Knockdown of Receptor-Interacting Serine/Threonine Protein Kinase-2 (RIPK2) Affects EMT-associated Gene Expression in Human Hepatoma Cells

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Abstract. *Background: Receptor-interacting serine/ threonine protein kinase-2 (RIPK2) has been reported to be an important regulator of tumor proliferation, differentiation and wound repair. We investigated the effects of RIPK2 knockdown in human hepatoma cells on epithelial-to-mesenchymal transition (EMT)-associated gene expression. Materials and Methods: HepG2 cells stably expressing RIPK2-shRNA (HepG2-shRIPK2) were generated after puromycin selection. Total RNAs from HepG2-shRIPK2 and from HepG2-shcontrol cells were isolated and PCR-based arrays were performed to compare the 84 EMT-associated gene expressions. Results: We observed that knockdown of RIPK2 down-regulated mRNA expression of jagged 1 (JAG1); plasminogen activator inhibitor-1 (PAI1); regulator of G-protein signalling 2, 24 kDa (RGS2); E-cadherin (CDH1); fibroblast growth factor binding protein 1 (FGFBP1); snail homolog 2 (SNAI2); protein tyrosine phosphatase type IVA, member 1 (PTP4A1); keratin 19 (KRT19); vimentin (VIM); and survival of motor neuron protein-interacting protein 1 (SIP1). Conclusion: We found that knockdown of RIPK2 down-regulated nuclear factor kappa B (NF-κB)-dependent PAI1 and VIM gene expressions. RIPK2 might play an important role in hepatic cell migration. These findings could shed new light on carcinogenesis and on liver regeneration.*

Receptor-interacting protein (RIP) family kinases have emerged as essential sensors of cellular stress (1). RIP kinases (RIPKs) are closely related to members of the interleukin-1-receptor-associated kinase (IRAK) family. To date, five RIPKs are known (2). They play important roles in situations of cellular stress caused by different factors, such as pathogen infection, inflammation, cellular differentiation programs and DNA damage, and eventually lead to the activation of transcription factors, such as nuclear factor kappa B (NF-κB), the induction of apoptotic processes, or activation of mitogen-activated protein kinase (MAPK) (1, 2).

Our group as well as others showed that receptor-interacting serine/ threonine protein kinase-2 (RIPK2, also known as RIP2, RICK or CARDIAK), a caspase-recruitment domain-containing kinase, plays an important role in cell migration and wound healing in keratinocytes as well as hepatocytes (2, 3). RIPK2 is also involved in the Toll-like receptor (TLR)-signalling pathway and plays an important role in the production of inflammatory cytokines through NF-κB activation (4, 5). RIPK2 also plays an important role in nucleotide-binding oligomerization domain containing 1 (NOD1) ligand-induced NF-κB activation in hepatocytes (3).

The extracellular matrix (ECM), which consists of collagens, glycoproteins, proteoglycans and glycosaminoglycans, provides cells with positional information and a mechanical scaffold for adhesion and migration. Chronic fibrogenesis can be regarded as a continuous wound-healing process that results in scar formation (6). Dynamic interactions between growth factors and the ECM are integral to wound healing (7, 8). Wound healing, and inflammatory processes, as well as changes in the tumor microenvironment through remodeling of the ECM, are important for cancer metastasis (8). The epithelial-to-mesenchymal transition (EMT) now takes center stage as the convergence point between inflammation and the progression of degenerative fibrotic diseases and cancer (9). EMT includes many processes associated with differentiation and development, morphogenesis, cell growth and proliferation, migration and motility, cytoskeleton formation, ECM and cell adhesion, and related signalling pathways, as well as transcription factors. The NF-κB family of transcription factors plays pivotal roles in both promoting and maintaining the cell phenotype (9, 10). Inflammation is necessary for EMT (11), and NF-κB plays an important role in the induction of inflammation (12).

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Key Words: EMT, HCC, NF-κB, RIPK2, hepatoma cells.

In the present study, we uncovered a novel function of endogeneous RIPK2, which is located upstream of NF-κB. Knockdown of *RIPK2* in human hepatoma cells affected EMT-associated gene expression.

Materials and Methods

Cell culture. Human hepatoblastoma HepG2 cells were maintained in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum under 5% CO_2, at 37˚C.

Transfection of cells. HepG2 cells were transfected with plasmid control-shRNA or RIPK2-shRNA (Santa Cruz Biotechnology, CA, USA). After 48 h of transfection, cells were split and treated with puromycin for selection of antibiotic-resistant colonies. Individual colonies were picked-up and examined for expression of endogenous RIPK2 by western blotting with specific antibodies against RIPK2 (3), and clones HepG2-shC and HepG2-shRIPK2 [HepG2-shRIPK2-3 (3)], in which *RIPK2* expression was knocked-down, were selected for subsequent studies.

RNA extraction. Cells were seeded into 6-well plates, and total cellular RNA was extracted 48 h later, using the RNeasy Mini kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. RNA samples were then stored at –80˚C until use. RNA quality was examined using the A_{280}/A_{260} ratio (Pharmacia Biotech, Bedford, MA, USA).

cDNA synthesis and real-time polymerase chain reaction (PCR). cDNA synthesis was performed using RT2 First Strand Kit (SABiosciences, Frederick, MD, USA). Each 1 μg of RNA was subjected to one reaction. The cDNA synthesis reaction was performed as follows: incubation at 42˚C for 15 min and then reaction stoppage by heating at 95˚C for 15 min. RNA quantification was conducted by real-time PCR with SyBr Green I, as described previously (12, 13). Gene quantification was determined using an ABI Prism 7300 instrument from Applied Biosystems (Foster City, CA, USA). Thermal cycling conditions were 95˚C for 10 min, followed by 40 cycles at 95˚C, 15 s for denaturation, and 1 min at 60˚C for annealing and extension. All primers for examining human EMT-associated gene expression were purchased from SABiosciences. EMT-associated genes examined in the present study are listed in Table I. Gene expression was normalized to that of house-keeping genes (beta-2-microglobulin, hypoxanthine phosphoribosyltransferase 1, ribosomal protein L 13a, glyceraldehyde-3-phosphate dehydrogenase and beta-actin) to determine the fold-change in gene expression between test (HepG2-shRIPK2) and control (HepG2-shC) samples by the 2^{-ddCT} (comparative cycle threshold) method (13). We performed each of these experiments in triplicate. Genes were annotated by Entrez Gene (NCBI, Bethesda, MD, USA).

Statistical analysis. Data were analyzed with RT^2 prolifer PCR array data analysis software (http://www.superarry.com/pcrarraydata analysis.php).

Results

Down-regulated genes among EMT-associated genes in RIPK2-knockdown HepG2 cells. RIPK2 functions as a signal transducer for both the innate and adaptive immune activation pathways (14). In our previous study, we observed that RIPK2 plays an important role in cell migration and wound healing in hepatocytes (3). We examined EMT-associated gene expression profiles using real-time PCR-based focused microarrays. A comparison of EMT-associated genes between HepG2-shC and HepG2-shRIPK2 is shown in Tables II and III. Among the 84 EMT-associated genes examined, collagen, type 1, alpha 2 gene (*COL1A2*) was undetected in both types of samples and the average threshold cycle of 12 genes was relatively high (>30), meaning that their relative expression level was low. We thus excluded these 13 genes from further analysis. Out of the remaining 71 genes, 10 (14.0%) were significantly down-regulated by the knockdown of *RIPK2* ($p<0.05$; Table II). Among these 10 genes, six [jagged 1 (*JAG1*); plasminogen activator inhibitor-1 (*PAI1*); regulator of G-protein signalling 2, 24 kDa (*RGS2*); E-cadherin (*CDH1*); fibroblast growth factor binding protein 1 (*FGFBP1*); and snail homolog 2 (*SNAI2*)] were down-regulated by 1.5-fold or more in HepG2-shRIPK2 cells.

Up-regulated genes among EMT-associated genes in RIPK2-knockdown HepG2 cells. Out of the remaining 71 genes, six (8.4%) were significantly up-regulated by the knock-down of *RIPK2* ($p<0.05$; Table III). Out of these six genes, four [collagen, type V, alpha 2 (*COL5A2*); matrix metalloproteinase 2 (MMP2); MMP3; and goosecoid homeobox (*GSC*)] were up-regulated by 1.5-fold or more in HepG2-shRIPK2 cells.

Discussion

In the present study, we observed that 10 genes were significantly down-regulated in HepG2-shRIPK2 cells. Out of these 10 genes, *PAI1* and vimentin (*VIM*) are NF-κB-dependent genes (15, 16). We also observed 6 genes were significantly up-regulated in HepG2-shRIPK2 cells. It was reported that NF-κB is involved in the expression of the wingless-type mouse mammary tumor virus (MMTV) integration site family, member 5B (WNT5B), MMP2 and MMP3 (17-19). Our previous study (3) showed that knockdown of *RIPK2* has an effect on NOD1 ligand C12-iE-DAP-induced NF-κB activation in HepG2 cells, suggesting that RIPK2 plays an important role in NF-κB activation induced through NOD1 triggering in hepatocytes. We also observed that silencing of RIPK2 was associated with the reduction of interleukin-6 (IL6), IL8 and hepatic wound closure (3).

PAI1, a multifaceted proteolytic factor, plays an important role in the plasminogen/plasmin system as it is the main inhibitor of tissue-type and urokinase-type plasminogen activator (20). PAI1 also plays an important role in signal transduction, cell adherence and cell migration (21). It was reported that PAI1 is associated with poor prognosis in several types of cancers (21) and that it is associated with hepatocellular carcinoma (HCC) caused by hepatitis B and C (22). Recently, single-nucleotide polymorphism (SNP) of

Table I. *Genes associated with epithelial-to-mesenchymal transition (EMT) assessed by real-time RT-PCR in the present study.*

Genes up-regulated during EMT

Symbol	Name
AHNAK	AHNAK nucleoprotein
BMP1	Bone morphogenetic protein 1
CALD1	Caldesmon 1
CAMK2N1	Calcium/calmodulin-dependent protein kinase II inhibitor
CDH2	Cadherin 2, type 1, N-cadherin (neuronal)
COL1A2	Collagen, type I, alpha 2
COL3A1	Collagen type III, alpha 1
COL5A2	Collagen, type V, alpha 2
FN1	Fibronectin 1
FOXC2	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)
GNG11	Guanine nucleotide binding protein (G protein), gamma 11
GSC	Goosecoid homeobox
IGFBP4	Insulin-like growth factor binding protein 4
ITGA5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
ITGAV	Integrin, alpha V (vitronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
MMP2	Matrix metalloproteinase 2 (gelatinase A, 72 kDa gelatinase, 72 kDa type IV collagenase)
MMP3	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)
MMP9	Matrix metalloproteinase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase)
MSN	Moesin
PAI1	Plasminogen activator inhibitor type 1
SNAI1	Snail homolog 1 (*Drosophila*)
SNAI2	Snail homolog 2 (*Drosophila*)
SNAI3	Snail homolog 3 (*Drosophila*)
SOX10	SRY (sex-determining region Y)-box 10
SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)
STEAP1	Six transmembrane epithelial antigen of the prostate 1
TCF4	Transcription factor 4
TIMP1	Tissue inhibitor of metalloproteinases-1
TMEFF1	Transmembrane protein with EGF-like and two follistatin-like domains 1
TMEM132A	Transmembrane protein 132A
TWIST1	Twist homolog 1 (*Drosophila*)
VCAN	Versican
VIM	Vimentin
VPS13A	Vacuolar protein sorting 13 homolog A *(Saccharomyces cerevisiae)*
WNT5A	Wingless-type MMTV integration site family, member 5A
WNT5B	Wingless-type MMTV integration site family, member 5B

Genes down-regulated during EMT

Symbol	Name
CAV2	Caveolin 2
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)
DSP	Desmoplakin
FGFBP1	Fibroblast growth factor binding protein 1
IL1RN	Interleukin 1 receptor antagonist
KRT19	Keratin 19
MITF	Microphthalmia-associated transcription factor
MST1R	Macrophage stimulating 1 receptor (c-MET-related tyrosine kinase)
NUDT13	Nudix (nucleoside diphosphate linked moiety X)-type motif 13
OCLN	Occludin
PPPDE2	PPPDE peptidase domain containing 2
RGS2	Regulator of G-protein signalling 2, 24 kDa
SPP1	Secreted phosphoprotein 1
TFPI2	Tissue factor pathway inhibitor 2
TSPAN13	Tetraspanin 13

continued

Table I. *continued*.

Differentiation and development

Symbol	Name
AKT1	V-akt murine thymoma viral oncogene homolog 1
BMP1	Bone morphogenetic protein 1
BMP7	Bone morphogenetic protein 7
COL3A1	Collagen, type III, alpha 1
COL5A2	Collagen, type V, alpha 2
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa
DSP	Desmoplakin
ERBB3	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
F11R	F11 receptor
FOXC2	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)
FZD7	Frizzled homolog 7 (*Drosophila*)
GSC	Goosecoid homeobox
JAG1	Jagged 1 (Alagille syndrome)
KRT14	Keratin 14
MITF	Microphthalmia-associated transcription factor
MST1R	Macrophage-stimulating 1 receptor (c-MET-related tyrosine kinase)
NODAL	Nodal homolog (mouse)
NOTCH1	Notch homolog 1, translocation-associated (*Drosophila*)
PTP4A1	Protein tyrosine phosphatase type IVA, member 1
SMAD2	SMAD family member 2
SNAI1	Snail homolog 1 (*Drosophila*)
SNAI2	Snail homolog 2 (*Drosophila*)
SOX10	SRY (sex determining region Y)-box 10
TGFB2	Transforming growth factor, beta 2
TGFB3	Transforming growth factor, beta 3
TMEFF1	Transmembrane protein with EGF-like and two follistatin-like domains 1
TWIST1	Twist homolog 1 (*Drosophila*)
VCAN	Versican
WNT11	Wingless-type MMTV integration site family, member 11
WNT5A	Wingless-type MMTV integration site family, member 5A
WNT5B	Wingless-type MMTV integration site family, member 5B

Morphogenesis

Symbol	Name
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa
FOXC2	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)
JAG1	Jagged 1 (Alagille syndrome)
RAC1	RAS-related C3 botulinum toxin substrate 1 (Rho family, small GTP binding protein RAC1)
SMAD2	SMAD family member 2
SNAI1	Snail homolog 1 (*Drosophila*)
SOX10	SRY (sex-determining region Y)-box 10
TGFB1	Transforming growth factor, beta 1
TGFB2	Transforming growth factor, beta 2
TGFB3	Transforming growth factor, beta 3
TWIST1	Twist homolog 1 (*Drosophila*)
WNT11	Wingless-type MMTV integration site family, member 11
WNT5A	Wingless-type MMTV integration site family, member 5A

Cell growth and proliferation

Symbol	Name
AKT1	V-akt murine thymoma viral oncogene homolog 1
BMP1	Bone morphogenetic protein 1

continued

Table I. *continued*.

Symbol	Name
BMP7	Bone morphogenetic protein 7
CAV2	Caveolin 2
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa
EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
ERBB3	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
FGFBP1	Fibroblast growth factor binding protein 1
FOXC2	Forkhead box C2 (MEF-1, mesenchyme forkhead 1)
IGFBP4	Insulin-like growth factor binding protein 4
ILK	Integrin-linked kinase
JAG1	Jagged 1 (Alagille syndrome)
MST1R	Macrophage stimulating 1 receptor (c-MET-related tyrosine kinase)
NODAL	Nodal homolog (mouse)
PDGFRB	Platelet-derived growth factor receptor, beta polypeptide
TGFB1	Transforming growth factor, beta 1
TGFB2	Transforming growth factor, beta 2
TGFB3	Transforming growth factor, beta 3
TIMP1	Tissue inhibitor of metalloproteinases-1
VCAN	Versican
ZEB1	Zinc finger E-box binding homeobox 1

Migration and motility

Symbol	Name
CALD1	Caldesmon 1
CAV2	Caveolin 2
EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
FN1	Fibronectin 1
ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
JAG1	Jagged 1 (Alagille syndrome)
MSN	Moesin
MST1R	Macrophage stimulating 1 receptor (c-MET-related tyrosine kinase)
NODAL	Nodal homolog (mouse)
PDGFRB	Platelet-derived growth factor receptor, beta polypeptide
RAC1	RAS-related C3 botulinum toxin substrate 1 (Rho family, small GTP binding protein RAC1)
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)
TGFB1	Transforming growth factor, beta 1
VIM	Vimentin

Cytoskeleton

Symbol	Name
CAV2	Caveolin 2
KRT7	Keratin 7
MAP1B	Microtubule-associated protein 1B
PLEK2	Pleckstrin 2
RAC1	RAS-related C3 botulinum toxin substrate 1 (Rho family, small GTP binding protein RAC1)
VIM	Vimentin

Extracellular matrix and cell adhesion

Symbol	Name
BMP1	Bone morphogenetic protein 1
BMP7	Bone morphogenetic protein 7
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)
CDH2	Cadherin 2, type 1, N-cadherin (neuronal)

continued

Table I. *continued*.

Symbol	Name
COL1A2	Collagen, type I, alpha 2
COL3A1	Collagen, type III, alpha 1
COL5A2	Collagen, type V, alpha 2
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa
DSC2	Desmocollin 2
EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
ERBB3	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
F11R	F11 receptor
FN1	Fibronectin 1
FOXC2	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)
ILK	Integrin-linked kinase
ITGA5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
ITGAV	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
MMP2	Matrix metallopeptidase 2 (gelatinase A, 72 kDa gelatinase, 72 kDa type IV collagenase)
MMP3	Matrix metallopeptidase 3 (stromelysin 1, progelatinase)
MMP9	Matrix metallopeptidase 9 (gelatinase B, 92 kDa gelatinase, 92 kDa type IV collagenase)
PAI1	Plasminogen activator inhibitor type 1
PTK2	PTK2 protein tyrosin kinase 2
RAC1	RAS-related C3 botulinum toxin substrate 1 (Rho family, small GTP binding protein RAC1)
SPP1	Secreted phosphoprotein 1
TGFB1	Transforming growth factor, beta 1
TGFB2	Transforming growth factor, beta 2
TIMP1	Tissue inhibitor of metalloproteinases-1
VCAN	Versican

Signalling pathways

Symbol	Name
Estrogen Receptor	
CAV2	Caveolin 2
ESR1	Estrogen receptor 1
KRT19	Keratin 19
TGFB3	Transforming growth factor, beta 3
G-Protein Coupled Receptor	
AKT1	V-akt murine thymoma viral oncogene homolog 1
FZD7	Frizzled homolog 7 (*Drosophila*)
GNG11	Guanine nucleotide binding protein (G protein), gamma 11
RAC1	RAS-related C3 botulinum toxin substrate 1 (Rho family, small GTP binding protein RAC1)
RGS2	Regulator of G-protein signalling 2, 24 kDa
Integrin-Mediated	
COL3A1	Collagen, type III, alpha 1
ILK	Integrin-linked kinase
ITGA5	Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
ITGAV	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
PTK2	PTK2 protein tyrosine kinase 2
Notch	
FOXC2	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)
JAG1	Jagged 1 (Alagille syndrome)
NOTCH1	Notch homolog 1, translocation-associated (*Drosophila*)
Receptor Tyrosine Kinase	
EGFR	Epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
ERBB3	V-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)
PDGFRB	Platelet-derived growth factor receptor, beta polypeptide
RGS2	Regulator of G-protein signalling 2, 24 kDa
SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)

continued

Table I. *continued*.

Symbol	Name
TGFβ/BMP	
BMP1	Bone morphogenetic protein 1
BMP7	Bone morphogenetic protein 7
COL3A1	Collagen, type III, alpha 1
SMAD2	SMAD family member 2
TGFB1	Transforming growth factor, beta 1
TGFB2	Transforming growth factor, beta 2
TGFB3	Transforming growth factor, beta 3
WNT	
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa
FZD7	Frizzled homolog 7 (*Drosophila*)
GSK3B	Glycogen synthase kinase 3 beta
WNT11	Wingless-type MMTV integration site family, member 11
WNT5A	Wingless-type MMTV integration site family, member 5A
WNT5B	Wingless-type MMTV integration site family, member 5B

Transcription factors

Symbol	Name
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88 kDa
ESR1	Estrogen receptor 1
FOXC2	Forkhead box C2 (MFH-1, mesenchyme forkhead 1)
GSC	Goosecoid Homeobox
MITF	Microphthalmia-associated transcription factor
NOTCH1	Notch homolog 1, translocation-associated (*Drosophila*)
SIP1	Survival of motor neuron protein interacting protein 1
SMAD2	SMAD family member 2
SNAI2	Snail homolog 2 (*Drosophila*)
SNAI3	Snail homolog 3 (*Drosophila*)
SOX10	SRY (sex-determining region Y)-box 10
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)
TCF3	Transcription factor 3 (E2A immunoglobulin enhancer binding factors E12/E47)
TCF4	Transcription factor 4
TWIST1	Twist homolog 1 (*Drosophila*)
ZEB1	Zinc finger E-box binding homeobox 1
ZEB2	Zinc finger E-box binding homeobox 2

EGF: Epidermal growth factor; MMTV: mouse mammary tumor virus.

PAI1 was reported to be associated with treatment response in patients with chronic hepatitis C, treated with pegylated-interferon plus ribavirin (23).

VIM is a member of the intermediate filament family of proteins. VIM provides cellular integrity under mechanical stress *in vivo* with a resilience not related to microtubule or actin filament networks (24). It was reported that VIM interacts with hepatitis B or C viral proteins (25, 26). Our previous study (3) showed that hepatitis B virus e antigen inhibits RIPK2 expression and interacts with RIPK2, which might represent two mechanisms through which hepatitis B virus e antigen blocks NOD1 ligand-induced NF-κB activation in HepG2 cells. We also observed that hepatitis B virus e antigen inhibits cell migration (3).

Our hypothesis is that RIPK2 plays an important role in hepatic cell migration and wound repair in the liver, possibly due to: i) activation of NF-κB through RIPK2; ii) production of inflammatory cytokines IL6 and IL8, which are also important for regeneration of the liver through activation of NF-κB; and iii) up-regulation of EMT-associated NF-κB-dependent genes, such as *PAI1* and *VIM*. In the present study, we also observed up-regulated genes such as *MMPs*, especially important in ECM and EMT. Further studies are required to determine this.

In conclusion, we observed that knockdown of *RIPK2* down-regulated the expression of the NF-κB-dependent genes *PAI1* and *VIM*. RIPK2 might play an important role in hepatic cell migration. These findings could shed new light on carcinogenesis and regeneration of the liver.

Table II. *Genes significantly down-regulated by knockdown of Receptor-Interacting Serine/Threonine Protein Kinase-2 (RIPK2) in HepG2 cells.*

Gene name	Fold change	Biological process
JAG1	–3.49	Differentiation and development, morphogenesis, cell growth and proliferation, migration and motility, Notch singalling
PAI1	–1.92	Genes up-regulated during EMT, extracellular matrix and cell adhesion
RGS2	–1.64	Genes down-regulated during EMT, G-protein coupled receptor, and receptor tyrosine kinase
CDH1	–1.61	Genes down-regulated during EMT
FGFBP1	–1.59	Genes down-regulated during EMT, cell growth and proliferation
SNAI2	–1.55	Genes up-regulated during EMT, differentiation and development
PTP4A1	–1.46	Differentiation and development
KRT19	–1.38	Genes down-regulated during EMT, estrogen receptor
VIM	–1.29	Genes up-regulated during EMT, migration and motility, cytoskeleton
SIP1	–1.24	Transcription factors

Refer to Table I for full gene names. EMT: Epithelial-to-mesenchymal transition.

Table III. *Genes significantly up-regulated by knockdown of Receptor-Interacting Serine/Threonine Protein Kinase-2 (RIPK2) in HepG2 cells.*

Gene name	Fold change	Biological process
WNT11	1.34	Differentiation and development, morphogenesis, WNT signalling
WNT5B	1.38	Genes up-regulated during EMT, differentiation and development, WNT
GSC	1.63	Genes up-regulated during EMT, differentiation and development
MMP2	1.71	Genes up-regulated during EMT, extracellular matrix and cell adhesion
MMP3	2.00	Genes up-regulated during EMT, extracellular matrix and cell adhesion
COL5A2	3.10	Differentiation and development, extracellular matrix and cell adhesion

Refer to Table I for full gene names. EMT: Epithelial-to-mesenchymal transition; WNT, wingless-type mouse mammary tumor virus (MMTV) integration site family.

Declaration of Interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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