Endocr (2008) 33:135–143
DOI 10.1007/s12020-008-9065-y

ORIGINAL PAPER

Resistin is expressed in human hepatocytes and induces insulin resistance

Chun Hua Sheng · Jun Di · Yong Jin · Yu Cheng Zhang · Mei Wu · Ying Sun · Gui Zhen Zhang

Received: 29 January 2008 / Accepted: 14 April 2008 / Published online: 30 April 2008

Abstract Resistin, known as an adipocyte-specific secretory factor (ADSF), is implicated to modulate insulin resistance in rodents. However, the precise role of this factor for human insulin resistance has remained elusive. Here, we investigate the relationship between human resistin and insulin resistance in hepatocytes and the effect of Metformin on resistin. In this study, the expression of resistin in human hepatocytes and hepatic tissues was examined, and the human resistin eukaryotic expression vector was constructed and stably transfected in HepG2 cells. Data showed that resistin is expressed in human hepatocytes and hepatic tissues. Overexpression of human resistin impaired significantly insulin-stimulated glucose uptake and glycogen synthesis in HepG2 cells. It also decreased the expression of insulin receptor substrate 2 (IRS-2) and c-cbl associated protein (CAP), whereas increased the expression of glycogen synthetase kinase 3β (GSK-3β). The result suggested that human resistin induced insulin resistance in hepatocytes by blocking the two insulin signal transduction pathways of PI-3K/Akt and of CAP/c-cbl. We also concluded that Metformin reversed the effect of resistin and downregulated the expression of resistin in hepatocytes.

C. H. Sheng · J. Di · Y. C. Zhang · M. Wu · G. Z. Zhang (✉)
Department of Central Research, The Third Clinical College, Jilin University, Xiantai Street No. 126, Changchun 130033, China
e-mail: zhangguizhenjlu@yahoo.com

C. H. Sheng · Y. Jin
Department of Endocrin of 208 Hospital, Changchun, China

Y. Sun
King's College London, Division of Asthma, Allergy Lung Biology, MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, London, UK

Keywords Human resistin · HepG2 cells · Insulin resistance · Insulin signal transduction pathway · Metformin

Introduction

Resistin belongs to a gene family found in inflammatory zone (FIZZ) or in resistin-like molecule (RELM) [1]. Most researchers accept the effect of resistin of inducing insulin resistance in rodents [1–6]. However, it is controversial in the studies of evaluating resistin expression related with type 2 diabetes in humans [7–11]. Thus, it is necessary to investigate the effect of human resistin on target cells on which insulin acts. As we know, the liver, an important target of insulin, plays a crucial role in regulating glucose levels in vivo. Hepatic insulin resistance is presumed to be importantly responsible for the development of type 2 diabetes [12]. Current studies in rodents indicated that resistin impaired hepatic insulin sensitivity, increased liver sugar output [13, 14] and downregulated PI/3K insulin signal transduction pathway [5], which suggests that resistin may directly induce insulin resistance in the liver in rodents. Furthermore, it was reported that resistin mRNA was positively and independently correlated with insulin resistance and hepatic fat [15] in human. Therefore, we hypothesized that human resistin impairs insulin action in hepatocytes. In order to investigate the biological effect of resistin on hepatic glucose metabolism, we examined the expression of resistin in human hepatocytes and hepatic tissues, cultured human HepG2 cells with transfected resistin, and examined the effect of resistin on insulin-stimulated glucose utilization and on the expression of relevant genes. Metformin is a widely used anti-diabetic agent which improves insulin resistance in liver. It was

reported that Metformin had no effect on resistin level in serum [16], but downregulated the expression of resistin in adipocytes at the transcriptional level [17]. We made further experiments to test its action on human resistin at the post-transcriptional level.

Materials and methods

Construction of expression plasmids

The pGEM-T-human resistin (pGEM-T-hR) clone vector was a kindly gift from MRC & Asthma UK Centre in Allergic Mechanisms of Asthma (London, UK). Human resistin (HR) gene was obtained by PCR amplification from pGEM-T-hR clone vector. The primers were designed according to hR gene with the addition of leader sequences corresponding to the KpnI and EcoRI restriction enzymes to facilitate directional cloning into the PcDNA3.1 plasmids (Pc-3.1) (Invitrogen). Primer sequences Resistin1 and PCR conditions were shown in Table 1. The sequence of hR cDNA generated by PCR was confirmed by sequencing and inserted into plasmid Pc-3.1 following the instruction of manufacturer. The genotypes of the construction were named as PcDNA-hR (Pc-hR). Empty Pc-3.1 was used as control.

Cell culture and stable transfection

HepG2 and 7721 cells (American Type Culture Collection) were cultured in Dulbecco's minimal essential medium (DMEM) containing 25 mM glucose, 10% fetal bovine serum (FBS) (GIBCO), respectively.

The Pc-hR and Pc-3.1 expression vectors were stably transfected in HepG2 cells using lipofectamine 2000 reagent (Invitrogen) according to the supplier's manual. In order to obtain stable transfection in HepG2 cells, the medium was changed to the selection DMEM supplemented with 800 μg/ml G418 (GIBCO) 72 h after transfection. After three weeks, the monoclone cell was selected, amplified, and maintained with 400 μg/ml G418.

Immunocytochemistry staining

Immunocytochemical detection of hR expression was carried out in HepG2 cells as previously described [18] using mouse anti-human resistin monoclone antibody (R&D Systems, Inc, 1:50 dilution). The cells were visualized

Table 1 Primers and PCR conditions for semi-quantitative RT-PCR analysis

mRNA	Primer pair (5′→3′)	Annealing temperature (°C)	No. of cycles	Product size (bp)
Resistin-1		58	30	352
Forward	TCAGGTACCATGGCCATGAAAGCTCTCTGTCTCCTC			
Reverse	TCGGAATTCTCAGGGCTGCACACGACAGC			
Resistin-2		59	35	267
Forward	GGGGCTGTTGGTGTCTA			
Reverse	GCTCCGGTCCAGTCCAT			
GSK-3β		58	28	710
Forward	GACGCTCCCTGTGATTTATGT			
Reverse	TAGCCAGAGGTGGATTACTTG			
IRS-2		63	30	547
Forward	GGCCTCTGTGGAAAATGTCTC			
Reverse	CTGTGGCTTCCTTCAAGTGATG			
CAP		61	28	885
Forward	TACATCGAAGGGGAGAAAGTGG			
Reverse	TCTTTATCATCGTGCCGTCTCC			
β-actin		59	25	632
Forward	GATGGTGGGTATGGGTCAGAAGGA			
Reverse	GCTCATTGCCGATAGTGATGACCT			
GADPH		58	28	258
Forward	AGAAGGCTGGGGCTCATTTG			
Reverse	AGGGGCCATCCACAGTCTTC			

using a microscope (Olympus) and the immunoreactivity was identified as brown staining in cytoplasm. The grey scale value of resistin expression was measured from randomly selected microscope fields from five different wells of Pc-3.1-transfected and Pc-hR-transfected HepG2 cells, respectively, and analyzed using image analytical system HPIAS-1000. At least five fields were selected per well.

Measurement of human resistin secretion

HepG2 cells, HepG2 cells transfected with Pc-3.1 or Pc-hR plasmids were seeded in 24-well plates (1×10^5 cells/well), respectively. After incubation with 600 μl of fresh serum-free medium in the absence or presence of Metformin (200 μM) for 24 h, the medium was used to measure the amount of resistin secreted by cells by enzyme-linked immunosorbent assay (ELISA), using a commercial kit (BioVendor Laboratory Medicine, Inc. Czech Republic).

Glucose uptake assays

The measurements for glucose uptake were performed as described by Nakamori [19]. Briefly, the HepG2 cells transfected with Pc-hR and Pc-3.1 plasmids were grown in serum-free DMEM with 0.2% BSA in the absence or presence of Metformin (200 μM) for 24 h. Cells were then incubated with Krebs-Ringer's Hepes buffer (pH 7.4) with or without insulin (100 nM) for 30 min at 37°C. Glucose transport was initiated by addition of 2-Deoxy-D- [2, 6–^{3}H] glucose (Amersham Biosciences, Barcelona Spain) to a final assay concentration of 50 μM for 10 min at 37°C. Assays were terminated by three washes with ice-cold phosphate buffered solution (PBS). Cells were lysed in 0.4 ml PBS containing 0.1% Triton X-100 for 45 min. Nonspecific uptake was assessed in the presence of 10 μM cytochalasin B (Sigma-Aldrich) and subtracted from all of the measured values. Radioactivity was counted by liquid scintillation spectroscopy using a Beckman LS 5000 TD instrument and normalized to protein amount measured with a Micro BCA protein assay kit (Pierce). Every experiment set up five reduplicate wells and was repeated thrice times.

Periodic acid-Schiff (PAS) staining

After fixation (85% of absolute alcohol, 5% of glacial acetic acid 5% and 10% of formaldehyde), HepG2 cells were incubated in periodic acid solution for 15 min, followed by 20 min' incubation in Schiff's reagent. Cells were counterstained for 2 min with methyl green [20]. The grey scale value was measured and analyzed using image analytical system HPIAS-1000 from randomly selected microscope fields from five different wells of control and Pc-hR-transfected HepG2 cells, respectively. At least five fields were selected per well.

Semi-quantitative RT-PCR analysis of mRNA expression

HepG2 cells, HepG2 cells transfected with Pc-3.1 or Pc-hR plasmids were treated with or without Metformin (200 μM) for 24 h. Total RNA was extracted from cells with the use of Trizol reagent (GIBCO). RNA (1 μg) was reverse transcribed using Revert Aid H Minus M-MuL V reverse transcriptase (Helena Biosciences, Europe, Sunderland, UK) and random hexamers in 20 μl reaction volume, according to the manufacturer's instructions. The products (1 μl of cDNA) were subjected to PCR with ExTaq and primers. Primer sequences and PCR conditions were shown in Table 1. The PCR products were separated by electrophoresis on agarose gel, visualized by ethidium bromide staining, and quantitated with gel Image Systems. The abundance of each specific mRNA was normalized on the basis of that of β-actin or GADPH mRNA.

In experiments evaluating resistin expression in hepatocytes and liver tissue, total RNA was extracted from HepG2 cells, 7721 cells, normal hepatic tissue, cirrhosis tissue and liver cancer tissue and was performed RT-PCR. For each PCR reaction, a negative control with the same volume of each RNA instead of cDNA was used, and meanwhile, amplification was conducted to control DNA contamination. The positive control for PCR reaction was performed using pGEM-T-human resistin clone plasmids as template instead of cDNA. The housekeeping gene was amplified, i.e. β-actin, as internal reference for gene expression.

Human resistin primer sequences Resistin2 and PCR conditions were shown in Table 1. Human resistin mRNA has four exon. The forward primer was designed in exon2 and the reverse primer was designed in exon4. The hepatic tissues were provided by liver surgery patients. Normal liver tissue (three samples) was obtained during surgical liver resection for hemangiomas of liver. Liver cancer tissue (three samples) and cirrhosis tissue (two samples) was collected in the liver transplantation for end-stage liver cancer or cirrhosis. The tissue histology was assessed by routine examination. The Human Study Committee of The Third Clinical College of Ji Lin University approved this study. Informed consent was obtained either from donors or from their legal representatives.

Western blot analysis

HepG2 cells, HepG2 cells transfected with Pc-3.1 or Pc-hR plasmids were treated with or without Metformin (200 μM) for 24 h. Then they were lysed and performed for Western

blot as described [21]. In brief, the protein were subjected to SDS-polyacrylamide gel electrophoresis, electroblotted onto Immobilon polyvinylidene difluoride membranes (Millipore) and immunodetected using mouse anti-human resistin monoclone antibody, rabbit polyclonal antibodies to insulin receptor substrate (IRS)-2, to glycogen synthetase kinase (GSK)-3β and to c-cbl associated protein (CAP) (Upstate Biotechnology, Lake Placid, NY), respectively. The immune complexes were detected with horseradish peroxidase–conjugated secondary antibodies. The intensity was determined using densitometry (Genesnap, Syngene, UK) and each specific protein expression was confirmed and normalized on the basis of that of β-actin (The Binding Site, Birmingham, UK).

Statistical analysis

Analyses were carried out using SPSS (SPSS Inc.12.0, Woking, UK) software. All qualitative data are representative of at least three independent experiments, with at least four wells per group per experiment. Quantitative data are presented as means ± SEM and were compared with Student's t test. $P < 0.05$ was considered statistically significant.

Results

Expression of resistin in human hepatocytes and hepatic tissues

Resistin expression in human normal hepatic tissue, hepatic cirrhosis tissue, hepatoma tissue and human hepatic cell lines including HepG2 cell line and 7721 cell line was evaluated using RT-PCR analysis. Products of the expected sizes were reproducibly observed in positive control, tissues and cell lines above (Fig. 1). No product was detected in the negative control.

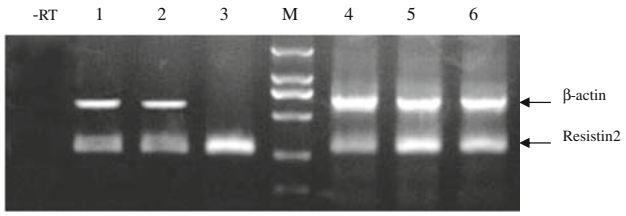

Fig. 1 Expression of resistin in human hepatocytes and hepatic tissues**.** Representative mRNA expression of resistin in HepG2 cells (1), 7721 cells (2), Normal Hepatic Tissue (4), Hepatoma Tissue (5) and Hepatic Cirrhosis Tissue (6). −RT: Negative control. 3: Positive control (pGEM-T-human resistin clone plasmids). M: Marker (DL2000)

Construction of recombinant human resistin expression vector and overexpression of human resistin in HepG2 cells transfected with recombinant plasmids

Full length of cDNA encoding human resistin (hR) coding sequences (327 bp) was cloned into PcDNA3.1 (Pc-3.1) vectors (Fig. 2A). The recombinant plasmids PcDNA-hR (Pc-hR) and the control plasmids Pc-3.1 were stably transfected into HepG2 cells. The expression of recombinant human resistin at mRNA and protein levels in HepG2 cells were identified by semi-quantitative RT-PCR analysis and immunocytochemistry staining, respectively. Compared with the control cells (Fig. 2B and C-a), the expression of human resistin mRNA and protein was significantly increased in HepG2 cells which were transfected with Pc-hR plasmids (Fig. 2B and C-b). Moreover, the amount of resistin secreted in the medium was quantitated by ELISA. As shown in Fig. 2D, the amount of resistin secreted in the medium of HepG2 cells transfected with Pc-hR plasmids was much higher than that in the control medium. The results suggested that the resistin concentration in the medium reflected the intracellular levels of resistin.

Effects of hR on insulin action in hepatocytes

As shown by the data in Fig. 3A, there was a 33.7% decrease ($P < 0.01$) in the basal glucose transport rate in HepG2 cells overexpressing resistin compared with the control cells. This effect was more evident after stimulation with insulin (100 nM). Overexpression of resistin reduced insulin-stimulated glucose uptake to the extent of 48.2% ($P < 0.001$) in HepG2 cells (Fig. 3A). Similar effect of resistin overexpression was observed in glycogen synthesis assays. Compared with the control cells, glycogen synthesis of HepG2 cells transfected with Pc-hR vectors was decreased in the absence of insulin ($P < 0.05$) (Fig. 3 B-a, b, e) and significantly decreased in the presence of insulin (100 nM) ($P < 0.01$) (Fig. 3 B-c, d, e). These results suggested that resistin disturbed glucose metabolism and impaired insulin responsiveness directly.

Then, we investigated the effect of overexpression of resistin on insulin signal transduction pathway. The mRNA and protein expression of insulin receptor substrate 2 (IRS-2), glycogen synthetase kinase 3β (GSK-3β) and c-cbl associated protein (CAP) were evaluated by semi-quantitative RT-PCR analysis and Western blot analysis. As shown by the data in Fig. 4A, compared with the control cells, mRNA expression of IRS-2 and CAP was down-regulated ($P < 0.01$, respectively) while mRNA expression of GSK-3β was upregulated ($P < 0.01$) in HepG2 cells overexpressing resistin. In consistency with the data of semi-quantitative RT-PCR analysis, Fig. 4B showed,

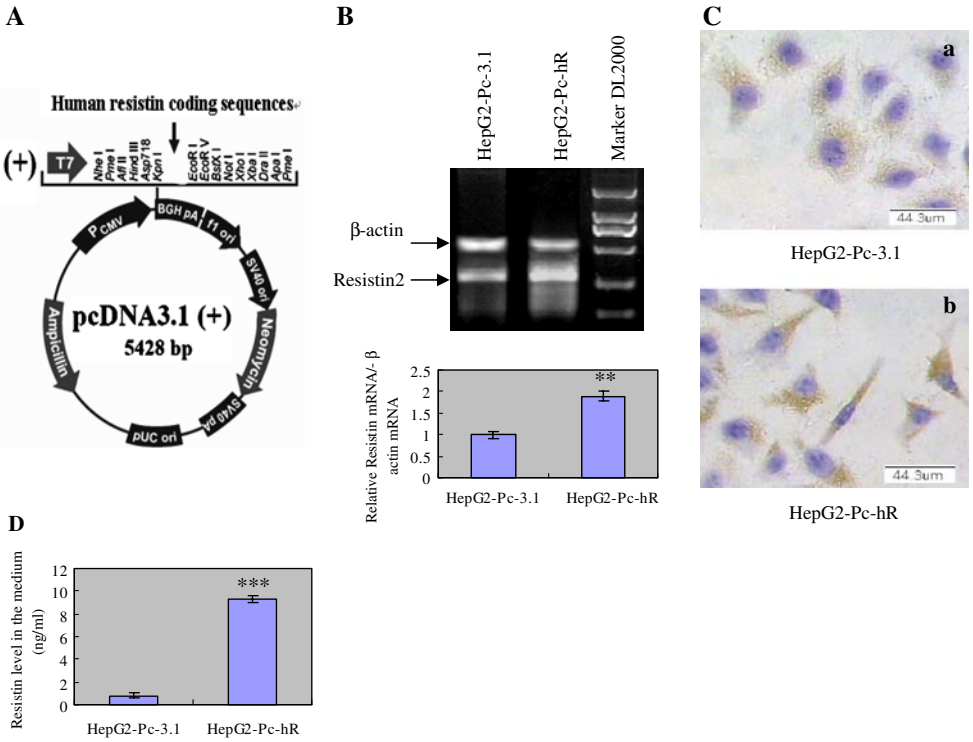

Fig. 2 Construction of recombinant human resistin expression vector and overexpression of human resistin in HepG2 cells transfected with recombinant plasmids. (**A**) A full length of human resistin (hR) coding sequence was inserted into PcDNA-3.1(Pc-3.1) plasmid. (**B**) The expression of hR mRNA in HepG2 cells transfected with Pc-3.1 or Pc-hR plasmids (semi-quantitative RT-PCR analysis). (**C**) Representative expression of hR protein in HepG2 cells transfected with Pc-3.1 (a) or Pc-hR (b) plasmids (immunocytochemistry staining). (**D**) Quantitative analysis of resistin secretion by HepG2 cells transfected with Pc-3.1 or Pc-hR plasmids by enzyme-linked immunosorbent assay (ELISA). Data are means ± SEM of values from four different preparations. HepG2-Pc-3.1: HepG2 cells transfected with Pc-3.1 plasmids; HepG2-Pc-hR: HepG2 cells transfected with Pc-hR plasmids. $^{**}P < 0.01$ versus HepG2 cells transfected with Pc-3.1 plasmids. $^{***}P < 0.001$ versus the medium of HepG2 cells transfected with Pc-3.1 plasmids

compared with the control cells, protein expression of IRS-2 and CAP was downregulated ($P < 0.001$, respectively) while protein expression of GSK-3β was upregulated ($P < 0.01$) in HepG2 cells overexpressing resistin.

Effect of Metformin on action and expression of resistin in hepatocytes

The data indicated the insulin-stimulated glucose uptake of HepG2 cells overexpressing resistin was significantly enhanced with Metformin (200 μM) treatment ($P < 0.01$) (Fig. 3A). Moreover, compared with the HepG2 cells overexpressing resistin, Metformin treatment increased expression of IRS-2, CAP and decreased expression of GSK-3β (Fig. 4A and B). These results suggested that Metformin reversed the influence of resistin in hepatocytes.

Furthermore, we examined effect of Metformin on the expression of resistin in HepG2 cells. As shown in Fig. 5, compared with the control cells, Metformin treatment decreased the expression of resistin in HepG2 cells at both transcription level (Fig. 5A) and protein level (Fig. 5B). In consistency with the results above, the amount of resistin secreted in medium was cut down by Metformin treatment (Fig. 5C).

Discussion

Resistin is a low molecular weight cysteine-rich protein (murine 114 amino acids, human 104 amino acids) secreted from adipose tissue and it is considered as a link between obesity and insulin resistance [22]. Even though originally claimed to be adipose-specific in rodents [1], more recent reports have suggested that, in human, resistin is also expressed in multiple other tissues. It is expressed at very low levels in preadipocytes, endothelial cells, and vascular smooth muscle cells but abundantly expressed in peripheral mononuclear cells and bone marrow [23]. The results are discrepant in the investigations on whether resistin is expressed in human liver tissue. Lisa Patel [23] and Tatiana Ort [10] reported that resistin was expressed in fetal liver tissue but not expressed in adult liver tissue. However, Bertolani C and his colleagues [24] demonstrated that resistin is expressed in human normal liver tissue and it is

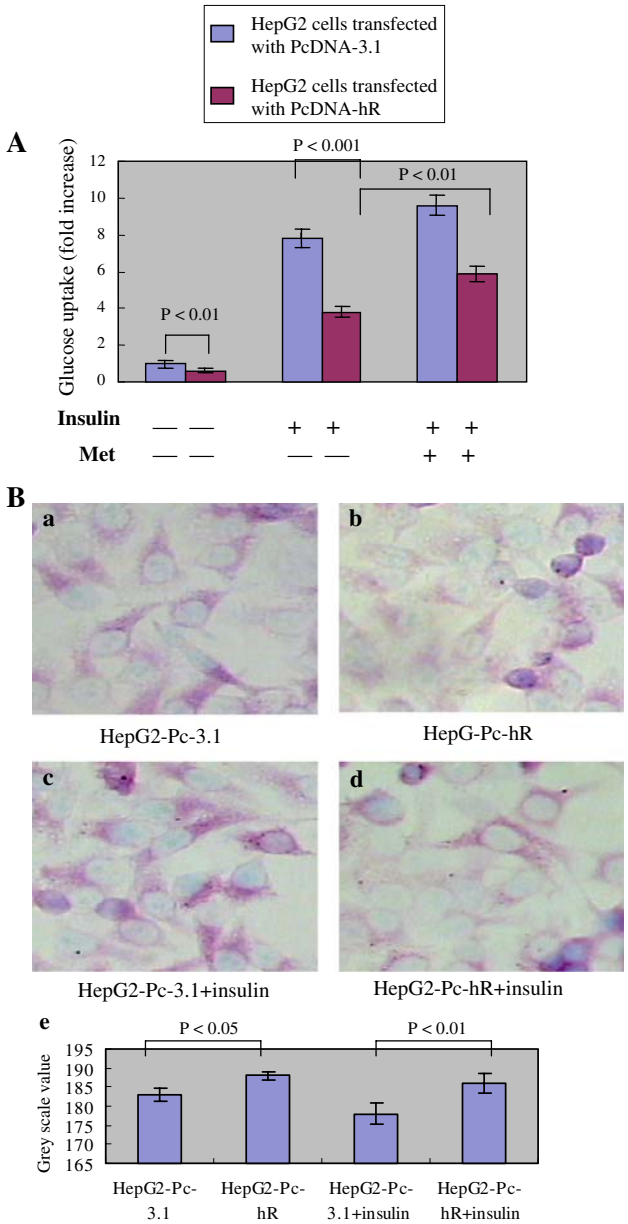

Fig. 3 Effects of human resistin on glucose uptake and glycogen synthesis in HepG2 cells**.** (**A**) Glucose uptake assays showing effects of hR and Metformin on glucose uptake in HepG2 cells. Data are means ± SEM of five experiments and are expressed as fold increase of glucose uptake. (**B:** a–e): PAS staining showing effect of hR on glycogen synthesis in HepG2 cells (magnification 200×). Met: Metformin. The other abbreviations are the same as Fig. 2

increased in condition of severe fibrosis. Resistin is expressed low in human normal liver tissue [24] and investigation methods used were different, which may contribute to different results in various study groups. In agreement with the work of Bertolani C [24], our results suggested that resistin is expressed in human normal hepatic tissue, hepatic cirrhosis tissue and hepatoma tissue. As we know, besides hepatocytes, liver tissue contains other cell types such as Kupffer cells and so on. They all possibly contribute to expression of resistin. Here, we indicated for the first time that resistin is expressed in hepatocyte lines including HepG2 and 7721 cell lines at transcription level (Fig. 1). Further investigations confirmed that resistin is expressed in HepG2 cells at protein level and is secreted in medium (Fig. 2 and Fig. 5). That the finding of resisin is expressed in hepatic parenchymal cells is perhaps helpful to understand the mechanism of resistin inducing insulin resistance in hepatocytes.

The resistin found at present is a secretory factor and it should act on target organs in vivo in an endocrine or paracrine fashion [1]. However, some reports considered that the activity of recombinant human resistin may be lower than that of the endogenous one [10, 25, 26], so here we mimicked the status by transfecting human resistin into HepG2 cells, which originated from hepatocyte and retains many characteristics of hepatocytes, to investigate its biological action on glucose metabolism in hepatocytes. The results demonstrated that overexpression of human resistin impaired glucose uptake and glycogen synthesis in HepG2 cells at the baseline and especially marked after stimulation with insulin (Fig. 3). This indicated that human resistin directly impaired insulin action in hepatocytes.

It is known that the insulin mediated transposition of GLUTs mainly depended on two pathways including PI-3K/Akt and CAP/c-cbl insulin signalling. IRS-2 and GSK-3β are crucial factors with opposing functions in PI-3K/Akt insulin signalling pathway. IRS-2 expression improves insulin sensitivity, but GSK-3β is known as an insulin signalling inhibitor. CAP is an adaptation protein which binds cbl, known as one of the substrates of insulin receptors, to form the complex to assist transduction of insulin signaling and offers secondary signal paralleled with PI-3K pathway for transposition of glucose transporter. The two pathways not only act independently but also interact to regulate transportation of GLUTs in common. Obviously, the expression variation of these genes would influence the action of insulin. Therefore, the mRNA and protein levels of the above genes were measured in the current study. In agreement with the work of Lei et al. [27], we found that GSK-3β mRNA and protein expression was up-regulated due to overexpression of resistin in HepG2 cells (Fig. 4), which indicated that resistin downregulated PI-3K/Akt insulin signalling pathway. Interestingly, our data also show that overexpression of resistin decreased the mRNA and protein expression of CAP as well as those of IRS-2 (Fig. 4) in HepG2 cells, which suggested that resistin also downregulated CAP/c-cbl insulin signalling pathway. These results demonstrated that the two insulin signal transduction pathway may be all involved in the impairment of insulin action caused by resistin in hepatocytes.

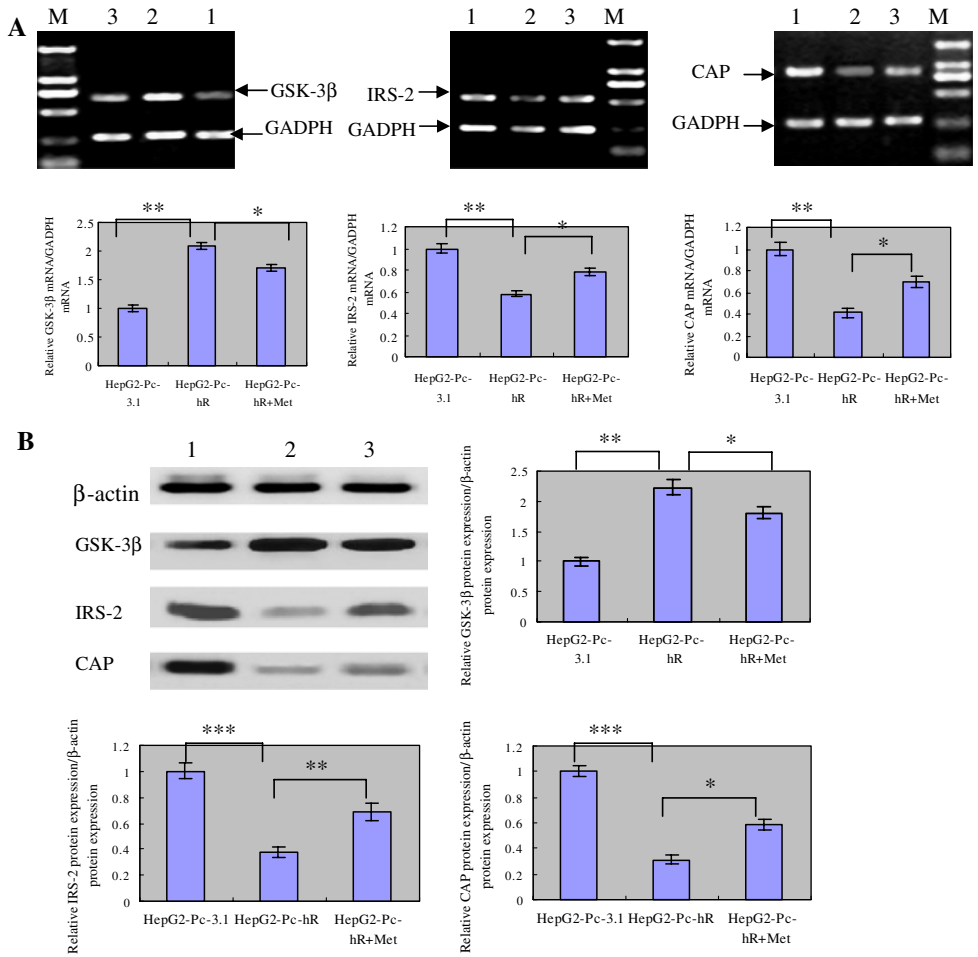

Fig. 4 Effects of human resistin and Metformin on the expression of relevant genes of insulin signal transduction pathway. (**A**) Representative mRNA expression of relevant genes in HepG2 cells transfected with Pc-3.1 or Pc-hR plasmids (semi-quantitative RT-PCR analysis). The amount of each target mRNA was normalized by that of GADPH mRNA and was expressed relative to the abundance of the target mRNA in cells transfected with Pc-3.1 plamids. (**B**) Representative protein expression of relevant genes in HepG2 cells transfected with Pc-3.1 or Pc-hR plasmids (Western blot analysis). The amount of each target protein was normalized by that of β-actin protein and was expressed relative to the expression of the target protein in cells transfected with Pc-3.1 plamids. Data are means ± SEM of values from three to five independent experiments. CAP: c-cbl associated protein. IRS-2: insulin receptor substrate 2. GSK-3β: glycogen synthetase kinase 3β. Met: Metformin. 1: i.e. HepG2-Pc-3.1, HepG2 cells transfected with Pc-3.1 plasmids. 2: i.e. HepG2-Pc-hR, HepG2 cells transfected with Pc-hR plasmids. 3: i.e. HepG2-Pc-hR + Met, Metformin treated HepG2 cells transfected with Pc-hR plasmids. M: Marker DL2000. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

It is recognized that altered regulation of the expression and secretion of resistin might be important in the development of insulin resistance. In fact, the various reports have shown that resistin expression is modulated by a variety of factors including glucose [22] and other mediators known to affect insulin sensitivity, including insulin [28], TNF-α [29], dexamethasone [30] as well as rosiglitazone [17, 22]. There is a great deal of evidence that rosiglitazone, an insulin sensitizer, downregulated resistin level in serum [16] and expression of resistin in vitro [17, 22], and antagonized the effect of resistin on insulin sensitivity [5]. Metformin, known as a widely used antidiabetic agent for the treatment of type 2 diabetes, was also reported that it decreased mRNA level of resistin in vitro [17]. Moreover, Metformin enhances insulin action in hepatocytes [31]. Therefore, its relationship with resistin was examined in this study. Data showed that Metformin could reverse the effects of resistin on insulin signalling in HepG2 cells (Fig. 4) and improved glucose uptake of HepG2 cells overexpressing human resistin (Fig. 3). Furthermore, Merformin was also implicated to downregulate the expression of resistin in HepG2 cells at transcription and translation level (Fig. 5). It maybe contributes to the mechanism of Metformin resisting the effect of resistin.

In conclusion, this study has demonstrated that human resistin overexpression impairs glucose transport and glycogen synthesis in hepatocytes. We propose that the potential mechanism for these effects is resistin-mediated

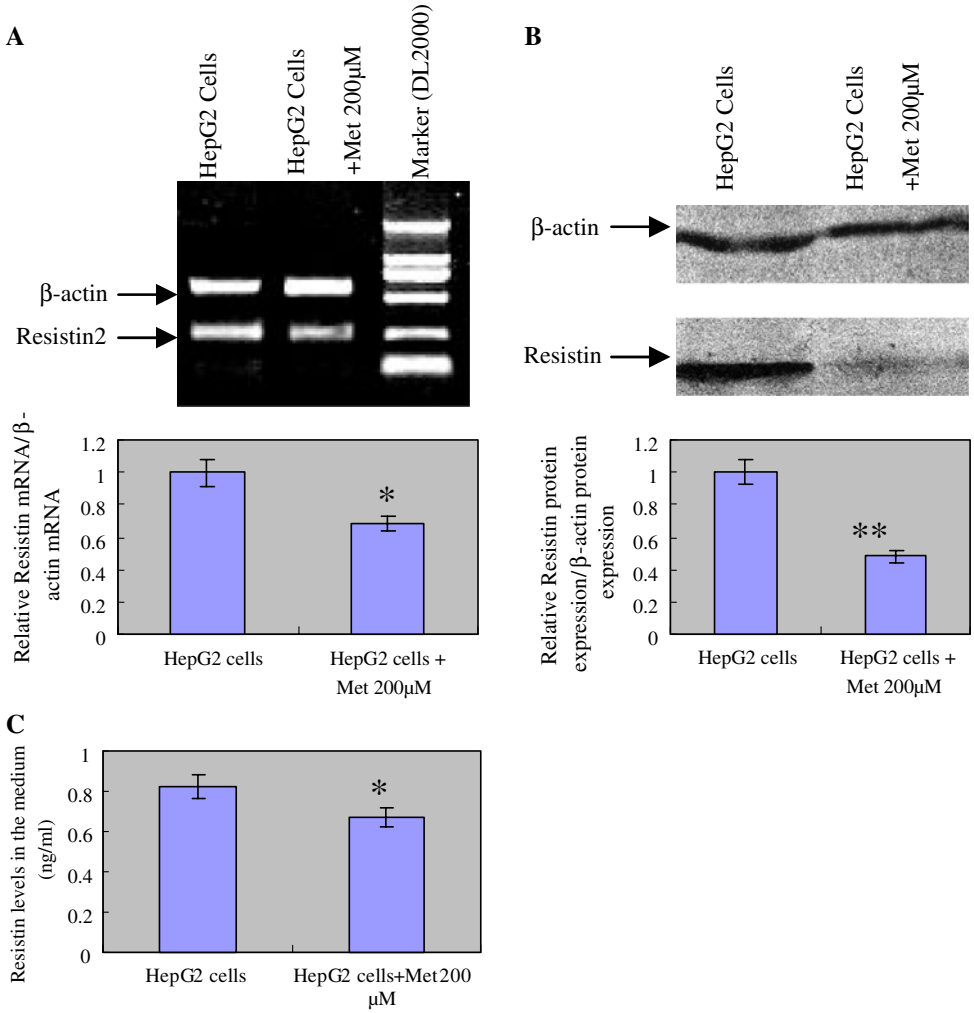

Fig. 5 Effect of Metformin on expression of human resistin in hepatocytes**.** (**A**) Semi-quantitative RT-PCR analysis showing expression of resistin at transcriptional level in HepG2 cells in the absence or presence of Metformin. The amount of resistin mRNA was normalized by that of β-actin mRNA and was expressed relative to the expression of resistin mRNA in HepG2 cells. (**B**) Western blot analysis showing expression of resistin at protein level in HepG2 cells in the absence or presence of Metformin. The amount of resistin protein was normalized by that of β-actin protein and was expressed relative to the expression of resistin protein in HepG2 cells. Data are means ± SEM of values from three to five independent experiments. (**C**) Quantitative analysis of resistin secretion by HepG2 cells in the absence or presence of Metformin by enzyme-linked immunosorbent assay (ELISA). Data are means ± SEM of values from four different preparations. Met: Metformin. $^{*}P < 0.05$, $^{**}P < 0.01$ versus HepG2 cells

impairment of both PI-3K/Akt and CAP/c-cbl insulin signalling pathway. Metformin inhibits resistin gene expression and attenuates its action in hepatocytes.

Acknowledgements Thanks to Professor PEI YIN ZHANG (Jilin University) for his great help in assays of glucose uptake. This work was supported by the National Natural Science Foundation of China (No. 39870667) and program for Scientific Research Foundation of Jilin Province (No.20020704).

Reference

1. C.M. Steppan, S.T. Bailey, S. Bhat, E.J. Brown, R.R. Banerjee, C.M. Wright, H.R. Patel, R.S. Ahima, M.A. Lazar, Nature **109**, 307–312 (2001)
2. H.Q. Fan, N. Gu, F. Liu, L. Fei, Acta. Pharmacol. Sin. **28**(3), 410–416 (2007)
3. K.H. Kim, Z. Ling, M. Yangsoo, Kang Chulho, H.S. Sul, PNAS **101**, 6780–6785 (2004)
4. E.D. Muse, T.K. Lam, P.E. Scherer, L. Rossetti, J. Clin. Invest. **117**(6), 1670–1678 (2007)
5. L. Zhou, Y. Li, T. Xia, S. Feng, X. Chen, Z. Yang, Eur. Cytokine. Netw. **17**(3), 189–195 (2006)
6. C. Graveleau, V.G. Zaha, A. Mohajer, R.R. Banerjee, N. Dudley-Rucker, C.M. Steppan, M.W. Rajala, P.E. Scherer, R.S. Ahima, M.A. Lazar, E.D. Abel, J. Biol. Chem. **280**(36), 31679–31685 (2005)
7. P.G. McTernan, F.M. Fisher, G. Valsamakis, R. Chetty, A. Harte, C.L. McTernan, P.M. Clark, S.A. Smith, A.H. Barnett, S. Kumar, J. Clin. Endocrinol. Metab. **88**(12), 6098–6106 (2003)
8. M.S. Farvid, T.W. Ng, D.C. Chan, P.H. Barrett, G.F. Watts, Diabetes Obes. Metab. **7**(4), 406–413 (2005)
9. J. Janowska, B. Zahorska-Markiewicz, M. Olszanecka-Glinianowicz, Metabolism **55**(11), 1495–1499 (2006)
10. T. Ort, A.A. Arjona, J.R. MacDougall, P.J. Nelson, M.E. Rothenberg, F. Wu, A. Eisen, Y.D. Halvorsen, Endocrinology **146**(5), 2200–2209 (2005)
11. R. Rea, R. Donnelly, Obes. Metab. **8**, 105–109 (2006)
12. C.M. Taniguchi, K. Ueki, R.J. Kahn, Clin. Invest. **115**, 718–727 (2005)
13. M.W. Rajala, S. Obici, P.E. Scherer, L. Rossetti, J. Clin. Invest. **111**, 225–230 (2003)
14. E.D. Muse, T.K. Lam, P.E. Scherer, L. Rossetti, J. Clin. Invest. **117**(6), 1670–1678 (2007)
15. S.R. Smith, F. Bai, C. Charbonneau, L. Janderova, G. Argyropoulos, Diabetes **52**(7), 1611–1618 (2003)
16. H.J. Kim, E.S. Kang, D.J. Kim, S.H. Kim, C.W. Ahn, B.S. Cha, M. Nam, C.H. Chung, K.W. Lee, C.M. Nam, H.C. Lee, Clin. Endocrinol. (Oxf) **66**(2), 282–289 (2007)

17. R. Rea, R. Donnelly, Diabetes Obes. Metab. **8**(1), 105–109 (2006)
18. L. Hari, V. Brault, M. Kleber, H.T. Lee, F. Ille, R. Leimeroth, C. Paratore, U. Suter, R. Kemler, L. Sommer, J. Cell. Biol. **159**(5), 867–880 (2002)
19. Y. Nakamori, M. Emoto, N. Fukuda, A. Taguchi, S. Okuya, M. Tajuri, M. Miyagishi, K. Taira, Y. Wada, Y. Tanizawa, J. Cell. Biol. **173**(5), 665–671 (2006)
20. T.J. Fairchild, P.A. Fournier, Med. Sci. Sports Exerc. **36**(12), 2053–2058 (2004)
21. C.M. Kusminski, N.F. da Silva, S.J. Creely, F.M. Fisher, A.L. Harte, A.R. Baker, S. Kumar, P.G. McTernan, J. Clin. Endocrinol. Metab. **92**(1), 270–276 (2007)
22. N. Shojima, H. Sakoda, T. Ogihara, M. Fujishiro, H. Katagiri, M. Anai, Y. Onishi, H. Ono, K. Inukai, M. Abe, Y. Fukushima, M. Kikuchi, Y. Oka, T. Asano, Diabetes **51**, 1737–1744 (2002)
23. L. Patel, A.C. Buckels, I.J. Kinghorn, P.R. Murdock, J.D. Holbrook, C. Plumpton, C.H. Macphee, S.A. Smith, Biochem. Biophys. Res. Commun. **300**(2), 472–476 (2003)
24. C. Bertolani, P. Sancho-Bru, P. Failli, R. Bataller, S. Aleffi, R. DeFranco, B. Mazzinghi, P. Romagnani, S. Milani, P. Ginés, J. Colmenero, M. Parola, S. Gelmini, R. Tarquini, G. Laffi, M. Pinzani, F. Marra, Am. J. Pathol. **169**(6), 2042–2053 (2006)
25. S.D. Patel, M.W. Rajala, L. Rossetti, P.E. Scherer, L. Shapiro, Science **304**, 1154–1158 (2004)
26. B. Aruna, S. Ghosh, A.K. Singh, S.C. Mande, V. Srinivas, R. Chauhan, N.Z. Ehtesham, Biochemistry **42**, 10554–10559 (2003)
27. L. Zhou, H. Sell, K. Eckardt, Z. Yang, J. Eckel, FEBS Lett. **581**, 4303–4308 (2007)
28. F. Haugen, A. Jorgensen, C.A. Drevon, P. Trayhurn, FEBS Lett **507**, 105–108 (2001)
29. M. Fasshauer, J. Klein, S. Neumann, M. Eszlinger, R. Paschke, FEBS Lett **500**, 60–63 (2001)
30. H. Raff, E.D. Bruder, Endocrine **29**(2), 341–344 (2006)
31. N.F. Wiernsperger, C.J. Bailey, *The antihyperglycaemic effect of metformin, therapeutic and cellular mechanisms* (Academic, London, 1999)