J Mol Med (2009) 87:139–144
DOI 10.1007/s00109-008-0429-9

RAPID COMMUNICATION

The role of HSP70 on ENPP1 expression and insulin-receptor activation

Antonella Marucci · Giuseppe Miscio · Libera Padovano · Watip Boonyasrisawat · Jose C. Florez · Alessandro Doria · Vincenzo Trischitta · Rosa Di Paola

Received: 29 April 2008 / Revised: 10 November 2008 / Accepted: 26 November 2008 / Published online: 16 December 2008

Abstract Ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) inhibits insulin-receptor (IR) signaling and, when over-expressed, induces insulin resistance in vitro and in vivo. Understanding the regulation of *ENPP1* expression may, thus, unravel new molecular mechanisms of insulin resistance. Recent data point to a pivotal role of the *ENPP1* 3'UTR, in modulating *ENPP1* mRNA stability and expression. We sought to identify *trans-acting* proteins binding the *ENPP1*-3'UTR and to investigate their role on ENPP1 expression and on IR signaling. By RNA electrophoresis mobility shift analysis and tandem mass spectrometry, we demonstrated the binding of heat shock protein 70 (HSP70) to *ENPP1*-3'UTR. Through this binding, HSP70 stabilizes *ENPP1* mRNA and increases *ENPP1* transcript and protein levels. This positive modulation of ENPP1 expression is paralleled by a reduced insulin-induced IR and IRS-1 phosphorylation. Taken together these data suggest that HSP70, by affecting ENPP1 expression, may be a novel mediator of altered insulin signaling.

Keywords Inhibitors of insulin signaling · Insulin resistance · 3′ untranslated regions · Tyrosine-kinase receptors

Vincenzo Trischitta and Rosa Di Paola equally supervised the study.

Antonella Marucci and Giuseppe Miscio contributed equally to the study.

Electronic supplementary material The online version of this article (doi:10.1007/s00109-008-0429-9) contains supplementary material, which is available to authorized users.

A. Marucci · G. Miscio · L. Padovano · V. Trischitta (✉) · R. Di Paola (✉)
Research Unit of Diabetes and Endocrine Diseases,
Scientific Institute,
Poliambulatorio Giovanni Paolo II, Viale Padre Pio,
71013 San Giovanni Rotondo, Italy
e-mail: vincenzo.trischitta@uniroma1.it
e-mail: r.dipaola@operapadrepio.it

W. Boonyasrisawat · A. Doria
Research Division, Joslin Diabetes Center,
Boston, MA, USA

W. Boonyasrisawat · J. C. Florez · A. Doria
Department of Medicine, Harvard Medical School,
Boston, MA, USA

J. C. Florez
Diabetes Unit/Center for Human Genetic Research,
Massachusetts General Hospital,
Boston, MA, USA

J. C. Florez
Program in Medical and Population Genetics,
Broad Institute of Harvard and MIT,
Cambridge, MA, USA

V. Trischitta
Department of Clinical Sciences, "Sapienza" University,
Rome, Italy

V. Trischitta
"CSS-Mendel" Institute,
Rome, Italy

Introduction

Insulin resistance is pathogenic for type 2 diabetes and cardiovascular disease [1]. Unraveling the molecular mechanisms underlying this syndrome is urgently needed.

Ectoenzyme nucleotide pyrophosphate phosphodiesterase 1 (ENPP1) has been proposed as a pathogenic factor for insulin resistance [2]. ENPP1 affects insulin signaling by binding to IR α-subunit and inhibiting receptor β-subunit autophosphorylation [3, 4]. ENPP1 expression is increased in tissues of insulin-resistant individuals [5, 6]. In addition, ENPP1 over-expression causes insulin resistance in rodents. [7, 8]. Finally, several data indicate that gain of function of *ENPP1* (as induced by the missense K121Q polymorphism) contribute to insulin resistance and type 2 diabetes [7–9]. Thus, determining the mechanisms whereby *ENPP1* is over-expressed may help develop strategies to counteract and possible reverse some forms of insulin resistance. Recent data point to a pivotal role of the *ENPP1*-3'UTR [10, 11], suggesting the existence of *trans*-acting proteins that affect *ENPP1* mRNA stability. Our aim was to identify these proteins and investigate their role in the modulation of ENPP1 expression and insulin signaling.

Material and methods

Preparation of RNA probes as well as cell culture and solubilization are described in the online appendix methods

RNA electrophoresis mobility shift analysis (REMSA)

Fifty microgram of HEK293 lysates were incubated with the ^{32}P-labeled-RNA probe for 20 min at room temperature (RT) [12]. Following incubation with heparin (5 mg/ml), gel electrophoresis was carried out at RT on 5% non-denaturing PAGE and visualized by autoradiography on Typhoon 8600 (Amersham). For supershift analysis, HEK293 lysates were incubated with ^{32}P-labeled-RNA before adding either heat shock protein 70 (HSP70) specific antibody (SPA-812, Stressgen) or total IgG (Santa Cruz Biotechnology) for 30 min at RT.

Isolation of ENPP1-RNA binding protein

REMSA was carried as described above. Four gels of 15 lanes each were loaded. High molecular weight complexes, located by a 1-h exposure to X-ray film at −80°C, were excised and eluted from the gel [12]. Proteins were pooled, concentrated by acetone precipitation and resolved on 10% SDS-PAGE. The only band present in the gel with an apparent molecular weight of 70 kD was excised, and washed twice with 50% HPLC-grade acetonitrile before subsequent analysis. After proteolytic digestion, peptide composition analysis was performed at the Harvard Microchemistry Facility, Harvard University, (Cambridge, MA USA; http://www.mcb.harvard.edu/microchem/) by microcapillary reverse-phase HPLC nano-electrospray tandem mass spectrometry (μLC/MS/MS) on a Finnigan LCQ DECA XP Plus quadrupole ion trap mass spectrometer.

RNA extraction, cDNA synthesis, and gene expression analysis

Total RNA was isolated from cells using RNAEasy Quick kit (Qiagen). cDNA was generated by reverse transcription with M-MLV Reverse Transcriptase (Promega) and used as template in the subsequent analyses. Gene Expression Assay on Demand Kit Reagents (Applera) were used to quantify relative gene expression levels of *ENPP1* and *GAPDH* on ABI-PRISM 7500 (Applera). Expression levels of *ENPP1* were normalized against *GAPDH* using the comparative C_t method, and expressed as percentage of control.

siRNA, cell transfections

Cells were seeded in six-well plates and grown in DMEM/F12 complete medium for 48 h. To down-regulate HSP70 expression, 150 nmol/L of siRNA targeted against *HSP70* mRNA (Ambion ID number: 202680 was the only oligonucleotide used in our experiments) were either cotransfected or not with *ENPP1* cDNAs by using TransMessanger Transfection Reagent (Qiagen) according to the manufacturer's instructions.

Cell lines stably over-expressing *IR* cDNA (HEK293-IR) were generated by co-transfection of the prk5-*IR* (provided by Dr. Axel Ulrich, Martinsried Germany) and prk-5neo followed by geneticin selection IR expression was evaluated by western blot (WB) as described below. HEK293-IR were transiently transfected with prk7-*ENPP1* and/or with pCMVSport6-*HSP70* plasmid (ATCC) by using FuGENE6 (Roche).

Western blot analysis

Cell lysates were separated by SDS-PAGE and transferred to nitrocellulose membrane (Amersham Pharmacia Biotech). Blots were probed with following antibodies: anti-HSP70 (SPA-812, Stressgen), anti-IRβ-subunit (C19, Santa Cruz Biotechnology), anti-PY (PY99 HRP, Santa Cruz Biotechnology), anti-ENPP1 (N-20, Santa Cruz Biotechnology) and anti-IRS-1 (A-19, Santa Cruz Biotechnology). Alternatively, cell lysates were immunoprecipitated with anti-PY antibody (4G10, Millipore) and analyzed by WB using IRβ-subunit or IRS-1 antibodies. Immunocomplexes were detected with the ECL Western Blotting System (Amersham Pharmacia Biotech).

ENPP1 mRNA stability

ENPP1 mRNA stability was evaluated by adding Actinomycin D (5 μg/ml) 60 h after *HSP*70 silencing. RNA extraction was performed at different times as described above, and *ENPP1* expressions determined as described.

Insulin stimulation

Insulin (10 nmol/L for 5' at 37°C) was added to cells and total cell lysates were either immunoprecipitated or not before SDS-PAGE. IRβ-subunit and IRS-1 phosphorylation were evaluated by WB as described.

Results

Identification and characterization of an ENPP1-3'UTR protein complex

REMSA was performed by incubating HEK293 cell extracts with a 395-bp probe corresponding to the *ENPP1*-3'UTR showing a high degree of conservation between human and mouse genomes (Supplementary Figure 1). RNA–protein complex was indicated by band-shift observed in the presence of the probe (Supplementary Figure 2, lanes 1–2). SDS-PAGE of the eluted shifted-band revealed a single protein in the RNA-protein complex. This was identified by tandem mass spectrometry as the 70 kDa HSP70 encoded by the *HSPA1B* isoform (Supplementary Figure 3). Addition of increasing amount of HSP70 antibody to the RNA-cell extract mixture induced a gradual loss of the high molecular complex (Supplementary Figure 2, lanes 3-10), confirming the specific interaction between HSP70 and *ENPP1*-3'UTR.

Effect of HSP70 on ENPP1 expression

We evaluated the effect of HSP70 down-regulation on *ENPP1* mRNA stability and expression by transfecting HEK293 cells with *HSP70*-siRNA. Exposure to the siRNA decreased HSP70 by approximately 80% (Fig. 1a). After transcription inhibition by Actinomycin D, a progressive reduction in *ENPP1* mRNA levels was observed (Fig. 1b);

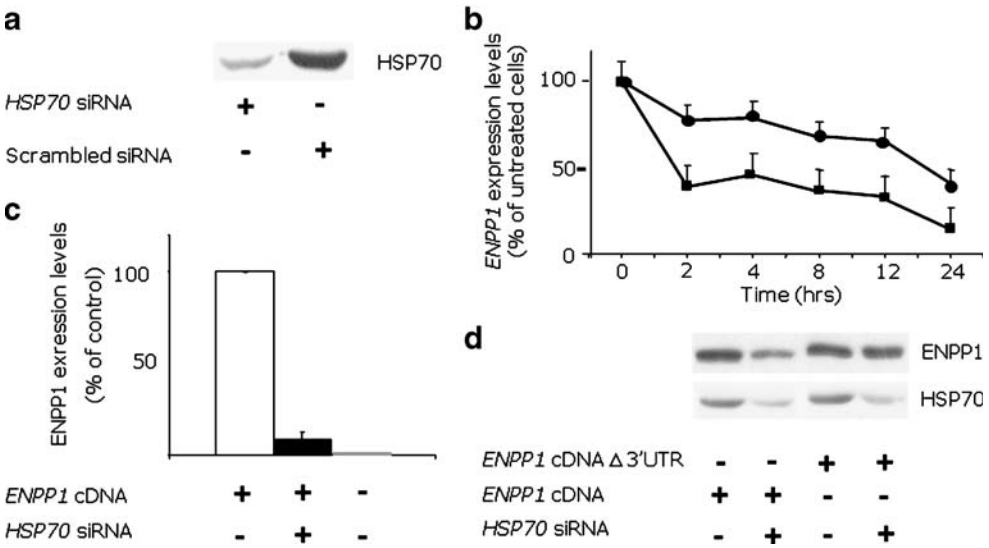

Fig. 1 HSP70 down-regulation and ENPP1 expression. **a** *HSP70* siRNA. HEK293 cells were either transfected with *HSP70* siRNA (*lane 1*) or with scrambled siRNA (*lane 2*). HSP70 expression was evaluated by western blot by using HSP70 SPA-812 specific antibody. **b** *ENPP1* mRNA stability. *ENPP1* mRNA content was determined by real time PCR (after transcription inhibition by 5 μg/ml Actinomycin-D) in HEK293 cells either treated (*square*) or not (*circle*) with *HSP70* siRNA. A significantly greater reduction of mRNA content over time (p=0.04, by two-way ANOVA) was observed in HSP70 down-regulated cells as compared to control cells. Expression levels of *ENPP1* were normalized against GAPDH using the comparative C_t method, expressed as percentage of untreated cells at time 0 (Endogenous levels of *ENPP1* expression were detectable with Ct values being 26.4±1.3, as compared to those of GAPDH being 15.7±1.9). Data are mean±SD (n=4 experiments in each group). **c** *ENPP1* levels in transfected HEK293 cells. *ENPP1* mRNA content was determined by real-time PCR in HEK293 cells transfected with *ENPP1* cDNA and treated (*black bar*) or not (*white bar*) with *HSP70* siRNA. The grey bar represents *ENPP1* levels in untransfected cells. HSP70 down-regulation caused 80% significant reduction (p= 0.016 by paired sample t-test) of *ENPP1* transcript. Expression levels of *ENPP1* were normalized against GAPDH using the comparative C_t method, expressed as percentage of untreated cells (*white bar*). Data are means±SD (n=3 experiments in each group). **d** ENPP1 protein content. HEK293 cells transfected with either *ENPP1* cDNA or with *ENPP1* cDNA lacking 3'UTR (*ENPP1* Δ,3'UTR lanes 3–4) were treated (+) or not (−) with *HSP70* siRNA. Total cell lysates were then used to measure HSP70, (by using HSP70 SPA-812 specific antibody) and ENPP1 (by using ENPP1 N-20 specific antibody) protein content. HSP70 down-regulation had no effect on ENPP1 protein content when *ENPP1* Δ3'UTR cDNA was used

the reduction, however, was significantly greater in HSP70 down-regulated than control cells ($p<0.01$; Fig. 1b). We also evaluated the effect of *HSP70*-siRNA on steady state levels of *ENPP1* mRNA and protein in cells transfected with *ENPP1* cDNA. Both mRNA (Fig. 1c) and protein (Fig. 1d, lanes 1–2) contents were lower following HSP70 down-regulation. This was not observed when *ENPP1* cDNA lacked the 3'UTR (Fig. 1d, lanes 3–4). We then evaluated the effect of HSP70 over-expression (i.e. by *HSP70* cDNA transfection) on ENPP1 protein content in cells co-transfected with *ENPP1* cDNA. ENPP1 protein levels were approximately 30% higher in co-transfected cells as compared to HEK293 transfected with *ENPP1* cDNA alone (Fig. 2), thus confirming that HSP70 levels affects ENPP1 expression.

Effect of HSP70 down-regulation on insulin signaling

When HSP70 expression was down-regulated in HEK293-IR cells, insulin-stimulated IR autophosphorylation was 2.5- to threefold higher than in control cells (Fig. 3a: lanes 2 vs. lane 1; Fig. 3b: second bar vs. first bar). In contrast, in cells transfected with *ENPP1* cDNA, insulin-induced IR autophosphorylation was markedly inhibited (Fig. 3a, lanes 3 vs. lane 1; Fig. 3b: third bar vs. first bar). This inhibition was partially abolished by HSP70 down-regulation (Fig. 3a lane 4 vs. lane 3; Fig. 3b: fourth bar vs. third bar), a rescue which was paralleled by a reduction of ENPP1 over-expression (Fig. 3a lanes 3–4). Neither the partial rescue of IR autophosphorylation (Fig. 3a lanes 5–6 and Fig. 3b sixth bar vs. fifth bar) nor the reduced ENPP1 expression (Fig. 3a lanes 5–6) was observed when cells were transfected with *ENPP1* cDNA lacking the 3'UTR. In human liver HepG2 cells, *HSP70* down-regulation increased insulin-induced IR and IRS-1 phosphorylation (Fig. 3c lane 3 vs. lane 2). As compared to control cells, insulin-induced IRβ-subunit autophosphorylation and IRS-1 phosphorylation were higher in cells treated with *HSP70* siRNA (153% and 274% increase, respectively, mean of two different experiments).

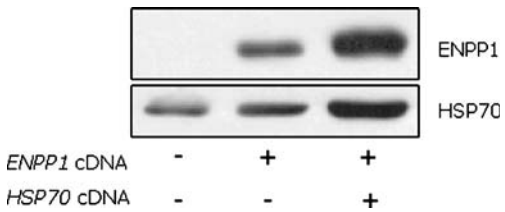

Fig. 2 HSP70 over-expression and ENPP1 protein content. HEK293 cells were either transfected with *ENPP1* cDNA (*lane 2*) or with both *ENPP1* and *HSP70* cDNAs (*lane 3*). Total cell lysates were then used to measure HSP70 (*bottom panel*) and ENPP1 (*upper panel*) protein content, by western blot analysis. HSP70 up-regulation (*bottom panel*, *lane 3*) increased by approximately 30% ENPP1 protein content (*lane 3* vs. *lane 2*). A representative experiment is shown

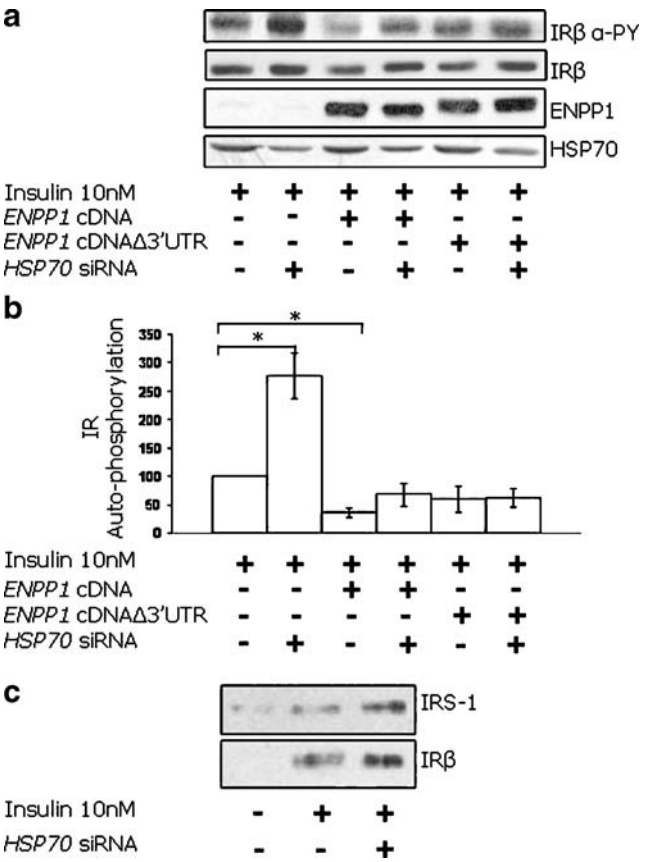

Fig. 3 HSP70 and insulin signaling. **a** HEK293-IR cells were either transfected (*lanes 3–6*) or not (*lanes 1–2*) with either *ENPP1* cDNA (*lanes 3–4*) or with *ENPP1* cDNA lacking the 3'UTR (Δ3'UTR, *lanes 5–6*) and then treated (*lanes 2*, *4* and *6*) or not (*lanes 1, 3* and *5*) with *HSP70* siRNA. Sixty hrs after silencing, cells were stimulated with 10 nmol/L insulin for 5 min at 37°C and IRβ-subunit autophosphorylation evaluated. IRβ-subunit autophosphorylation was assessed with anti-PY antibody. Blot was then stripped and reprobed with anti IRβ subunit. HSP70 protein content was evaluated with specific SPA-812 antibody. IR and HSP70 bands are from the same blot, but were cropped because of their different motilities. A representative experiment is shown. (**b**) IRβ-subunit autophosphorylation in each condition was calculated as percentage of that of control untransfected cells (first bar, from the left). IRβ-subunit autophosphorylation was almost 3 times higher in cells treated with *HSP70* siRNA (second bar) and 70% lower in cells transfected with *ENPP1* cDNA (third bar). As compared to that of cells transfected with *ENPP1* cDNA (third bar), IRβ-subunit autophosphorylation was doubled in those co-transfected, with *HSP70* siRNA (fourth bar), so that an almost complete rescue of IRβ-subunit autophosphorylation was observed. This rescue was not observed when HSP70 expression was down-regulated in cells transfected with *ENPP1* cDNA Δ 3'UTR (lanes 5–6). Data shown are mean ± SEM of 3 experiments. * $p<0.04$ vs. control cells. (**c**) HepG2 cells were either treated (lane 3 or not lane 2) with *HSP70* siRNA. Sixty hrs after silencing, cells were stimulated with 10 nmol/L insulin for10 min at 37°C and cell lysates were immunoprecipitated with anti-PY antibody. Blots were probed with both IRS-1 and IRβ-subunit antibodies, respectively. IRβ-subunit and IRS-1 bands are from the same blot, but were cropped because of their different motilities. A representative experiment is shown

Discussion

The two major messages of our study are as follows 1) by binding *ENPP1*-3'UTR, HSP70 stabilizes *ENPP1* mRNA and eventually increases *ENPP1* transcript and protein levels; 2) through the modulation of ENPP1 expression, HSP70 affects insulin-induced IR and IRS–1 phosphorylation, thus becoming a new potential modulator of insulin resistance. Similar data were observed in two different cell lines, thus suggesting this phenomenon occurs in several insulin target tissues. To the best of our knowledge, this is the first report elucidating a molecular mechanism which modulates ENPP1 expression and, consequently, affects IR signaling.

Most of the molecular mechanisms of insulin resistance so far described are located at the downstream 'post-receptor level'. However, recent functional, metabolic [5, 13, 14] and genetic studies [9–11, 15–23] have suggested that an additional mechanism of insulin resistance resides at the 'receptor level' and is mediated by ENPP1[2]. In this context, we propose HSP70 as a new potential contributor to insulin resistance through up-regulation of ENPP1 expression and subsequent inhibition of IR-autophosphorylation. Of note, HSP70 is known to bind and modulate other 3'UTRs of yeast and human mRNAs [24, 25].Very recently an opposite role of HSP70 on insulin signaling has been reported in transgenic animal models [26]. The proposed mechanism is mediated by HSP70-inhibition of JNK activation, a negative modulator of IRS-1 signaling. These discrepancies are difficult to reconcile and might be due to relevant differences intrinsic to the study models utilized (i.e. in vivo obese rodents vs. in vitro human cells) or variable tissue-specific expression. Further studies are clearly needed to acquire deeper insights on the potential role of HSP70 in both cellular and human insulin resistance.

Acknowledgements This research was supported by Italian Ministry of Health Grants: RC2004 (G.M) C2005 (R.D.P. and G.M.), RC2006 (G.M.), RC2007 (R.D.P.), RF05ED01 (R.D.P.); Italian Ministry of University and Research Grant: RBNE01N4Z9_009; Telethon Grants: E1239 (V.T.), and GGP02423 (R.D.P.). National Institutes of Health Grants HL73168 and DK55523 (A.D.) and DK36836 (Genetics Core of the Diabetes & Endocrinology Research Center at the Joslin Diabetes Center), and a National Institutes of Health Research Development Career Award K23 DK65978-04 (J.C.F.).

References

1. Reaven GM (1988) Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595–1607
2. Goldfine ID, Maddux BA, Youngren JF, Reaven G, Accili D, Trischitta V, Vigneri R, Frittitta L (2008) The role of membrane glycoprotein plasma cell antigen 1/ectonucleotide pyrophosphatase phosphodiesterase 1 in the pathogenesis of insulin resistance and related abnormalities. Endocr Rev 29:62–75
3. Maddux BA, Goldfine ID (2000) Membrane glycoprotein PC-1 inhibition of insulin receptor function occurs via direct interaction with the receptor alpha-subunit. Diabetes 49:13–19
4. Costanzo BV, Trischitta V, Di Paola R, Spampinato D, Pizzuti A, Vigneri R, Frittitta L (2001) The Q allele variant (GLN121) of membrane glycoprotein PC-1 interacts with the insulin receptor and inhibits insulin signaling more effectively than the common K allele variant (LYS121). Diabetes 50:831–836
5. Frittitta L, Youngren JF, Sbraccia P, D'Adamo M, Buongiorno A, Vigneri R, Goldfine ID, Trischitta V (1997) Increased adipose tissue PC-1 protein content, but not tumour necrosis factor-alpha gene expression, is associated with a reduction of both whole body insulin sensitivity and insulin receptor tyrosine–kinase activity. Diabetologia 40:282–289
6. Maddux BA, Sbraccia P, Kumakura S, Sasson S, Youngren J, Fisher A, Spencer S, Grupe A, Henzel W, Stewart TA et al (1995) Membrane glycoprotein PC-1 and insulin resistance in non-insulin-dependent diabetes mellitus. Nature 373:448–451
7. Dong H, Maddux BA, Altomonte J, Meseck M, Accili D, Terkeltaub R, Johnson K, Youngren JF, Goldfine ID (2005) Increased hepatic levels of the insulin receptor inhibitor, PC-1/NPP1, induce insulin resistance and glucose intolerance. Diabetes 54:367–372
8. Maddux BA, Chang YN, Accili D, McGuinness OP, Youngren JF, Goldfine ID (2006) Overexpression of the insulin receptor inhibitor PC-1/ENPP1 induces insulin resistance and hyperglycemia. Am J Physiol Endocrinol Metab 290:E746–E749
9. McAteer JB, Prudente S, Bacci S, Lyon HN, Hirschhorn JN, Trischitta V, Florez JC (2008) The Enpp1 K121q polymorphism is associated with type 2 diabetes in European populations: evidence from an updated meta-analysis in 42,042 subjects. Diabetes 57:1125–1130
10. Frittitta L, Ercolino T, Bozzali M, Argiolas A, Graci S, Santagati MG, Spampinato D, Di Paola R, Cisternino C, Tassi V, Vigneri R, Pizzuti A, Trischitta V (2001) A cluster of three single nucleotide polymorphisms in the 3′-untranslated region of human glycoprotein PC-1 gene stabilizes PC-1 mRNA and is associated with increased PC-1 protein content and insulin resistance-related abnormalities. Diabetes 50:1952–1955
11. Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoeur C, Vatin V, Ghoussaini M, Wachter C, Hercberg S, Charpentier G, Patsch W, Pattou F, Charles MA, Tounian P, Clement K, Jouret B, Weill J, Maddux BA, Goldfine ID, Walley A, Boutin P, Dina C, Froguel P (2005) Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. Nat Genet 37:863–867
12. Heaton JH, Dlakic WM, Dlakic M, Gelehrter TD (2001) Identification and cDNA cloning of a novel RNA-binding protein that interacts with the cyclic nucleotide-responsive sequence in the Type-1 plasminogen activator inhibitor mRNA. J Biol Chem 276:3341–3347
13. Frittitta L, Spampinato D, Solini A, Nosadini R, Goldfine ID, Vigneri R, Trischitta V (1998) Elevated PC-1 content in cultured skin fibroblasts correlates with decreased in vivo and in vitro insulin action in nondiabetic subjects: evidence that PC-1 may be an intrinsic factor in impaired insulin receptor signaling. Diabetes 47:1095–1100
14. Stentz FB, Kitabchi AE (2007) Transcriptome and proteome expressions involved in insulin resistance in muscle and activated T-lymphocytes of patients with type 2 diabetes. Genomics Proteomics Bioinformatics 5:216–235
15. Abate N, Chandalia M, Satija P, Adams-Huet B, Grundy SM, Sandeep S, Radha V, Deepa R, Mohan V (2005) ENPP1/PC-1 K121Q polymorphism and genetic susceptibility to type 2 diabetes. Diabetes 54:1207–1213

16. Bacci S, Ludovico O, Prudente S, Zhang YY, Di Paola R, Mangiacotti D, Rauseo A, Nolan D, Duffy J, Fini G, Salvemini L, Amico C, Vigna C, Pellegrini F, Menzaghi C, Doria A, Trischitta V (2005) The K121Q polymorphism of the ENPP1/PC-1 gene is associated with insulin resistance/atherogenic phenotypes, including earlier onset of type 2 diabetes and myocardial infarction. Diabetes 54:3021–3025
17. Baratta R, Di Paola R, Spampinato D, Fini G, Marucci A, Coco A, Vigneri R, Frittitta L, Trischitta V (2003) Evidence for genetic epistasis in human insulin resistance: the combined effect of PC-1 (K121Q) and PPARgamma2 (P12A) polymorphisms. J Mol Med 81:718–723
18. Frittitta L, Baratta R, Spampinato D, Di Paola R, Pizzuti A, Vigneri R, Trischitta V (2001) The Q121 PC-1 variant and obesity have additive and independent effects in causing insulin resistance. J Clin Endocrinol Metab 86:5888–5891
19. Gu HF, Almgren P, Lindholm E, Frittitta L, Pizzuti A, Trischitta V, Groop LC (2000) Association between the human glycoprotein PC-1 gene and elevated glucose and insulin levels in a paired-sibling analysis. Diabetes 49:1601–1603
20. Hamaguchi K, Terao H, Kusuda Y, Yamashita T, Hazoury Bahles JA, Cruz LM, Brugal VL, Jongchong WB, Yoshimatsu H, Sakata T (2004) The PC-1 Q121 allele is exceptionally prevalent in the Dominican Republic and is associated with type 2 diabetes. J Clin Endocrinol Metab 89:1359–1364
21. Kubaszek A, Pihlajamaki J, Karhapaa P, Vauhkonen I, Laakso M (2003) The K121Q polymorphism of the PC-1 gene is associated with insulin resistance but not with dyslipidemia. Diabetes Care 26:464–467
22. Pizzuti A, Frittitta L, Argiolas A, Baratta R, Goldfine ID, Bozzali M, Ercolino T, Scarlato G, Iacoviello L, Vigneri R, Tassi V, Trischitta V (1999) A polymorphism (K121Q) of the human glycoprotein PC-1 gene coding region is strongly associated with insulin resistance. Diabetes 48:1881–1884
23. Tasic I, Milojkovic M, Sunder-Plassmann R, Lazarevic G, Tasic NM, Stefanovic V (2007) The association of PC-1 (ENPP1) K121Q polymorphism with metabolic syndrome in patients with coronary heart disease. Clin Chim Acta 377:237–242
24. Henics T, Nagy E, Oh HJ, Csermely P, von Gabain A, Subjeck JR (1999) Mammalian Hsp70 and Hsp110 proteins bind to RNA motifs involved in mRNA stability. J Biol Chem 274:17318–17324
25. Laroia G, Cuesta R, Brewer G, Schneider RJ (1999) Control of mRNA decay by heat shock-ubiquitin–proteasome pathway. Science 284:499–502
26. Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, Lancaster GI, Southgate RJ, Bruce CR, Duffy SJ, Horvath I, Mestril R, Watt MJ, Hooper PL, Kingwell BA, Vigh L, Hevener A, Febbraio MA (2008) HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci U S A 105:1739–1744