

Original Article

DNA damage induces down-regulation of *PEPCK* and *G6P* gene expression through degradation of PGC-1 α

Hyun-Ju Kim[†], Hye Jin Jee[†], and Jeanho Yun^{*}

Department of Biochemistry, College of Medicine, Dong-A University, Busan 602-714, South Korea

[†]These authors contributed equally to this work.

^{*}Correspondence address. Tel: +82-51-240-2919; Fax: +82-51-241-0778; E-mail: yunj@dau.ac.kr

Hepatic gluconeogenesis plays a crucial role in glucose homeostasis. Although it is well established that various cellular processes are modulated by DNA damage, whether the DNA damage signaling pathway regulates gluconeogenesis has not yet been studied. In this study, we found that mRNA levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P), key enzymes for gluconeogenesis, were dramatically decreased upon IR- and UV-irradiation. *PEPCK* and *G6P* promoter activities were also suppressed by IR- and UV-irradiation, suggesting that *PEPCK* and *G6P* gene transcription are down-regulated upon DNA damage. We also found that the protein level of PGC-1 α , which is a critical transcription factor for *PEPCK* gene expression, is decreased upon UV-irradiation. The decreased PGC-1 α protein level was abolished by MG132, a potent proteasome inhibitor, suggesting that PGC-1 α is degraded through the ubiquitin-proteasome pathway upon UV-irradiation. These results reveal a novel link between glucose metabolism and the DNA damage signaling pathway and suggest a possible role for PEPCK and G6P in the DNA damage response.

Keywords DNA damage response; PEPCK; G6P; PGC-1 α ; gluconeogenesis; transcription regulation

Received: January 31, 2011 Accepted: April 7, 2011

Introduction

The cellular genome is constantly exposed to both internal and external DNA damaging agents such as reactive oxygen species (ROS), ionizing radiation, UV light, and various chemical agents. Mammalian cells respond to genomic insults by activating a highly conserved, complex signaling pathway initiating the DNA damage response [1]. The eukaryotic DNA damage response ensures the maintenance of genomic integrity and suppression of tumorigenesis in

the presence of DNA damage through the regulation of various cellular responses, including DNA repair and arrest of cell cycle progression [2]. Coordinated regulation of the expression of a subset of genes that play important roles in these responses is a key step in DNA damage response. To achieve precise control of the expression of these genes, the stability and activity of transcriptional activators is tightly regulated through different mechanisms including phosphorylation, acetylation, and ubiquitination. It has been demonstrated that many important transcription factors, such as p53 [3], E2F [4], and NF- κ B [5], are degraded by the ubiquitin-proteasome pathway and these degradation is essential for a proper DNA damage response.

Glucose concentrations are strictly maintained under physiological conditions. Glucose homeostasis is maintained through a balance between glucose uptake by skeletal muscle and adipose tissue, and production by liver. Hepatic gluconeogenesis is strictly controlled by the activities of rate-limiting enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P). The expression of PEPCK and G6P genes are regulated at the transcriptional level by a complex network of transcription factors and cofactors including cAMP responsive element-binding protein (CREB), hepatocyte nuclear factor 4 α (HNF-4 α), and forkhead factor (Foxo1) [6–10]. Particularly, recent studies demonstrated that the transcription cofactor peroxisome proliferator-activated receptor- γ (PPAR- γ) coactivator-1 α (PGC-1 α) acts as a key modular in the regulation of *PEPCK* and *G6P* gene expression [11,12]. Although the mechanisms involved in regulation of gluconeogenesis genes are well understood, the possible link between gluconeogenesis and the DNA damage response has not been studied.

In this study, we investigated whether the key enzymes of gluconeogenesis such as PEPCK and G6P are regulated upon DNA damage. We describe our findings that PEPCK transcription is repressed by the DNA damage checkpoint upon DNA damage. In addition, we found DNA damage induced

PGC-1 α degradation through the ubiquitin-proteasome pathway, suggesting that the gluconeogenesis pathway is repressed by the DNA damage checkpoint pathway.

Materials and Methods

Cell culture and treatments

HepG2 human liver carcinoma cells and HEK293 cells were grown in Dulbecco modified Eagle medium (DMEM) containing 10% FBS. For DNA damage, cells were treated with gamma irradiation (IR) using a ^{131}Cs source, or with UV using a germicidal UV lamp and UV radiometer (UVX-25; Ultra-Violet Products Ltd., Upland, USA) at the indicated dosage. Caffeine and MG132 were obtained from Sigma (St. Louis, USA).

Semi-quantitative reverse transcriptase-polymerase chain reaction

For semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analyses, total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. cDNA was obtained using Moloney murine leukemia virus reverse transcriptase (MMLV-RT) and oligo-dT primers (Promega, Madison, USA). PCR was performed with AccuPower PCR premix (Bioneer Co., Taejeon, Korea) using cDNA as a template. PCR products were separated by electrophoresis on 2% agarose gels. PCR conditions were as follows: one cycle at 95°C for 5 min, followed by 22 cycles at 95°C for 45 s, 55°C for 1 min, and 72°C for 1 min. The primers employed in RT-PCR were as follows: *PEPCK* forward primer 5'-AAGAGACACAGTGTCCCATCC-3', *PEPCK* reverse primer 5'-ACGTAGGGTGAATCCGTCAG-3', *G6P* forward primer 5'-GAGACTGGCTCAACCTCGTC-3', *G6P* reverse primer 5'-CCTGGTCCAGTCTCACAGGT-3', actin forward primer 5'-ATGGATGATGATATCGCCGCG-3', and actin reverse primer 5'-TCTCCATGTCGTCCAGTTG-3'. Experiments were repeated at least twice and relative band intensities were calculated after normalization to the actin signal. Band intensities were quantified by densitometry using AlphaEaseFC 4.0 software (Alpha Innotech, Randburg, Gauteng, South Africa). Relative band intensities were calculated based on the band intensity of untreated samples after normalization to the actin signal.

Plasmids, transfection, and reporter gene assays

PEPCK- and *G6P*-promoter-luciferase plasmids were kindly provided by Dr Akiyoshi Fukamizu [13]. Flag-PGC-1 α was generated previously [14] by subcloning PGC-1 α cDNA (a gift from Bruce Spiegelman) into the pCMV-Tag2B vector (Stratagene, La Jolla, USA). DNA transfections were performed using Lipofectin reagent (Invitrogen) according to the manufacturer's instructions.

To examine the effect of DNA damage on the *PEPCK* and *G6P* promoters, HepG2 cells were transfected with 1 μg of the pPEPCK- or pG6P-promoter-luciferase plasmids. The pSV40- β -galactosidase plasmid was included in each transfection sample as a control for the efficiency of transfection. Cells were treated with IR- or UV-irradiation 24 h after transfection and were further incubated for another 24 h. To examine the effect of PGC-1 α , cells were cotransfected with 2 μg of Flag-PGC-1 α plasmid. Cells were lysed with Reporter Lysis Buffer (Promega). Firefly luciferase activity was quantified with Luciferase Assay System (Promega) using the GloMax luminometer (Promega). β -galactosidase activity was measured using the β -galactosidase activity Enzyme Assay System (Promega). Luciferase activities were normalized according to β -galactosidase activity and the relative luciferase activity is presented as the mean value plus standard deviation. Each experiment was repeated at least three times.

Western blot analysis

Cells were lysed in RIPA buffer and subjected to Western blot analysis as described previously [14]. Briefly, cells were harvested with RIPA lysis buffer [50 mM Tris (pH 7.5), 50 mM NaCl, 0.5 % Triton X-100, 1 mM EDTA, 1 mM DTT containing phosphatase and protease inhibitors]. The protein concentration of each cell lysate was determined using the Bio-Rad protein assay kit (Bio-Rad). Thirty micrograms of lysate was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane (Amersham, Piscataway, USA). Immunoblotting was performed with Enhanced Chemiluminescence Western blotting detection reagents (Amersham). Antibodies for PGC-1 α were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Antibodies for phospho-Thr68 Chk2 and phospho-Ser317 Chk1 were obtained from Cell Signaling Technology Inc. (Danvers, USA). Anti-Flag antibodies were obtained from Sigma. Levels of actin were monitored as an internal loading control using anti-actin (Sigma) antibodies.

Results

PEPCK and *G6P* gene expression are down-regulated upon DNA damage

To investigate whether the expression of gluconeogenesis genes is regulated by a DNA damage checkpoint, we first examined mRNA levels of *PEPCK* and *G6P* after DNA damage in HepG2 human liver carcinoma cells. Because IR and UV irradiation are representative treatments used to activate the DNA damage checkpoint [2], HepG2 cells were treated with either IR (10 Gy) or UV (50 J/m 2), and mRNA levels were examined 18 h later by semi-

quantitative RT-PCR. The activation of DNA damage checkpoint upon IR- and UV-irradiation was confirmed by the strong phosphorylation of both Chk1 and Chk2, two essential effector kinases in the DNA damage signaling pathway [2] (Supplementary Fig. S1). Interestingly, we found that mRNA levels of *PEPCK* and *G6P* were remarkably decreased upon IR- and UV-irradiation [Fig. 1(A)].

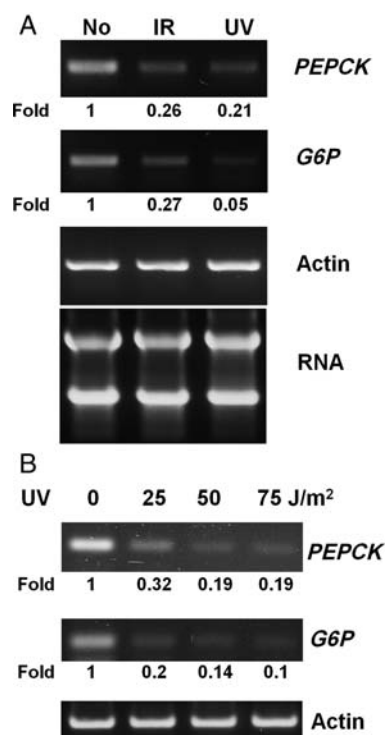


Figure 1 Down-regulation of *PEPCK* and *G6P* mRNA levels by IR- and UV-irradiation in HepG2 cells (A) HepG2 cells were treated with either IR (10 Gy) or UV (50 J/m²) and harvested 18 h later. Total RNA was prepared and semi-quantitative RT-PCR was performed using the indicated primers as described in the 'Materials and Methods' section. The data shown are representative of three independent experiments. (B) HepG2 cells were treated with various dose of UV-irradiation and harvested 18 h later. RT-PCR analysis was performed as in (A).

The RT-PCR results indicated that *PEPCK* mRNA levels were decreased to ~26 and 21% of their original levels after IR and UV-irradiation, respectively. *G6P* mRNA levels were also decreased in similar ratios upon IR- and UV-irradiation. RT-PCR analysis of cells after treatment with various doses of UV-irradiation revealed that mRNA levels of *PEPCK* and *G6P* were decreased in a dose-dependent manner [Fig. 1(B)]. These results suggest that the expression of gluconeogenesis genes is down-regulated during the DNA damage response.

DNA damage represses promoter activity of *PEPCK* and *G6P*

To further substantiate the down-regulation of gluconeogenesis genes upon DNA damage, we next examined *PEPCK* and *G6P* promoter activity upon DNA damage using a reporter assay. HepG2 cells were transfected with *PEPCK*- or *G6P*-promoter-luciferase constructs and were then treated with IR (10 Gy) or UV (50 J/m²). As expected from the semi-quantitative RT-PCR results (Fig. 1), IR- and UV-irradiation led to substantial decreases in *PEPCK* and *G6P* promoter activity, whereas the *pGL2* control promoter was unchanged [Fig. 2(A)]. Similar to the RT-PCR results, *PEPCK* and *G6P* promoter activity was also decreased from 12 to 25% after IR or UV-irradiation. These results confirm that *PEPCK* and *G6P* gene transcription is repressed by DNA damage signals.

We next examined whether IR- and UV-induced *PEPCK* repression are mediated by the DNA damage signaling pathway using caffeine, a potent chemical inhibitor of the DNA damage checkpoint pathway [15]. As shown in Fig. 2(B), caffeine treatment significantly inhibited ($P < 0.05$) both IR- and UV-induced *PEPCK* promoter repression, although it did not completely suppress the reduction of *PEPCK* promoter activity upon IR and UV-irradiation. Statistical analyses revealed that caffeine treatment inhibited UV- and IR-induced *PEPCK* promoter

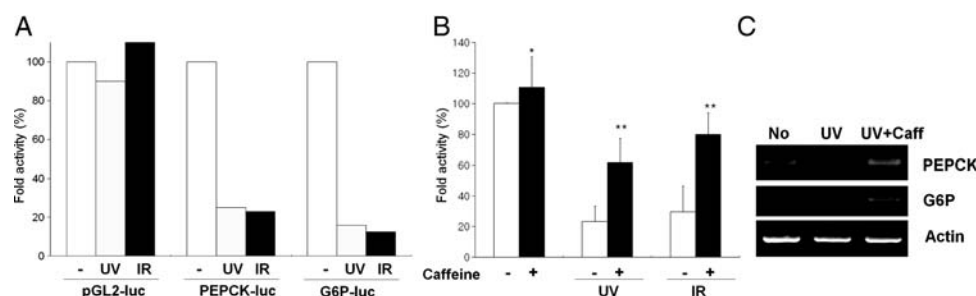


Figure 2 Repression of the *PEPCK* promoter upon IR and UV-irradiation through the DNA damage checkpoint pathway (A) HepG2 cells were transfected with either pGL2-luc control, *PEPCK*-(*PEPCK*-luc) or *G6P*-promoter-luciferase (*G6P*-luc) plasmids. Twenty-four hours after transfection, cells were treated with IR (10 Gy) or UV (50 J/m²) and harvested 24 h later. Luciferase activities were determined as described in 'Materials and Methods'. (B) HepG2 cells were transfected with *PEPCK*-luc and DNA damage was treated as (A) in the presence or absence of caffeine (5 mM). Cells were harvested 24 h later and luciferase activities were determined. * $P > 0.05$; ** $P < 0.05$ by Student's *t*-test. (C) HepG2 cells were treated with UV (50 J/m²) and cultured in the presence or absence of caffeine (5 mM). Cells were harvested 18 h later and RT-PCR analysis was performed using the indicated primers as described in the 'Materials and Methods' section.

activity reduction from 62 to 80%, respectively. Furthermore, we observed that IR- and UV-induced decrease of endogenous mRNA levels of *PEPCK* and *G6P* were also inhibited by caffeine treatment [Fig. 2(C)]. These results suggest that the gene expression of *PEPCK* and *G6P* is down-regulated through the DNA damage checkpoint pathway.

UV-irradiation suppresses the transactivation function of PGC-1 α

It has been previously shown that the transcriptional coactivator PGC-1 α is an essential transcription factor for the transcription of *PEPCK* and *G6P* [11,12]. Cotransfection of a PGC-1 α expression plasmid with either *PEPCK*- or *G6P*-promoter-luciferase constructs substantially increased the promoter activity of *PEPCK* and *G6P* (Fig. 3A), confirming that PGC-1 α plays an important role in transcription activation of *PEPCK* and *G6P* in HepG2 cells. To address whether DNA damage modulates the function of PGC-1 α , we examined the effect of UV-irradiation on the activation of *PEPCK* by PGC-1 α . We found that PGC-1 α -mediated activation of the *PEPCK* promoter was completely inhibited by UV-irradiation (Fig. 3B). These results suggest that the transcription transactivation function of PGC-1 α is suppressed by UV-irradiation.

PGC-1 α is degraded upon UV-irradiation through the ubiquitin-proteasome pathway

To examine whether PGC-1 α is involved in the DNA damage-induced down-regulation of *PEPCK* and *G6P* transcription, we examined endogenous PGC-1 α levels after

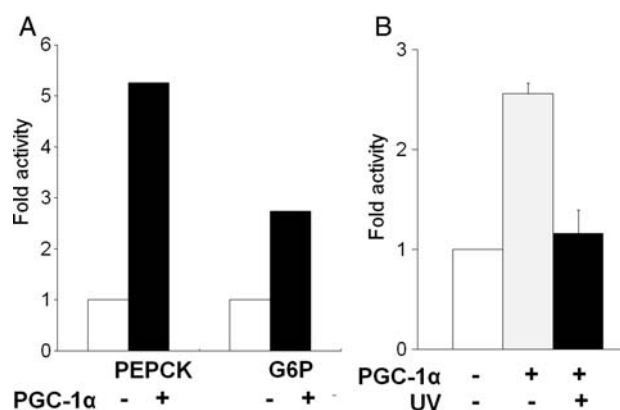


Figure 3 Suppression of the transcription transactivation function of PGC-1 α by UV-irradiation (A) HepG2 cells cotransfected with 1 μ g of *PEPCK*-luc or *G6P*-luc plasmid together with or without 2 μ g of Flag-PGC-1 α . Cells were harvested 48 h after transfection and luciferase activities were determined as described in the 'Materials and Methods' section. (B) HepG2 cells were transfected with the *PEPCK*-luc plasmid. Twenty-four hours after transfection, cells were treated with UV (50 J/m²) and cultured for 24 h in the presence or absence of caffeine (5 mM). Luciferase activities were determined as in (A).

UV-irradiation. Interestingly, the protein level of PGC-1 α decreased upon UV-irradiation [Fig. 4(A)]. To explore this potentially significant observation, we transiently expressed Flag-tagged PGC-1 α in HEK293 cells and examined the Flag-PGC-1 α level after UV-irradiation. Consistent with the endogenous PGC-1 α findings, the protein level of exogenous Flag-PGC-1 α also decreased after UV-irradiation [Fig. 4(B)]. These results suggest that PGC-1 α protein is degraded in response to DNA damage.

Rapid protein degradation in the cell is usually mediated by the ubiquitin-proteasome pathway [16,17]. To test whether UV induces PGC-1 α degradation through this pathway, we examined the effect of MG132, a potent proteasome inhibitor. As shown in Fig. 4(C), UV-induced degradation of PGC-1 α was completely abolished by MG132 treatment. These results suggest that PGC-1 α is degraded upon UV-irradiation through the ubiquitin-proteasome pathway.

Discussion

In this study, we found that the expression of gluconeogenesis genes is regulated by the DNA damage signaling pathway. Although previous studies showed that DNA damage regulates the expression of many genes, whether the expression of gluconeogenesis genes is regulated by DNA damage has never been examined. We found that *PEPCK* and *G6P* mRNAs were reduced upon IR- and UV-irradiation in HepG2 cells (Fig. 1). The promoter activity of *PEPCK* and *G6P* was also decreased upon UV-irradiation [Fig. 2(A)]. These results suggest that gluconeogenesis gene expression is down-regulated during the DNA damage response. Moreover, inhibition of UV-induced repression of *PEPCK* and *G6P* mRNA by caffeine [Fig. 2(B)], an inhibitor of the DNA damage checkpoint, suggests that the caffeine-sensitive DNA damage

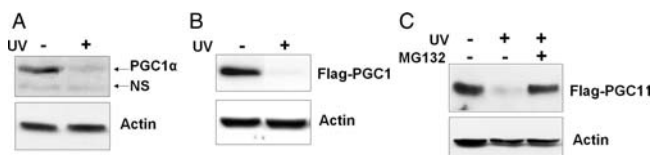


Figure 4 Degradation of PGC-1 α through the ubiquitin-proteasome pathway by UV-irradiation (A) HepG2 cells were treated with UV (50 J/m²) and harvested 12 h later. Cell lysates were subjected to Western blotting using anti-PGC-1 α and anti-actin antibodies. The non-specific band (NS) is indicated. (B) HEK293 cells were transiently transfected with Flag-PGC-1 α expressing plasmid. Twenty-four hours after transfection, cells were treated with UV (50 J/m²) and harvested 12 h later. Cell lysates were then subjected to Western blotting using anti-Flag and anti-actin antibodies. (C) HEK293 cells were transiently transfected with Flag-PGC-1 α expressing plasmid. Twenty-four hours after transfection, cells were treated with both UV (50 J/m²) and MG132 (20 μ M), then harvested 12 h later. Cell lysates were immunoblotted with anti-Flag antibody or anti-actin antibody.

checkpoint pathway is involved in DNA damage-induced repression of gluconeogenesis genes.

The results presented in this study suggest a possible link between glucose metabolism and cellular DNA damage checkpoint function. One possible explanation for DNA damage-induced regulation of gluconeogenesis genes is that it may be necessary to inhibit, gluconeogenesis, an energy-consuming process, to conserve energy for the DNA damage response. Notably, the alteration of energy expenditure and cellular metabolism are well known hallmarks of cancer. Glucose metabolism is the most apparently changed pathway in tumor cells. Glycolysis has been shown to be elevated in almost all cancers, referred to as the 'Warburg effect' [18]. Recently, Bi *et al.* reported that colorectal cancer tissues exhibit various aberrant metabolic changes, including elevated glycolysis, decreased glucuronate metabolism, impaired tricarboxylic acid cycle, and down-regulated gluconeogenesis [19]. *PEPCK* and *G6P* are two rate-limiting enzymes that are widely used as markers for gluconeogenesis [20]. Interestingly, Mandal and Davie also reported that gluconeogenesis gene expression is decreased in breast cancer [21]. Additionally, down-regulation of the *PEPCK* gene has been reported in human colorectal cancer [19]. Whether *PEPCK* and *G6P* play a role in tumorigenesis and how DNA damage-induced down-regulation of gluconeogenesis gene contribute to this process remains to be addressed in future studies.

In addition, the results presented in this study revealed that PGC-1 α , which is an essential transcription activator for *PEPCK* and *G6P*, is degraded upon UV-irradiation [Fig. 4(A,B)]. Complete inhibition of UV-induced PGC-1 α reduction by MG132, a potent proteasome inhibitor [Fig. 4(C)], indicates that PGC-1 α is degraded upon UV-irradiation through the ubiquitin-proteasome pathway. These results suggest that DNA damage may inhibit gluconeogenesis gene expression through the degradation of PGC-1 α . It has been shown that PGC-1 α expression was reduced in breast and colon cancer tissues [22,23]. Jiang *et al.* showed that low expression of PGC-1 α in cancer tissues is associated with poor clinical outcomes for breast cancer patients [24]. These studies suggest that the aberrant expression of PGC-1 α is implicated in tumor formation and progression. Importantly, we have recently reported that PGC-1 α is directly phosphorylated by Chk1 and Chk2 [14]. Chk1 and Chk2 are activated upon DNA damage by upstream kinase, ATM and ATR, and act as key signal transducers for proper DNA damage response [2]. Thus, it is possible that Chk1/2-dependent phosphorylation could promote PGC-1 α degradation and subsequently result in down-regulation of *PEPCK* and *G6P* gene expression. Whether PGC-1 α degradation is responsible for *PEPCK* repression following DNA damage and whether Chk1/2-dependent phosphorylation indeed induces PGC-1 α degradation remain to be determined.

In conclusion, this study revealed that *PEPCK* gene expression is down-regulated upon DNA damage. In conjunction with our previous study, these current results suggest that *PEPCK* gene expression is actively regulated through the modulation of PGC-1 α protein stability. Although the exact mechanism of *PEPCK* gene repression and PGC-1 α degradation needs to be explored, further studies should provide important insight for understanding the role of gluconeogenesis in the DNA damage response and provide efficient strategies for cancer treatment.

Supplementary Data

Supplementary data is available at *ABBS* online.

Acknowledgements

We thank Dr Akiyoshi Fukamizu for providing *PEPCK* and *G6P* promoter plasmids.

Funding

This work was supported by Dong-A university research fund in 2007.

References

- 1 Zhou BB and Elledge SJ. The DNA damage response: putting checkpoints in perspective. *Nature* 2000, 408: 433–439.
- 2 Bartek J and Lukas J. Mammalian G1- and S-phase checkpoints in response to DNA damage. *Curr Opin Cell Biol* 2001, 13: 738–747.
- 3 Maki CG, Huibregtse JM and Howley PM. *In vivo* ubiquitination and proteasome-mediated degradation of p53(1). *Cancer Res* 1996, 56: 2649–2654.
- 4 Hofmann F, Martelli F, Livingston DM and Wang Z. The retinoblastoma gene product protects E2F-1 from degradation by the ubiquitin-proteasome pathway. *Genes Dev* 1996, 10: 2949–2959.
- 5 Palombella VJ, Rando OJ, Goldberg AL and Maniatis T. The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 1994, 78: 773–785.
- 6 Hanson RW and Reshef L. Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annu Rev Biochem* 1997, 66: 581–611.
- 7 Roesler WJ. What is a cAMP response unit? *Mol Cell Endocrinol* 2000, 162: 1–7.
- 8 Hall RK, Sladek FM and Granner DK. The orphan receptors COUP-TF and HNF-4 serve as accessory factors required for induction of phosphoenolpyruvate carboxykinase gene transcription by glucocorticoids. *Proc Natl Acad Sci U S A* 1995, 92: 412–416.
- 9 Puigserver P, Rhee J, Donovan J, Walkey CJ, Yoon JC, Oriente F and Kitamura Y, *et al.* Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1 α interaction. *Nature* 2003, 423: 550–555.
- 10 Hall RK, Yamasaki T, Kucera T, Waltner-Law M, O'Brien R and Granner DK. Regulation of phosphoenolpyruvate carboxykinase and insulin-like growth factor-binding protein-1 gene expression by insulin. The role of winged helix/forkhead proteins. *J Biol Chem* 2000, 275: 30169–30175.

- 11 Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J and Adelmant G, *et al.* Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature* 2001, 413: 131–138.
- 12 Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A and Rudolph D, *et al.* CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature* 2001, 413: 179–183.
- 13 Yamagata K, Daitoku H, Shimamoto Y, Matsuzaki H, Hirota K, Ishida J and Fukamizu A. Bile acids regulate gluconeogenic gene expression via small heterodimer partner-mediated repression of hepatocyte nuclear factor 4 and Foxo1. *J Biol Chem* 2004, 279: 23158–23165.
- 14 Kim MA, Kim HJ, Brown AL, Lee MY, Bae YS, Park JI and Kwak JY, *et al.* Identification of novel substrates for human checkpoint kinase Chk1 and Chk2 through genome-wide screening using a consensus Chk phosphorylation motif. *Exp Mol Med* 2007, 39: 205–212.
- 15 Sarkaria JN, Busby EC, Tibbetts RS, Roos P, Taya Y, Karnitz LM and Abraham RT. Inhibition of ATM and ATR kinase activities by the radiosensitizing agent, caffeine. *Cancer Res* 1999, 59: 4375–4382.
- 16 Herskho A and Ciechanover A. The ubiquitin system. *Annu Rev Biochem* 1998, 67: 425–479.
- 17 Pickart CM. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 2001, 70: 503–533.
- 18 Warburg O. On the origin of cancer cells. *Science* 1956, 123: 309–314.
- 19 Bi X, Lin Q, Foo TW, Joshi S, You T, Shen HM and Ong CN, *et al.* Proteomic analysis of colorectal cancer reveals alterations in metabolic pathways: mechanism of tumorigenesis. *Mol Cell Proteomics* 2006, 5: 1119–1130.
- 20 Sutherland C, O'Brien RM and Granner DK. New connections in the regulation of PEPCK gene expression by insulin. *Philos Trans R Soc Lond B Biol Sci* 1996, 351: 191–199.
- 21 Mandal S and Davie JR. An integrated analysis of genes and pathways exhibiting metabolic differences between estrogen receptor positive breast cancer cells. *BMC Cancer* 2007, 7: 181.
- 22 Feilchenfeldt J, Brundler MA, Soravia C, Totsch M and Meier CA. Peroxisome proliferator-activated receptors (PPARs) and associated transcription factors in colon cancer: reduced expression of PPARgamma-coactivator 1 (PGC-1). *Cancer Lett* 2004, 203: 25–33.
- 23 Watkins G, Douglas-Jones A, Mansel RE and Jiang WG. The localisation and reduction of nuclear staining of PPARgamma and PGC-1 in human breast cancer. *Oncol Rep* 2004, 12: 483–488.
- 24 Jiang WG, Douglas-Jones A and Mansel RE. Expression of peroxisome-proliferator activated receptor-gamma (PPARgamma) and the PPARgamma co-activator, PGC-1, in human breast cancer correlates with clinical outcomes. *Int J Cancer* 2003, 106: 752–757.