Dual Specificity MAPK Phosphatase 3 Activates PEPCK Gene Transcription and Increases Gluconeogenesis in Rat Hepatoma Cells*

Haiyan Xu, Qing Yang, Minhui Shen, Xueming Huang, Marlene Dembski, Ruth Gimeno, Louis A. Tartaglia, Rosana Kapeller, and Zhidan Wu

From Millennium Pharmaceuticals Incorporated, Cambridge, Massachusetts 02139

Insulin is a key hormone that controls glucose homeostasis. In liver, insulin suppresses gluconeogenesis by inhibiting the transcriptions of phosphoenolpyruvate carboxylase (PEPCK) and glucose-6-phosphatase (G6Pase) genes. In insulin resistance and type II diabetes there is an elevation of hepatic gluconeogenesis, which contributes to hyperglycemia. To search for novel genes that negatively regulate insulin signaling in controlling metabolic pathways, we screened a cDNA library derived from the white adipose tissue of ob/ob mice using a reporter system comprised of the PEPCK promoter placed upstream of the alkaline phosphatase gene. The mitogen-activated dual specificity protein kinase phosphatase 3 (MKP-3) was identified as a candidate gene that antagonized insulin suppression on PEPCK gene transcription from this screen. In this study, we showed that MKP-3 was expressed in insulin-responsive tissues and that its expression was markedly elevated in the livers of insulin-resistant obese mice. In addition, MKP-3 can activate PEPCK promoter in synergy with dexamethasone in hepatoma cells. Furthermore, ectopic expression of MKP-3 in hepatoma cells by adenoviral infection increased the expression of PEPCK and G6Pase genes and led to elevated glucose production. Taken together, our data strongly suggests that MKP-3 plays a role in regulating gluconeogenic gene expression and hepatic gluconeogenesis. Therefore, dysregulation of MKP-3 expression and/or function in liver may contribute to the pathogenesis of insulin resistance and type II diabetes.

Type 2 diabetes mellitus is increasing at an alarming rate and affects ~6% of the adult population in the Western society (1). Insulin resistance, characterized by elevated circulating insulin level and impaired glucose tolerance, usually precedes the development of diabetes and is closely associated with obesity. In the past decade, a tremendous amount of effort has been made trying to understand the underlying mechanism of insulin resistance. Our approach toward this same goal is to identify genes that antagonize insulin action in insulin-responsive tissues and develop small molecule inhibitors for these targets, hoping to find drugs that cannot only treat type 2 diabetes but also improve insulin sensitivity and ultimately cure the disease.

Insulin is an anabolic hormone that controls many aspects of metabolism. The mitogen-activated protein kinase (MAPK)2 cascade and the PI 3-kinase pathway are two of the signaling networks activated by insulin upon binding to its receptor. The activation of ERK by insulin seems to play a major role in insulin-mediated mitogenesis, whereas PI 3-kinase has been implicated as a key player of the metabolic arm. Defects in the insulin-stimulated metabolic cascade lead to insulin resistance. Therefore, to identify additional genes that negatively regulate insulin-stimulated metabolic pathway(s), we employed an expression cloning approach using the promoter of the PEPCK gene placed upstream of the secreted alkaline phosphatase gene as a surrogate reporter system for the metabolic effects of insulin as described previously (2). Like many other genes, PEPCK, a key enzyme in gluconeogenesis, is highly regulated by insulin at transcription level (3–5). Therefore, genes blocking the inhibitory effect of insulin on PEPCK transcription are also likely to attenuate the metabolic signaling pathways elicited by insulin.

Using this approach, we identified two MAPK phosphatases, MKP-3 and MKP-4. Both of these proteins belong to the family of dual specificity protein phosphatase. Previously we reported that MKP-4 potentially played a role in insulin resistance in adipose tissue (2). In this study we showed that the expression of MKP-3 was markedly elevated in the livers from the insulin-resistant animal models. We further investigated the role of MKP-3 in the regulation of gluconeogenic gene expression in the absence or presence of insulin as well as hepatic glucose production.

MATERIALS AND METHODS

Cells and DNA Constructs—The rat hepatoma H4IIIE and Fao cells were cultured in Dulbecco’s modified Eagle’s medium with 5% fetal bovine serum and RPMI 1640 with 10% fetal bovine serum, respectively. The PEPCKp600-SEAP (which includes the secreted placental alkaline phosphatase) was constructed as described previously (2). The PEPCKp600-Luc was generated by subcloning the PEPCK promoter (−558 to +73) into the pGL3-basic vector at the MluI and BgIII sites. To construct adenovirus expression vectors for MKP-3 and GFP, the two genes were first cloned into a shuttle vector containing the cytomegalovirus promoter (CMV), then both genes and the CMV promoter were excised with I-cceI and PI-sceI digestion and ligated into the Adeno-X expression vector as described in the user’s manual (Clontech). The Adeno-X expression constructs were linearized with PacI digestion before being transfected into HEK293 cells with Lipofectamine 2000 reagent (Invitrogen) for virus production. Crude cell lysates were obtained 2 weeks after the initial transfection and then used to infect a larger amount of HEK293 cells for further virus amplification and purification.

Construction of cDNA Library— Epididymal adipose tissues were dissected from 10 week-old male ob/ob mice, and total RNA was extracted. cDNAs were generated from total RNA by reverse transcription using MRP, MAPK phosphatase; PECK, phosphoenolpyruvate carboxylase; PI, phosphatidylinositol; SEAP, secreted placental alkaline phosphatase.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed: Novartis Inst. for Biomedical Research, Inc., 100 Technology Square, Cambridge, MA 02139. Tel.: 617-871-7345; Fax: 617-871-7051; E-mail: zhidan.wu@pharma.novartis.com.

2 The abbreviations used are: MAPK, mitogen-activated kinase; Dex, dexamethasone; ERK, extracellular signal-regulated protein kinase; GFP, green fluorescent protein;
MKP-3 and Gluconeogenesis

oligo(dT) primer. The cDNAs were then cloned into the pMET7 expression vector (6) at the Sall and NotI sites. The individual clones were sequenced from the 5′-end. The cDNA clones were plated into 96-well plates (1 clone/well), and duplicate plates were made to be used for the functional screening (125 ng DNA/clone/well).

Functional Screening and PEPCk Reporter Gene Assays in H4IIE Cells—In the functional screening, individual clones in the ob/ob white adipose tissue cDNA library were cotransfected with the PEPCk600-SEAP in H4IIE cells. Cells were seeded at 20,000 per well on 96-well plates a day prior to transfection. The next morning, 125 μg of the PEPCk reporter construct, PEPCk600-SEAP (2), was added to each well containing individual cDNA clones (125 ng/well) on 96-well plates. FuGENE 6 (0.38 μl/well) (Roche Applied Science) was added to the DNAs, and the entire mixture was transferred to the cells. The dominant negative 85-kDa subunit of PI 3-kinase, DNp85, was cotransfected with PEPCk600-SEAP into H4IIE cells and served as a positive control. Cells were serum-starved for 5 h in Dulbecco’s modified Eagle’s medium containing 0.5% fetal calf serum at 16 h post-transfection followed by treatment with dexamethasone (1 μM), 8-bromo-cAMP (1 mM), and insulin (100 nM) for an additional 16 h. Medium was then collected and assayed for alkaline phosphatase activity as described previously (2). The cDNA clones that resulted in higher alkaline phosphatase activity were tested again in the same assay, and the confirmed clones’ identities were determined by DNA sequencing. To determine the effect of MKP-3 on the PEPCk gene transcription, H4IIE cells were seeded at 20,000 per well on a 96-well plate. Cells were cotransfected overnight with either an empty vector or the MKP-3 expression vector (100 ng) along with PEPCk600-Luc (80 ng) and phRL-SV40 (an internal control; Promega) using FuGENE 6. Cells were then changed to Dulbecco’s modified Eagle’s medium containing 0.1% fetal bovine serum for 16 h and subsequently treated with various stimuli as indicated in TABLE ONE. Twenty-four hours after treatment, cells were harvested and subjected to luciferase assay using Dual-Glo luciferase assay system (Promega).

RNA Extraction, Northern Blot, and TaqMan Analysis—RNA samples were extracted using Trizol reagent (Invitrogen). RNA samples were denatured and loaded onto a 1% agarose gel containing 3% formaldehyde. After electrophoresis, RNAs were transferred to a Biotran membrane (ICN Pharmaceuticals), UV light cross-linked, and baked at 80 °C for 1 h. Hybridization with [32P]dCTP (PerkinElmer Life Sciences) labeled cDNA probes and subsequent washings were done as described previously (7). Photographs of ethidium bromide gels were presented for loading adjustment. The gene expression profiles of MKP-3, G6Pase, and PEPCk in Fao cells were measured using real time PCR (TaqMan analysis). Total RNA was extracted from cells using Trizol reagent. The first strand cDNA was synthesized from total RNA using reverse transcriptase reagents (Applied Biosystems). The TaqMan assay was performed in a 12.5-μl reaction containing 1× TaqMan Universal PCR master mix (Applied Biosystems), 10 ng cDNA, 900 nM primers, 250 nM FAM-labeled probe for the target genes, and 1× of both the 18 S ribosomal RNA (rRNA) primers and VIC-labeled 18 S rRNA TaqMan probe (Applied Biosystems) in 384-well clear plates (Applied Biosystems). PCR conditions were 50 °C for 2 min followed by 95 °C for 10 min for 1 cycle and then 95 °C for 15 s followed by 60 °C for 1 min for 40 cycles. The relative mRNA expression levels were calculated by comparing the target gene versus 18 S rRNA. The sequences for the primers and probe sets for mouse and rat MKP-3, rat G6Pase, and rat PEPCk were as follows: 5′-GCTAGACGACCCCTTGTGACA-3′ (forward), 5′-TGAGGGGCTGTTGAAGTAGTAC-3′ (reverse), and 5′-GACTCCAGCAGAGATGTACC-3′ (reverse), and 5′-6FAM-CCGGGTCCCCACTCCGCAGC-TAMRA-3′ (probe) for mouse MKP-3; 5′-CCCTCCACCAAATGCTTACAA-3′ (forward), 5′-ACACTCCGGCTAGAAAGAGG-3′ (reverse), and 5′-6FAM-TGGACTCCCTGCATTCTAGTGGAA-3′ (probe) for rat MKP-3; 5′-GCCAGCTAGATCCGTGACAGAT-3′ (forward), 5′-GGACAAGGCTTCTTTTTTACC-3′ (reverse), and 5′-6FAM-ACTGTCGACATCCAGCGCCGA-3′ (probe) for rat MKP-3; 5′-GGCAACCTCTAGTGAAGTAC-3′ (forward), 5′-TTTTCTTTTACC-3′ (reverse), and 5′-6FAM-AATCCCGAGGCCATTAA-GACCTACCT-TAMRA-3′ (probe) for rat PEPCk.

Western Blot Analysis—Livers were dissected from 9 week-old ob/ob mice and their lean controls that were either fasted overnight or fed ad libitum. For diet-induced obese mice, male C57Bl/16 mice were fed with either a low fat (10% fat) or high fat diet (45% fat) for 4 months. Mice were fasted overnight, and livers were dissected. The isolated livers were homogenized in a radioimmunoprecipitation assay buffer containing protease inhibitors. Cells were washed with ice-cold phosphate-buffered saline once and lysed with cell lysis buffer (Passive lysis buffer from Promega plus protease inhibitors). One hundred micrograms of liver extract or 60 μg of cell lysate from each sample was used for Western blot analysis according to the manufacturer’s protocol (Invitrogen). Briefly, following SDS-PAGE the resolved proteins were transferred onto nitrocellulose. Membranes were blocked in 5% nonfat dry milk for 1 h and then incubated with the appropriate primary antibodies in the presence of 5% nonfat dry milk followed by incubation with anti-mouse (Amersham Biosciences) or anti-goat (Alpha Diagnostic International) horseradish peroxidase-linked whole antibody. Protein bands were detected by ECL Western blotting detection reagent (Amersham Biosciences). MKP-3 antibody was purchased from Santa Cruz Biotechnology and used at 1:200. Tubulin antibody was obtained from ABCam and used at 1:3000 dilution. The secondary antibodies, sheep anti-mouse IgG-horseradish peroxidase and rabbit anti-goat IgG-horseradish peroxidase were used at 1:5000.

Glucose Output—Fao cells were seeded in 12-well plates at 1.5 million/well in growth medium (RPMI 1640 plus 10% fetal bovine serum). The next day, cells were transduced with either MKP-3 or GFP adenovirus at a multiplicity of infection of 50. Twenty-four hours post-transduction, cells were washed once with RPMI 1640 and incubated in RPMI 1640 containing 0.5% bovine serum albumin, 1 μM dexamethasone, and 1 μM 8-bromo-cAMP for 5 h. Cells were then incubated in 0.35 ml (per well) of phenol red-free, glucose-free Dulbecco’s modified Eagle’s medium containing 2 mM pyruvate and 20 mM lactate containing dexamethasone (1 μM) and 8-bromo-cAMP (1 μM) in the presence or absence of insulin (100 nM). Medium was collected 3 h later and subjected to glucose measurement using the Amplex® Red glucose/glucose oxidase assay kit (Invitrogen). Cells were lysed, and protein concentration was determined. The glucose output rate was normalized by cellular protein concentration and expressed as microgram of glucose per milligram of protein per 3 h.

RESULTS

Identification of MKP-3 as a Candidate Gene for Insulin Resistance—To find negative modulators of insulin signaling, a cDNA library derived from the white adipose tissue of ob/ob mice was cotransfected with a PEPCk promoter (−558 to +73 bp) construct into H4IIE cells (8), an insulin-responsive rat hepatoma cell line. The PEPCk promoter containing an insulin response unit, a glucocorticoid response unit, and a cAMP response unit (4, 9–11), was placed upstream of the secreted placental alkaline phosphatase gene (PEPCk600-SEAP). The transcription of the PEPCk promoter has been well characterized in response to a number of hormones and stimuli.
Dexamethasone (Dex) and 8-bromo-cAMP strongly stimulate PEPCK gene transcription. In contrast, insulin suppresses transcription of PEPCK (4, 5). Using SEAP as a reporter, the rate of transcription is reflected by the activity of alkaline phosphatase in the conditioned media. The cDNA clones that resulted in high alkaline phosphatase activity in the presence of insulin were selected and further confirmed in the secondary assays. A dominant negative form of PI 3-kinase's regulatory subunit p85, DNp85, was used as a positive control (12). As shown in Fig. 1, insulin strongly antagonized the activation of the PEPCK gene transcription induced by cAMP and Dex (lane 2 versus lane 1). DNp85, a negative modulator of the insulin signaling pathway, released insulin’s suppression on the PEPCK transcription (lane 3 versus lane 2) as expected. Among the 10,000 clones screened in this system, MKP-3 and MKP-4 (2), two dual specificity protein phosphatase family members, were found to antagonize the insulin effect on PEPCK promoter as indicated by restoration the alkaline phosphatase activity (Fig. 1, lane 4 versus lane 2). MKP-4 was reported previously (2) to interfere with the insulin-stimulated glucose uptake in adipocytes, which may contribute to insulin resistance in adipose tissue. The role of MKP-3 in glucose metabolism and insulin resistance is unknown and, therefore, is the focus of this study.

Expression of MKP-3 Is Markedly Elevated in Insulin-resistant Mouse Livers—The expression of MKP-3 mRNA was examined previously in a small number of rat tissues and during mouse embryogenesis (13–15). The expression of this gene in adult human and mouse tissues has not been reported. Fig. 2 showed that MKP-3 mRNA was widely expressed in human with the highest expression in placenta, trachea, and spleen (Fig. 2A). In mouse, MKP-3 was detected in all the tissues examined (Fig. 2B). To understand the relevance of this phosphatase to obesity and insulin resistance, the expression of MKP-3 was investigated in insulin-responsive tissues, including white adipose tissue, liver, and muscle, from two genetic obese and insulin-resistant mouse models, ob/ob and db/db mice, and their lean, insulin-sensitive controls (Fig. 3). MKP-3 mRNA is markedly elevated in the livers from ob/ob and db/db mice compared with their controls (Fig. 3B). Its expression is also significantly increased in white adipose tissue from the same mice (Fig. 3A). In contrast, MKP-3 expression in muscle does not vary regardless of decreased insulin sensitivity in this tissue of ob/ob and db/db mice (Fig. 3C). To determine whether MKP-3 protein level is also elevated in the livers from insulin-resistant mice, Western blot analysis was performed using liver extracts from ob/ob mice and their lean controls. Coincident with its mRNA level, the protein level of MKP-3 is much higher in the ob/ob liver compared with that in the control mice in both fasted and fed states (Fig. 3, D and E). Interestingly, MKP-3 protein level is higher in the fasted state than in the fed state in the insulin-sensitive livers. However, this regulation is lost in the insulin-resistant ob/ob livers (Fig. 3, D and E). To further determine whether this dysregulation of MKP-3 occurs in diet-induced obese and insulin-resistant animals, hepatic MKP-3 protein level was measured in mice fed with either a low fat or a high fat diet for 4 months. In contrast to ob/ob mice, MKP-3 protein level in liver is not significantly elevated in the diet-induced obese mice (Fig. 3F). It is worth noting that although both diet-induced obese and ob/ob mice are insulin-resistant, only the later is hyperglycemic. These results suggest that MKP-3 may play a role in modulating the hepatic gluconeogenic pathway.

Gluconeogenesis is one of the key metabolic pathways in the liver. Hepatic glucose production and output increase during fasting as an important mechanism for maintaining blood glucose within a normal range. In healthy individuals, gluconeogenesis is tightly regulated by glucagon and insulin in response to fasting and feeding. This regulation is disrupted in obesity because of decreased sensitivity to insulin in liver. Hepatic MKP-3 protein level is modulated by fasting and fed conditions, and the elevation of MKP-3 expression in ob/ob and db/db livers suggests that MKP-3 may be involved in this metabolic process. To investigate which gluconeogenic hormone(s) may increase the expression of MKP-3, Fao cells, an insulin sensitive rat hepatoma cell line, were treated with 8-bromo-cAMP and Dex for 6 h. MKP-3 mRNA level was measured by TaqMan analysis. MKP-3 mRNA was significantly elevated by cAMP treatment but not by cAMP (Fig. 3F). It is known that glucocorticoid promotes gluconeogenesis and that its level is elevated in the state of obesity and insulin resistance (16, 17). Based on these data, it is conceivable that the elevation of MKP-3 expression by glucocorticoid...
MKP-3 and Gluconeogenesis

MKP-3 Activates the PEPCK Gene Transcription in Synergy with Glucocorticoid—PEPCK, the rate-limiting enzyme controlling the gluconeogenic pathway, can be transcriptionally activated by glucocorticoid and glucaons but suppressed by insulin. The control of PEPCK on hepatic glucose production is exclusively regulated at the transcription level. Given the fact that MKP-3 expression was up-regulated by Dex treatment, we hypothesize that MKP-3 may affect the PEPCK transcription. To address this question, we used a PEPCK promoter-luciferase system to investigate the role of MKP-3 in activating the PEPCK gene transcription. H4IIE cells were transfected with the PEPCK promoter-luciferase plasmid PEPCKp600-Luc along with either the control vector or the MKP-3 expression vector. Cells were then treated with various stimuli as shown in TABLE ONE for 24 h and then harvested for luciferase measurement. Modulation of the PEPCK gene transcription was summarized in TABLE ONE. Overexpression of MKP-3 alone did not significantly increase PEPCK promoter activity. Treatment with Dex alone resulted in a 4.4-fold increase of PEPCK transcription. Dex treatment in combination with overexpression of MKP-3 led to a 62.4-fold induction on PEPCK transcription. This indicates that MKP-3 and Dex worked in synergy to strongly induce the PEPCK gene transcription. Treatment with cAMP alone did not significantly increase PEPCK transcription; however, cAMP and Dex had a synergistic effect in activating PEPCK transcription in both vector- and MKP-3-transfected cells (8.62 and 62.4-fold by Dex treatment alone). Insulin completely suppressed PEPCK promoter activity to the basal level in vector-transfected H4IIE cells treated with Dex or Dex in combination with cAMP (0.9-fold of baseline, respectively); but not in MKP-3-transfected H4IE cells under the same treatment, which has PEPCK transcription 7.42- and 5.84-fold of baseline, respectively. These results indicate that MKP-3 overexpression negatively impacted the effect of insulin on suppressing PEPCK transcrip-

![FIGURE 3. Regulation of MKP-3 expression in insulin-resistant mouse models. A–C, MKP-3 mRNA expression level in white adipose tissue (WAT) (A), liver (B), and skeletal muscle (C) from db/db, db/db diabetes mice (lanes 1 and 3) and the lean control mice fed ad libitum (lanes 2 and 4). 20 μg of each total RNA was subjected to Northern blot analysis. D–F, MKP-3 protein level in genetic and diet-induced obese mouse livers. One hundred micrograms of liver protein extract from each sample was subjected to Western blot analysis to determine the levels of MKP-3 protein and tubulin (served as a loading control). The luminescence signal was quantified and is shown in panels E and F. The y-axis shows the level of MKP-3 protein relative to that of tubulin (mean ± S.D.). *, p < 0.05 for ob versus lean; **, p < 0.05 for fasted versus fed from the same genotype; LF, low fat diet (10% fat); HF, high fat diet (45% fat). G, MKP-3 expression in Faoh hepatoma cells treated with 1 μM 8-bromo-cAMP or 1 μM Dex. Cells were serum-starved overnight and then treated with cAMP or Dex for 6 h. Total RNA was extracted, and the expression of MKP-3 mRNA was quantified by Q-PCR analysis with 18 S rRNA as an internal control. The y-axis shows the level of MKP-3 mRNA relative to that of 18 S rRNA. *, p < 0.05, n = 3, mean ± S.D.; NS, not significant; WT, wild type.](http://www.jbc.org/)

TABLE ONE

| Treatment | vector | MKP-3 |
|-----------|--------|-------|
| None      | 1.00 ± 0.02 | 2.20 ± 0.86 |
| Dex       | 4.41 ± 1.07 | 62.4 ± 11.4 |
| cAMP      | 1.33 ± 0.38 | 1.42 ± 0.50 |
| Dex + cAMP| 8.62 ± 4.75 | 176.95 ± 29.17 |
| Dex + Ins⁴ | 0.90 ± 0.14 | 7.42 ± 0.53 |
| cAMP + Ins⁵ | 1.51 ± 0.03 | 2.32 ± 1.56 |
| Dex + cAMP + Ins⁵ | 0.83 ± 0.19 | 5.84 ± 0.74 |

* p < 0.05 vector versus MKP-3 with the same treatment. * Insulin.
tion. These data, together with the fact that Dex induces MKP-3 expression, suggest that MKP-3 plays a role in mediating the effect of glucocorticoid on PEPCK gene transcription.

**Ectopic Expression of MKP-3 Increases Hepatic Glucose Production and Activates Gluconeogenic Gene Expression**—Given the fact that MKP-3 in combination with Dex markedly induced the PEPCK gene transcription and that its expression was elevated in ob/ob and db/db livers, which have increased gluconeogenesis, we wondered whether the elevation of MKP-3 expression in obese livers contributed to enhanced hepatic glucose production. To address this question, we infected Fao cells with adenovirus expressing either MKP-3 or GFP, stimulated them with Dex and cAMP in the absence or presence of insulin, and measured glucose output. Fig. 4A shows that the cells infected with the MKP-3 adenovirus resulted in a significant increase of MKP-3 protein compared with the GFP-infected cells. This increased expression of the MKP-3 protein led to an increase of glucose output as shown in Fig. 4B. Under all conditions, MKP-3 infected cells had a higher glucose output rate compared with GFP-infected cells. In the absence of any treatment, the basal glucose output in MKP-3 infected cells was increased to 159% of the GFP-infected cells. Treatments with Dex and cAMP led to increased glucose output in both GFP- and MKP-3-infected cells; the latter was 237% of the former. In the presence of insulin, glucose production was suppressed in both GFP- and MKP-3-infected cells. However, in the MKP-3-infected cells the effect of insulin in suppressing glucose production was partially reduced, because the glucose production in the MKP-3 cells was ~2-fold higher compared with the GFP cells. To investigate whether MKP-3 overexpression could increase glycogenolysis, which may contribute to elevated glucose production, glycogen content in Fao cells transduced with either adenoviral MKP-3 or GFP was measured before and after stimulation for measurement of gluconeogenesis. The glycogen content did not change in either cells (data not shown), suggesting that the rate of glycogenolysis was not altered by MKP-3 overexpression. Thus the effect of MKP-3 on glucose output is due to increased gluconeogenesis.

To investigate whether MKP-3 expression could induce the expression of gluconeogenic genes, which may account for the elevation of glucose production, we examined the expression of PEPCK and G6Pase, two key genes controlling gluconeogenic pathway. Consistent with the role of MKP-3 on activation of the PEPCK promoter, overexpression of MKP-3 in Fao cells significantly increased the mRNA level of PEPCK (Fig. 4C) and G6Pase (Fig. 4D) in all conditions examined. These results,
MKP-3 and Gluconeogenesis

together with the increased glucose production, strongly suggest that MKP-3 plays a role in modulating the hepatic gluconeogenic program.

DISCUSSION

Liver is a key target tissue of insulin action, which controls fasting blood glucose primarily by regulating the rate of gluconeogenesis. The rate of gluconeogenesis is controlled in a large part by changes in the transcription of the rate-limiting enzyme in this pathway, PEPCK (18, 19). A number of key hormones, such as glucagon, cAMP, and glucocorticoids, have been shown to activate the transcription of this enzyme. Insulin, on the other hand, suppresses PEPCK transcription. Under pathological conditions such as insulin resistance and type 2 diabetes, the effect of insulin in suppressing PEPCK transcription is diminished, which leads to enhanced hepatic glucose output. Although several factors have been identified in mediating insulin suppression on PEPCK gene transcription, the signaling pathways and regulators involved in hepatic insulin resistance and elevated gluconeogenesis are not yet well understood (20). Functional screening was conducted in search for novel genes that antagonize insulin action on PEPCK transcription, and MKP-3 was identified as a candidate.

MKP-3 belongs to the dual specificity phosphatase family with nine members identified to date (21). These phosphatases have different tissue distributions and substrate specificity, suggesting they may have distinct functions. In this family, MKP-1 and MKP-4 have been shown to play a role in regulating adipocyte differentiation and insulin-stimulated glucose uptake in adipocytes (22, 23). MKP-3 has been shown to be involved in modulating growth and apoptosis. However, the role of MKP-3 in insulin sensitivity and metabolism is unknown. In this study, we investigated the expression and regulation of MKP-3 in liver under physiological conditions such as fasting and feeding as well as pathological conditions such as insulin resistance and diabetes. We found that MKP-3 expression was markedly elevated in livers from insulin-resistant and diabetic animals. The MKP-3 protein level was induced by fasting and suppressed by feeding in mouse livers. This regulation is tantamount and diabetic animals. The MKP-3 protein level was induced by Dex, a glucocorticoid that promotes hepatic gluconeogenesis. In addition, MKP-3 dramatically activated the PEPCK gene transcription in synergy with glucocorticoid. Furthermore, ectopic expression of MKP-3 in Fao hepatoma cells significantly elevated both basal and stimulated glucose output and increased the expression of G6Pase and PEPCK genes in these cells.

It is interesting to note that MKP-3 inhibits insulin-mediated repression of the PEPCK promoter (Fig. 1 and TABLE ONE). MKP-3 is highly selective for inactivation of p42/44 MAPKs (24), and complete blockage of ERK-mediated signaling with kinase inhibitors and dominant negative mutants has no effect on PEPCK gene expression (25, 26). Therefore, it is unlikely that MKP-3 blocks insulin-mediated repression on the PEPCK promoter by inactivating ERKs. In contrast to the MAPK pathway, the PI 3-kinase-dependent pathway is required for repression of PEPCK transcription by insulin. To address the question whether MKP-3 antagonizes insulin regulation of the PI 3-kinase pathway, we investigated MKP-3 overexpression on the phosphorylations of several components in insulin signaling pathway upstream and downstream of PI-3kinase, including tyrosine phosphorylation of insulin receptor, insulin receptor substrate-1, and insulin receptor substrate-2 and serine phosphorylation of AKT in Fao cells transduced with either GFP or MKP-3 adenovirus. We found that insulin regulation of PI 3-kinase pathway was not impaired in the MKP-3-overexpressing Fao cells at the level of and upstream of AKT.

The evidence we provided in this study clearly demonstrates that MKP-3 plays a role in regulating the hepatic gluconeogenic program. MKP-3 strongly activated PEPCK transcription in synergy with Dex, a hormone that also induced MKP-3 expression. These data suggest that MKP-3 promotes gluconeogenesis by potentiating and/or mediating the effect of glucocorticoid signaling on hepatic glucose output. It is known that there is a glucocorticoid regulatory unit in the PEPCK promoter. It remains unknown whether activation of PEPCK transcription by MKP3 in synergy with Dex is mediated through the same glucocorticoid response unit or other regulatory element(s). This question will be addressed in future study via mutagenesis of PEPCK promoter. On the other hand, MKP-3 antagonized the effect of insulin on suppressing PEPCK transcription. Interestingly, the insulin-regulated PI 3-kinase pathway (i.e. AKT phosphorylation) was not impaired by MKP-3 overexpression. This suggests that other factors downstream of AKT or additional signaling pathway(s) might be involved. Our data, along with other studies (2, 23), indicate that the MAPK pathway can cross-talk with the metabolic pathways. Further studies are necessary to identify the substrates of MKP-3 in regulating these metabolic processes in liver and fat.

Acknowledgment—We thank Dr. Yang-Sheng Tseng for critical reading of the manuscript.

REFERENCES

1. Moller, D. E. (2001) Nature 414, 821–827
2. Xu, H., Dembski, M., Yang, Q., Yang, D., Mouriarty, A., Tayber, O., Chen, H., Kapeller, R., and Tartaglia, L. A. (2003) J. Biol. Chem. 278, 30187–30192
3. Sutherland, C., O’Brien, R. M., and Granner, D. K. (1996) Philos. Trans. R. Soc. Lond. B Biol. Sci. 351, 191–199
4. Hanson, R. W., and Reshel, L. (1997) Annu. Rev. Biochem. 66, 581–611
5. O’Brien, R. M., and Granner, D. K. (1990) Diabetes Care 13, 327–339
6. Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culepper, J., Devos, R., Richards, G. J., Campbell, L. A., Clark, F. T., Deeds, J., Muir, C., Sanker, S., Mouriarty, A., Moore, K. J., Smutko, J. S., Mays, G. G., Wool, E. A., Monroe, C. A., and Tepper, R. I. (1995) Cell 83, 1263–1271
7. Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M. (1993) Science 259, 89–91
8. Forest, C. D., O’Brien, R. M., Lucas, P. C., Magnuson, M. A., and Granner, D. K. (1990) Mol. Endocrinol. 4, 1302–1310
9. O’Brien R M., Streeter, R. S., Ayala, J. E., Stadelmaier, B. T., and Hornbuckle, L. A. (2001) Biochem. Soc. Trans. 29, 552–558
10. Wallner-Law, M., Duong, D. T., Daniels, M. C., Herzog, B., Wang, X. L., Prasad, R., and Granner, D. K. (2003) J. Biol. Chem. 278, 10427–10435
11. Olovyan, Y., Blum, B., Cassuto, H., Cohen, H., Biberman, Y., Hanson, R. W., and Reshel, L. (2003) J. Biol. Chem. 278, 12929–12936
12. Jascur, T., Gilman, J., and Mustell, T. (1997) J. Biol. Chem. 272, 14483–14488
13. Muda, M., Boschert, U., Dickinson, R., Martinou, J. C., Martinou, I., Camps, M., Schlegel, W., and Arkinstall, S. (1996) J. Biol. Chem. 271, 4319–4326
14. Klock, A., and Herrmann, B. G. (2002) Mech. Dev. 116, 243–247
15. Dickinson, R. J., Eblangie, M. C., Keyse, S. M., and Morrice-Kay, G. M. (2002) Mech. Dev. 113, 193–196
16. Kraus-Friedmann, N. (1984) Physiol. Rev. 64, 170–259
17. Barf, T. (2004) Mini Rev. Med. Chem. 4, 897–908
18. Hanson, R. W., and Patel, Y. M. (1994) Adv. Enzymol. Relat. Areas Mol. Biol. 69, 203–281
19. O’Brien, R. M., and Granner, D. K. (1996) Physiol. Rev. 76, 1109–1161
20. Barthel, A., and Schmoll, D. (2003) Am. J. Physiol. 285, E605–E609
21. Camps, M., Nichols, A., and Arkinstall, S. (2000) FASEB J. 14, 6–16
22. Sakaue, H., Ogawa, W., Nakamura, T., Mori, T., Nakamura, K., and Kasuga, M. (2004) J. Biol. Chem. 279, 39951–39957
23. Buzziene, M., Carlotti, F., Tafrechi, R. S., Hoeben, R. C., and Maassen, J. A. (2004) Mol. Endocrinol. 18, 1697–1707
24. Camps, M., Nichols, A., Gillieron, C., Antonsson, B., Muda, M., Chabert, C., Boschert, U., and Arkinstall, S. (1998) Science 280, 1262–1265
25. Sutherland, C., Wallner-Law, M., Granner, D. K. (1998) J. Biol. Chem. 273, 3198–3204
26. Gabbay, R. A., Sutherland, C., Grundi, L., Kahn, B. B., O’Brien, R. M., Granner, D. K., and Flier, J. S. (1996) J. Biol. Chem. 271, 1890–1897
Dual Specificity MAPK Phosphatase 3 Activates PEPCK Gene Transcription and Increases Gluconeogenesis in Rat Hepatoma Cells
Haiyan Xu, Qing Yang, Minhui Shen, Xueming Huang, Marlene Dembski, Ruth Gimeno, Louis A. Tartaglia, Rosana Kapeller and Zhidan Wu

J. Biol. Chem. 2005, 280:36013-36018.
doi: 10.1074/jbc.M508027200 originally published online August 26, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M508027200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 26 references, 11 of which can be accessed free at http://www.jbc.org/content/280/43/36013.full.html#ref-list-1