB-231 cells because they are major pathways activated by HGF (28–30). As shown in Figure 3A, treating MDA-MB-231 cells with Akt inhibitor Wortmannin markedly reduced the induction of LEF1 by HGF. One of the major pathways for activated Akt to transduce signals is via the cellular activation of NF-κB transcription factor (31). Activation of NF-κB by HGF was evidenced by nuclear accumulation of p65 subunit of NF-κB in the nucleus (Figure 3B). NF-κB inhibitor BAY-117082 attenuated the induction of LEF1 by HGF (Figure 3C). At the concentration used, no significant increase of apoptotic cell is detected, indicating the effect was not due to decreased cell viability (Supplementary Figure S2, available at Carcinogenesis online). A computer-aided analysis (by the AliBaba 2 software) of the proximal 3 kb of LEF1 promoter showed the presence of four putative consensus regions for NF-κB binding.

![Fig. 2. Induction of LEF1 by HGF is not due to accumulation and nuclear translocation of β-catenin. Treating MDA-MB-231 cells and HepG2 cells with HGF did not induce accumulation (A) or nuclear translocation (B) of β-catenin. (C) Activation of canonical β-catenin pathway by treatment with LiCl (40 μM) did not induce the expression of LEF1. (D) Inhibition of β-catenin pathway by treatment with XAV939 (1 μM) did not reduce the protein level of LEF1 in MDA-MB-231 cells. N, nuclear; C, cytosolic; Wt, wild-type β-catenin; Mt, mutant β-catenin. The numbers below the β-catenin lanes in (B) indicate the proportion of nuclear β-catenin.](image-url)
Transcriptional activation of LEF1

Figure 3. LEF1 is induced by HGF through the Akt/NF-κB pathway. (A) MDA-MB-231 cells were serum starved for 24 h and pretreated with PD98059 (20 μM) and Wortmannin (200 nM) for 2 h and then incubated with 40 ng/ml of HGF for additional 6 h. The cell lysates were subjected to western blot analysis. The induction of LEF1 by HGF was markedly inhibited by Wortmannin. (B) Nuclear and cytosolic fractions of HGF-treated MDA-MB-231 cells were extracted. p65 subunit of NF-κB accumulated in nuclei of HGF-treated cells. (C) MDA-MB-231 cells were serum starved for 24 h and pretreated with NF-κB inhibitors BAY-117082 (20–40 μM) for 2 h and then incubated with 40 ng/ml of HGF for additional 6 h. The induction of LEF1 by HGF was inhibited by NF-κB inhibitor. (D) The putative NF-κB-binding sites on LEF1 promoter. (E) ChIP analysis of NF-κB binding to the LEF1 promoter. NF-κB bound to site d and the binding was inhibited by NF-κB inhibitor BAY-117082. The numbers below the p65 lanes in (B) indicated the proportion of nuclear p65.

LEF1 expression is essential for HGF-induced tumor invasion

Five different lentiviral constructs carrying LEF1 shRNAs were used to transduce breast cancer cell line MDA-MB-231 to test whether the induction of LEF1 is essential for HGF-induced tumor invasion. Cells transduced with shRNAs #4 and #5 resulted in a dramatic reduction of LEF1 protein expression (Figure 4A) and were selected for subsequent studies. We first tested the effect of LEF1 knockdown on cell proliferation. 3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay showed that knockdown had no effect on cell survival and proliferation (data not shown), so its effect on subsequent studies cannot be simply accounted by cell proliferation. In the modified Boyden chamber assay, HGF enhanced the invasion of control shLacZ cells. However, knockdown of LEF1 dramatically inhibited tumor invasion induced by HGF (Figure 4B). Wounding assay also showed reduced cell migration in LEF1-knocked down cells in the presence of HGF. Previous studies showed overexpression of LEF1 induces EMT in normal epithelial cell lines (18). Therefore, we tested whether the expression of EMT-inducing transcriptional factors was affected by depletion of LEF1. As shown in Figure 4D, knockdown of LEF1 resulted in the reduction of protein levels of Slug and ZEB2 but not Snail.

LEF1 is overexpressed in several types of epithelial cancer

Immunohistochemical staining was performed to determine the expression of LEF1 in cancer and non-tumorous cells. LEF1 was not expressed in non-tumorous epithelial cells of breast, lung, kidney, stomach and liver. In oral mucosa, LEF1 was occasionally expressed in basal and parabasal cells of squamous epithelium. In contrast, LEF1 was expressed in various frequencies in breast (42%), lung (16%), oral (22%), renal (13%), gastric (13%) and liver (7%) cancers (Figure 5 and Table I). In the ovary, LEF1 was frequently expressed in serous carcinoma (67%) but rarely expressed in clear cell carcinoma (8%). The specificity of immunostaining was confirmed by negative control staining using rabbit non-immune immunoglobulin G instead of the primary antibody (Supplementary Figure S3, available at Carcinogenesis online). The results suggest that overexpression of LEF1 is one mechanism for cancer cells to promote invasion.

Discussion

It is well documented that cross talk between cancer cells and host stromal cells such as fibroblasts through cytokines has a great...
HGF is one of the proteins secreted by fibroblasts and it mediates tumor development and progression (1,2). One of the most important functions of HGF is promoting motility and tumor invasion (1). However, the mechanism accounting for its invasion-promoting activity is still poorly identified.

HGF/c-Met pathway cross talks with many other pathways, one of them being the Wnt/β-catenin pathway (11). Treating mammary epithelial cells with HGF leads to decrease in GSK-3 activity and increases the cytoplasmic pool of β-catenin (11). c-Met associates with β-catenin at the inner surface of the hepatocyte cell membrane. HGF induces nuclear translocation of β-catenin in hepatocytes by dissociating Met–β-catenin complex (33). Treating colon cancer stem cells with HGF induces nuclear translocation of β-catenin and this activity is essential for the maintenance of cancer stem cell phenotype (34). However, our results indicate that HGF cannot induce accumulation or nuclear translocation of β-catenin in breast or liver cancer cell lines, indicating that this pathway may be context dependent. Nuclear β-catenin is rarely seen in breast cancers and in those with nuclear β-catenin, only a small subset of cancer cells has nuclear accumulation (35). These observations suggest cancer may use other mechanisms to activate Wnt/β-catenin pathway.

In this study, we used microarray analysis to identify HGF-induced genes. Based on the similar roles of HGF/c-Met and Wnt/β-catenin pathways in tumor invasion, we proposed that the induction of LEF1 by HGF is a mechanism for activation of β-catenin signaling in tumor cells by stromal fibroblasts and LEF1 is indispensable for HGF-induced tumor invasion. It has been reported that LEF1 mediates metastasis in pulmonary adenocarcinoma (36). LEF1 also enhances growth and invasion in prostate cancer (37). Previous studies showed LEF1 is overexpressed in colorectal cancer, breast cancer and prostate cancer (16,17,37). We further found LEF1 is expressed at high frequency in ovarian cancer and oral cancer. The high basal level of LEF1 in cancer cells is probably to increase transcriptional activation of target genes without elevation of nuclear β-catenin level. It may also result in shifting of nuclear binding partner of β-catenin from other members of TCF/LEF family to LEF1, which may have different target genes. Of the candidate target genes, we tested EMT-inducing transcription factors and found Slug and ZEB2 are regulated by LEF1. Slug has been reported to be a direct target of LEF1 in osteoblasts and embryonic ectoderm (38,39). Further studies will be done to identify more targets of LEF1.

Employing specific inhibitors, we identified Akt/NF-κB pathway is the mechanism for inducing LEF1 expression by HGF. Yun et al. (40) found NF-κB regulates LEF1 expression in chondrocytes. A difference between our result and their finding is that they found NF-κB binds to conserved binding site at the 14 kb upstream of LEF1 transcription initiation site but we found a binding site just 2 kb upstream of LEF1 transcription initiation site. It is likely that NF-κB regulates LEF1 expression by binding to multiple sites in LEF1 promoter. Since Akt/NF-κB pathway is also frequently activated by other growth factors, induction of LEF1 expression may be a common pathway to promote motility of cancer cells by growth factors.

**Supplementary material**

Supplementary Figures S1–S3 can be found at http://carcin.oxfordjournals.org/.
Table I. Expression of LEF1 in various cancers

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>LEF1</th>
<th>Total case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell</td>
<td>54 (76%)</td>
<td>13 (18%)</td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal</td>
<td>48 (58%)</td>
<td>25 (30%)</td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>26 (83%)</td>
<td>4 (13%)</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal cell</td>
<td>33 (86%)</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td>21 (33%)</td>
<td>23 (36%)</td>
</tr>
<tr>
<td>Clear cell</td>
<td>21 (93%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>14 (93%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>14 (88%)</td>
<td>2 (13%)</td>
</tr>
</tbody>
</table>

- ‘−’, no staining; ‘+’, <10% cancer cells stained; ‘++’, 10–50% cancer cells stained; ‘+++’, >50% cancer cells stained.

References


Funding

National Science Council, Republic of China (101-2811-B-002-008-).

Acknowledgements

RNAi reagents were obtained from the National RNAi Core Facility located at the Institute of Molecular Biology/Genomic Research Center, Academia Sinica.

Conflict of Interest Statement: None declared.


Received December 9, 2011; revised February 29, 2012; accepted March 14, 2012