FEBS Letters 586 (2012) 4023–4030

FEBS Letters

journal homepage: www.FEBSLetters.org

NOXA contributes to the sensitivity of PERK-deficient cells to ER stress

Sanjeev Gupta [a,b,1], Zoltan Giricz [a,c,1,2], Alessandro Natoni [a,c], Neysan Donnelly [a,c], Shane Deegan [a,c], Eva Szegezdi [a,c], Afshin Samali [a,c,*]

[a] Apoptosis Research Centre, NUI Galway, Ireland
[b] School of Medicine, NUI Galway, Ireland
[c] School of Natural Sciences, NUI Galway, Ireland

ARTICLE INFO

Article history:
Received 26 July 2012
Revised 21 September 2012
Accepted 2 October 2012
Available online 12 October 2012

Edited by Quan Chen

Keywords:
PERK
NOXA
Apoptosis
ER stress
Unfolded protein response

ABSTRACT

PKR-like ER kinase (PERK) deficient mouse embryonic fibroblasts (MEFs) are hypersensitive to ER stress-induced apoptosis. However, the molecular determinants of increased sensitivity of PERK$^{-/-}$ MEFs are not clearly understood. Here we show that induction of several Unfolded Protein Response (UPR) target genes is attenuated in PERK$^{-/-}$ MEFs. We also report elevated expression of the BH3-only protein, NOXA in PERK$^{-/-}$ MEFs. Further, shRNA-mediated knockdown of NOXA rescued the hypersensitivity of PERK$^{-/-}$ MEFs to ER stress-induced apoptosis. Taken together our results suggest that compromised induction of UPR and increased NOXA expression contributes to hypersensitivity of PERK$^{-/-}$ MEFs to ER stress-induced apoptosis.

1. Introduction

The endoplasmic reticulum (ER) is the cellular site for Ca^{2+} storage and for synthesis, folding and maturation of most secreted and transmembrane proteins [1]. Accumulation of unfolded/misfolded proteins in the ER is toxic to the cell and causes a condition referred to as ER stress [2]. The cells' response to ER stress is the activation a set of signaling pathways termed the Unfolded Protein Response (UPR) [3]. This concerted and complex cellular response is mediated initially by at least three molecules, inositol requiring enzyme 1 (IRE1), activated transcription factor-6 (ATF6) and PKR-like-ER kinase (PERK) which are kept in an inactive state by the binding of the chaperone GRP78/BiP [1,4]. ER stress causes GRP78 to dissociate leading to the activation of IRE1, ATF6 and PERK.

Activated PERK phosphorylates translation initiation factor 2α (eIF2α), thereby reducing the rate of translation and the protein load on the ER [5]. In addition to its translational regulatory function, PERK directly elicits the activation of the pro-survival transcription factor NF-E2-related factor-2 (NRF2) [6]. PERK-mediated phosphorylation of NRF2 causes it to dissociate from its cytoplasmic regulator Kelch-like ECH-associated protein 1 (KEAP1) and migrate to the nucleus where it induces the expression of a battery of detoxifying enzymes that aim to restore redox homeostasis [7]. PERK activation during the UPR is transient and under a negative feedback regulation; p58IPK, a UPR-target gene, binds to the kinase domain of PERK and inhibits its activity [8]. In addition, protein phosphatase 1 (PP1), activated by ATF4-induced GADD34, dephosphorylates eIF2α to remove the translational block [9]. Despite the numerous reports on activation of cytoprotective signaling pathways downstream of PERK, the role of PERK in determining cell fate during the ER stress and UPR is still controversial. It has been shown that constitutive PERK signaling is in fact pro-apoptotic [10]. This may be due to the enhanced expression of the transcription factor C/EBP homologous protein (CHOP), whose induction strongly depends on PERK signaling during ER stress [11]. CHOP is thought to play an important role in ER stress-induced apoptosis in many scenarios; however CHOP is not necessary for cell death induced by ER stress. PERK$^{-/-}$ and catalytically inactive eIF2α (Ser51Ala) knock-in MEFs are hypersensitive to ER stress-induced apoptosis, although they fail to induce CHOP during ER stress [12].

The role for mitochondria in ER stress-induced apoptosis is well accepted [13]. Here we set out to address the question regarding the mechanisms by which PERK can promote cellular survival. We demonstrate that in the absence of PERK the cells experience constitutive oxidative stress that correlates with increased expression of the BH3 only protein, NOXA. Knock down of NOXA

* Corresponding author at: Apoptosis Research Centre, NUI Galway, Ireland. Fax: +353 91494596.
E-mail address: afshin.samali@nuigalway.ie (A. Samali).
[1] These authors contributed equally to this work.
[2] Current address: Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary.

0014-5793/$36.00 © 2012 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.febslet.2012.10.002

expression in PERK$^{-/-}$ MEFs by shRNA rescues their hypersensitivity to ER stress-induced apoptosis. Our findings provide evidence that the BH3-only protein, NOXA is the mediator of the apoptotic signaling triggered by ongoing oxidative stress in absence of PERK.

2. Materials and methods

2.1. Cell culture and treatments

PERK$^{+/+}$ and PERK$^{-/-}$ MEFs were a gift from Dr. David Ron (Institute of Metabolic Science, University of Cambridge, UK). Cells were maintained in DMEM medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, non-essential amino acid solution, 55 μM β-mercaptoethanol (βME), 1% penicillin/streptomycin at 37 °C, 5% CO_2 in a humidified incubator. To induce apoptosis, cells were treated with 0.01–1 μM thapsigargin (Tg), 0.01–1 μg/ml tunicamycin (Tm), 10–500 ng/ml doxorubicin, 0.01–1 μM etoposide, or 1–100 nM staurosporine for the time periods indicated. All reagents were from Sigma–Aldrich unless otherwise stated.

2.2. Annexin V and propidium iodide staining

Externalization of phosphatidylserine (PS) to the outer leaflet of the plasma membrane of apoptotic cells was assessed with

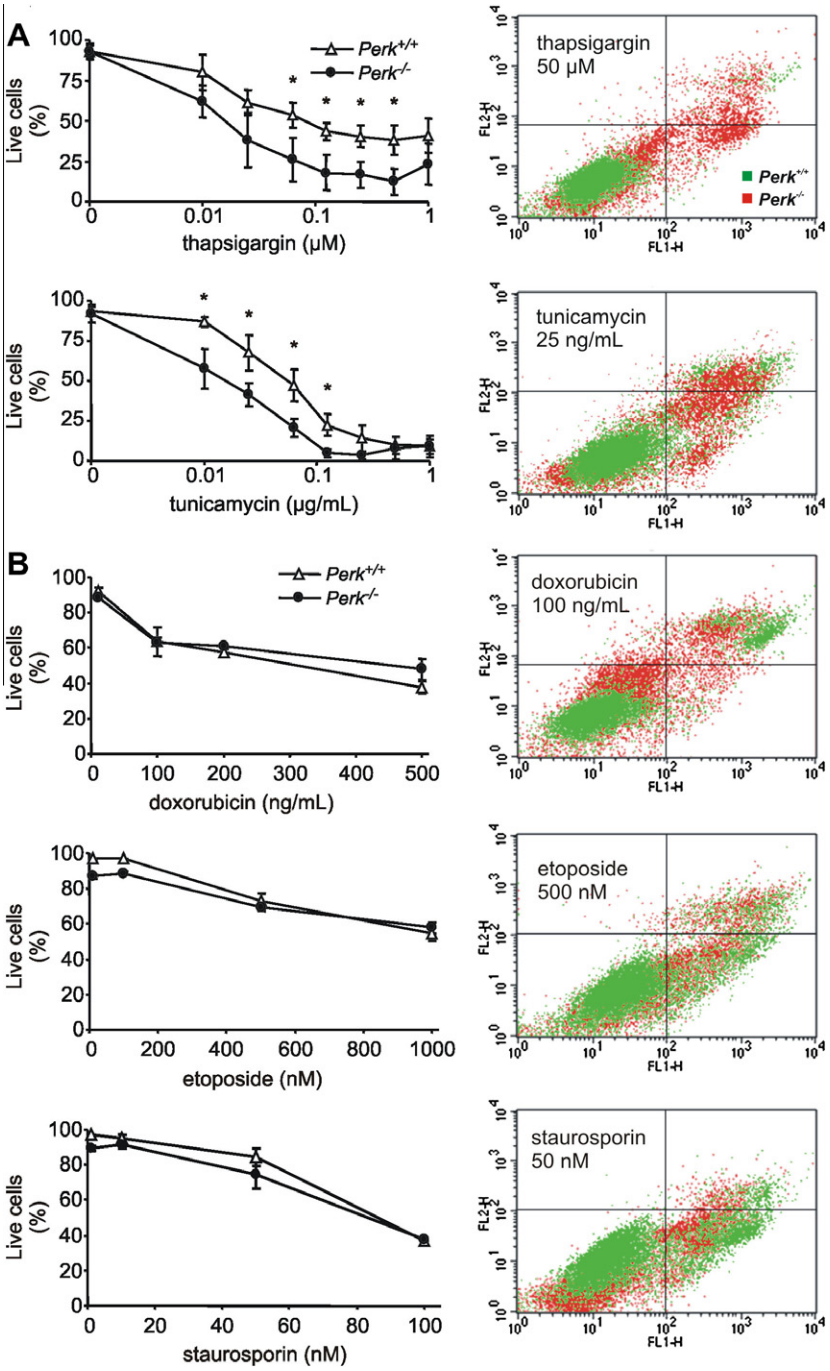

Fig. 1. PERK$^{-/-}$ MEFs are sensitive to ER stress-induced apoptosis. PERK$^{+/+}$ and PERK$^{-/-}$ MEFs were treated with indicated concentration of thapsigargin, tunicamycin, doxorubicin, etoposide, or staurosporine for 48 h. *Left panel*, Cells negative for annexin V-FITC staining are shown as live cells ($n = 3$), $^{*}P < 0.05$. *Right panel*, shows the dot plot of Annexin V/PI staining of PERK$^{+/+}$ (Green) and PERK$^{-/-}$ (Red) MEFs.

annexin V-FITC or annexin V-PE (ImmunoTools) as described previously [14].

2.3. Western blotting

Cells were lysed in a buffer containing 10% glycerol, 200 mM NaCl, 100 mM Tris pH 8.0, 5 mM EDTA, 1% Triton X-100, 100 μM PMSF, 2 μg/ml pepstatin A, 25 μM ALLN, 2.5 μg/ml aprotinin, 10 μM leupeptin, 1 mM Na_3VO_4, 100 mM NaF and 17.5 mM β-glycerophosphate. Cellular proteins were separated by electrophoresis on 8–12% SDS–PAGE and transferred onto nitrocellulose membranes. After blocking in 5% non-fat milk and 0.05% Tween-20 in PBS, blots were incubated with rabbit polyclonal antibodies to caspase-3 (1:1,000; Cell Signaling Technology), caspase-9 (1:1,000; Cell Signaling Technology), GRP78 (1:1,000; Stressgen), CHOP (1:1,000; Santa Cruz Biotechnology), XBP1 (1:2,000; Santa Cruz Biotechnology), actin (1:1,000) or mouse monoclonal antibodies to PARP (1:1,000; Biomol). For detection, the appropriate horseradish peroxidase-conjugated secondary antibodies (Pierce) were used at a 1:5,000 dilution. Protein bands were visualized with Super Signal Ultra Chemiluminescent Substrate (Pierce) on X-ray film (Agfa).

2.4. RNA extraction, RT-PCR and real time RT-PCR

Total RNA was isolated using the RNeasy kit (Qiagen) or TRI Reagent (Invitrogen) according to the manufacturers' instructions and has been previously described [15].

2.5. Analysis of caspase-3-like activity

DEVDase activity was determined using 50 μM of the caspase substrate carbobenzoxy-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (DEVD-AMC) as described previously [14].

2.6. Measurement of $\Delta\Psi m$

Mitochondrial membrane potential was determined by using the fluorescent probe tetramethylrhodamine ethyl ester (TMRE, Molecular Probes) as previously described [16].

2.7. Detection of ROS

$PERK^{+/+}$ and $PERK^{-/-}$ MEFs were plated at 7.5×10^4 cells/ml in 6 well plates in complete medium in the presence or absence of 55 μM of β-ME. After 36 h, cells were trypsinized and resuspended in 1 mL of complete medium and incubated with 25 μM of 2′,7′-dichlorofluorescin diacetate (DCFDA) for 30 min at 37 °C in a 5% CO_2 humidified incubator. After incubation, DCF fluorescence was measured by BD FACS Canto I using FITC channel.

2.8. Plasmid transfection

To generate cells expressing NOXA shRNA, $PERK^{-/-}$ MEFs (10^6 cells) were transfected with 2 μg pGIPZ lentiviral shRNAmir plasmid against NOXA (clone ID: V2LMM_41163, Open Biosystems) or 2 μg pGIPZ lentiviral empty vector (Open Biosystems) using MEF Nucleofector Kit 2 (Amaxa) and Nucleofector II device (Amaxa) with program A23. The stably transfected cells were selected using 2 μM puromycin. Individual clones expressing GFP were picked at day 7 and the expression of NOXA was determined with real time RT-PCR.

2.9. LDH-release assay

LDH release was determined from cell supernatant with the CytoTox-1 kit (Promega) according to the manufacturer's instructions.

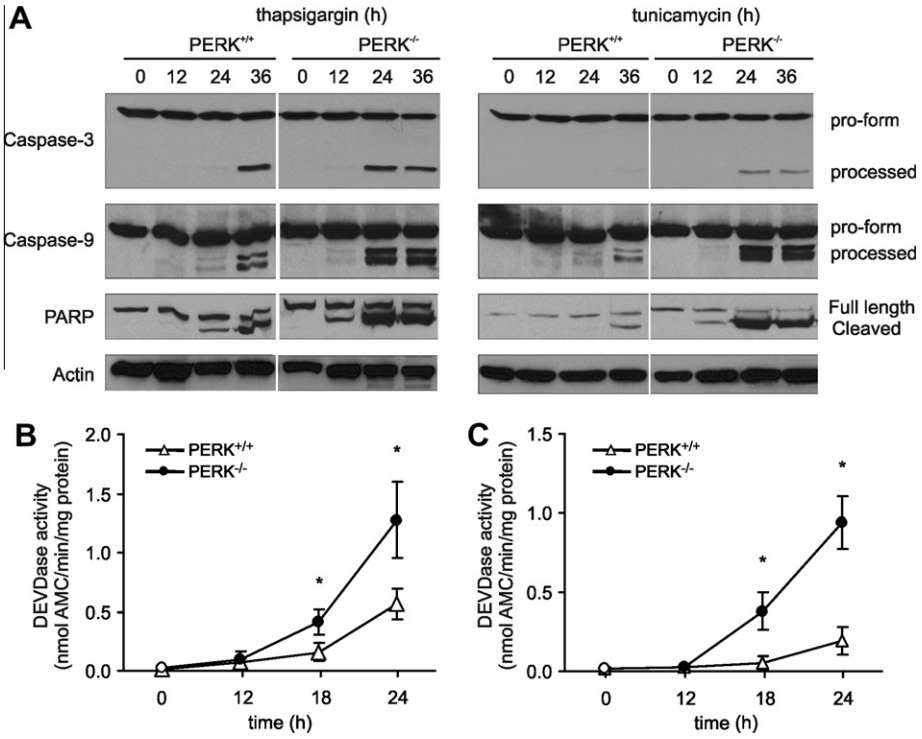

Fig. 2. Increased ER stress-induced caspase activation in $PERK^{-/-}$ MEFs. (A) $PERK^{+/+}$ and $PERK^{-/-}$ MEFs were treated with thapsigargin (Tg, 25 nM) or tunicamycin (Tm, 25 ng/ml) for 0–36 h. Immunoblotting of total protein was performed using indicated antibodies. The samples for caspase-3, caspase-9, and PARP were run on the same gel but not side-by-side. (B–C) $PERK^{+/+}$ and $PERK^{-/-}$ MEFs were treated with (B) Tg (25 nM) or (C) Tm (25 ng/ml) for indicated time points, and DEVD-AMC cleavage activity was measured in whole cell extracts. The DEVDase activity was normalized to the amount of protein, as determined by c Bradford assay. ($n = 3$), *= $P < 0.05$.

2.10. Statistical analysis

Differences between the treatment groups were assessed using one-way ANOVA with Tukey's post hoc test with a significance of $P < 0.05$. All statistics were carried out using SPSS 14.0 for Windows (SPSS).

3. Results

3.1. PERK$^{-/-}$ MEFs are more susceptible to ER stress-induced apoptosis

Earlier studies have reported that MEFs lacking PERK are more sensitive to ER stress-induced apoptosis [12]. In agreement with these findings, we observed that PERK$^{-/-}$ MEFs are more susceptible to ER stress-induced cell death (Fig. 1A). However, to evaluate whether this sensitisation is specific to ER stress, or PERK deficiency also influences the sensitivity to other apoptosis inducers, cells were treated with doxorubicin, etoposide, or staurosporine. We observed that cell death induced by doxorubicin, etoposide, or staurosporine was comparable in PERK$^{+/+}$ and PERK$^{-/-}$ MEFs (Fig. 1 B). The more pronounced apoptotic cell death in PERK$^{-/-}$ MEFs after exposure to ER stress-inducing agents was further corroborated by the earlier and increased processing of pro-caspase-3 and -9 and increased cleavage of PARP, a *bona fide* substrate of active caspase-3 (Fig. 2A). Furthermore, PERK$^{-/-}$ MEFs showed increased DEVDase activity as compared to PERK$^{+/+}$ cells (Fig. 2B and C). These results demonstrate that loss of PERK specifically sensitizes cells to ER stress-induced apoptosis.

3.2. Loss of PERK abrogates optimal induction of the UPR

To investigate the effect of PERK deficiency on the UPR, we compared the induction of key UPR target genes between PERK$^{+/+}$ and PERK$^{-/-}$ MEFs. A real-time RT-PCR analysis showed that induction of GRP78, HERP and CHOP was significantly compromised in PERK$^{-/-}$ MEFs as compared to PERK$^{+/+}$ cells upon exposure to ER stress (Fig. 3A; left panel). Furthermore, ER stress-mediated induction of other UPR-target genes such as p58IPK, WARS and HOX1 (Fig. 3A; right panel) was also mitigated in PERK$^{-/-}$ MEFs as compared to PERK$^{+/+}$ cells. We also observed reduced protein levels of GRP78, spliced XBP1 and CHOP in PERK$^{-/-}$ MEFs as compared to PERK$^{+/+}$ cells (Fig. 3B). Further we observed a reduction in the amount of total and spliced XBP1 mRNA in the PERK$^{-/-}$ MEFs (Fig. 3C). This is in agreement with previous reports where reduced levels of XBP1 mRNA and protein were reported in PERK$^{-/-}$ MEFs [18]. Thus, PERK$^{-/-}$ MEFs are compromised in the optimal induction of several key mediators of the UPR.

3.3. Expression of NOXA is increased in PERK$^{-/-}$ MEFs and is responsive to cellular redox state

Although the involvement of BCL-2 proteins in ER stress-induced cell death is clear [19], how they are regulated during

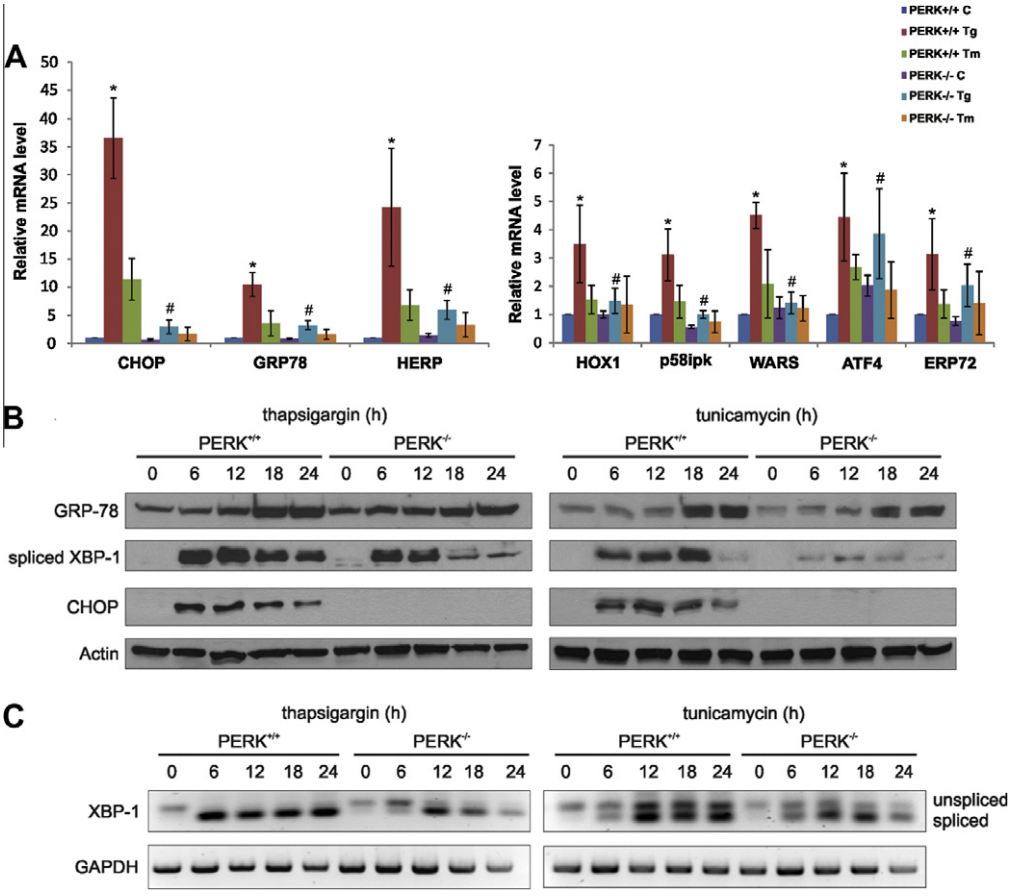

Fig. 3. PERK$^{-/-}$ MEFs are defective in induction of mediators of UPR. (A) PERK$^{+/+}$ and PERK$^{-/-}$ MEFs were treated with Tg (25 nM) or Tm (25 ng/ml) for 6 h and the expression levels of the indicated genes were quantified by real-time RT-PCR analysis of total RNA, normalizing against GAPDH expression. Ratios of indicated gene to GAPDH are plotted in arbitrary units ± SD with expression level from PERK$^{+/+}$ untreated control set at 1. ($n = 3$); *=vs. PERK$^{+/+}$ untreated control; #=vs. PERK$^{+/+}$ Tg; $P < 0.05$. (B) PERK$^{+/+}$ and PERK$^{-/-}$ MEFs were treated with Tg and Tm for 0–24 h and immunoblotting of total protein was performed using indicated antibodies. (C) PERK$^{+/+}$ and PERK$^{-/-}$ MEFs were treated as above and RT-PCR analysis of total RNA was performed to simultaneously detect both spliced and unspliced XBP1 mRNA and GAPDH. The image is presented inverted for greater clarity.

the UPR is less well understood. We reasoned that upregulation of some BH3-only proteins at the transcriptional level may lead to the increased sensitivity of PERK$^{-/-}$ MEFs. We determined the expression level of several BCL-2 family members by real-time quantitative RT-PCR in PERK$^{-/-}$ MEFs compared to PERK$^{+/+}$ cells (Supplementary Table 1). We found that while most BCL-2 genes were not regulated or even downregulated by mild ER stress in PERK expressing MEFs, expression of NOXA, a pro-apoptotic BH3 only member of the BCL-2 family was upregulated in both PERK$^{+/+}$ and PERK$^{-/-}$ MEFs (Fig. 4A). Additionally, the basal and ER stress-induced expression of NOXA was higher in PERK$^{-/-}$ MEFs as compared to PERK$^{+/+}$ MEFs. Despite repeated efforts with several commercially available mouse anti-NOXA antibodies we were unable to reliably detect NOXA at the protein level in lysates from MEFs and so we were limited to analyzing NOXA transcript levels.

It has been reported that PERK$^{-/-}$ cells experience increased levels of reactive oxygen species (ROS) and reducing substances are shown to counteract the accumulation of ROS in PERK$^{-/-}$ cells [17]. While it has been reported that β-ME can itself induce ER stress in the micromolar range [18], when added to PERK$^{-/-}$ MEFs at a concentration of 55 μM it has been shown to protect PERK$^{-/-}$ cells against tunicamycin-induced cell death [17]. Thus, we hypothesized that the higher levels of NOXA in PERK-deficient cells stem from higher levels of ROS. First, using the fluorescent marker, DCFDA we determined the levels of ROS in PERK$^{+/+}$ and PERK$^{-/-}$ MEFs when grown in the presence and absence of the reducing agent (55 μM β-ME). We observed a high intensity of DCFDA fluorescence in PERK$^{-/-}$ MEFs when grown in the absence of β-ME indicating high levels of ROS (Fig. 4B) as compared to β-ME-supplemented medium. However, there was no significant difference in DCFDA fluorescence in PERK$^{+/+}$ MEFs grown in the presence and absence of β-ME (Fig. 4B). We then explored the link between increased oxidative stress and increased expression of NOXA. We observed that PERK$^{-/-}$ MEFs grown in the absence of β-ME (Fig. 4C) led to had increased expression of NOXA compared to PERK$^{-/-}$ MEFs grown in β-ME -supplemented medium, indicating that oxidative stress in PERK$^{-/-}$ MEFs contribute to the higher expression levels of NOXA.

3.4. NOXA contributes to the sensitivity of PERK$^{-/-}$ MEFs to ER stress-induced apoptosis

This study and others have shown induction of NOXA under ER stress conditions [22]. We tested whether the increased expression of NOXA seen in PERK$^{-/-}$ MEFs contributes to the increased ER

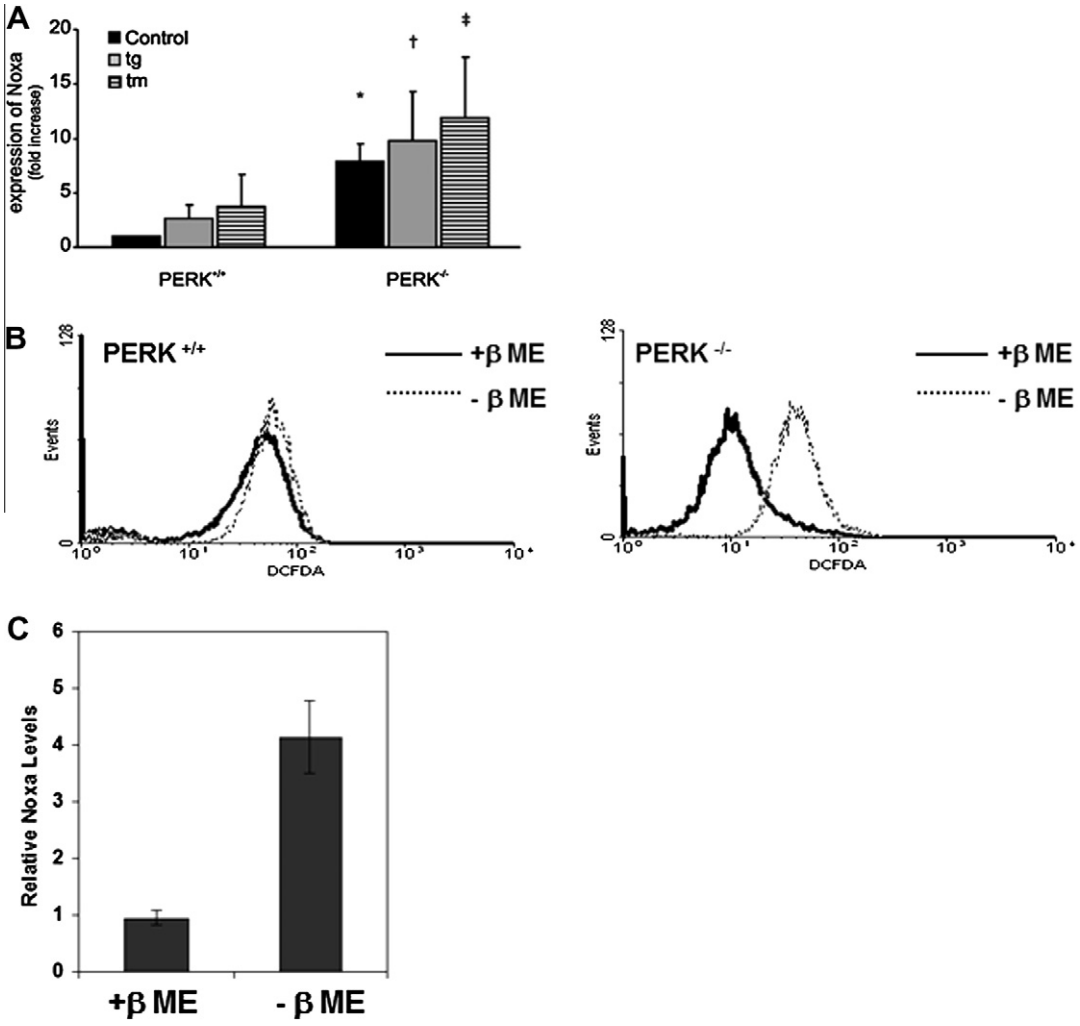

Fig. 4. Expression of NOXA is increased in PERK$^{-/-}$ MEFs and is sensitive to cellular redox state. (A) Total RNA was isolated from PERK$^{+/+}$ and PERK$^{-/-}$ MEFs either untreated or treated with Tg (25 nM) or Tm (25 ng/ml) for 6 h and the expression level of NOXA was quantified by real-time RT-PCR, normalizing against GAPDH expression; *= vs. PERK$^{+/+}$ control; †= vs. PERK$^{+/+}$ tg; ‡= vs. PERK$^{+/+}$ tm; $P < 0.05$. (B) FACS measurement of DCF fluorescence in PERK$^{+/+}$ (upper graph) and PERK$^{-/-}$ MEFs (lower graph) cultured in the presence or absence of β-mercaptoethanol for 36 h. A representative image of three independent experiments is shown. (C) PERK$^{-/-}$ MEFs were cultured as in B, and the expression level of NOXA was quantified by real-time RT-PCR, normalizing against GAPDH expression. Error bars represent mean ± SD from two independent experiments performed in triplicates.

stress-induced cell death. For this purpose, we stably expressed short hairpin RNA (shRNA) targeting NOXA in PERK$^{-/-}$ MEFs. After generating stable clones, we selected a NOXA shRNA expressing clone (PERK$^{-/-}$ NOXA shRNA), where the expression level of NOXA was comparable to its level in PERK$^{+/+}$ cells (Fig. 5A). The expression of NOXA-specific shRNA did not affect the viability of untreated cells, but it effectively reduced ER stress-induced cell death in PERK$^{-/-}$ MEFs (Fig. 5B). As shown in Fig. 5C and D, ER stress-induced cell death and caspase activity in NOXA shRNA expressing PERK$^{-/-}$ MEFs was comparable to PERK expressing MEFs, while ER stress-induced cell death and caspase activity in control vector-transfected PERK$^{-/-}$ MEFs did not change. To test whether increased expression of NOXA leads to higher levels of mitochondrial membrane depolarization in PERK$^{-/-}$ MEFs in response to ER stress inducing stimuli, we performed cytofluorimetric analysis after TMRE staining. We observed further dissipation of mitochondrial transmembrane potential in PERK$^{-/-}$ MEFs as compared to PERK$^{+/+}$ MEFs upon exposure to ER stress. However, NOXA shRNA-expressing PERK$^{-/-}$ MEFs were protected against ER stress-induced loss of mitochondrial membrane potential (Fig. 5E). Ectopic expression of wild-type mouse PERK into PERK$^{-/-}$ MEFs, reduced the NOXA mRNA levels (Fig. 6A) and rescued the hypersensitivity of PERK$^{-/-}$ MEFs to ER stress-induced cell death (Fig. 6B).

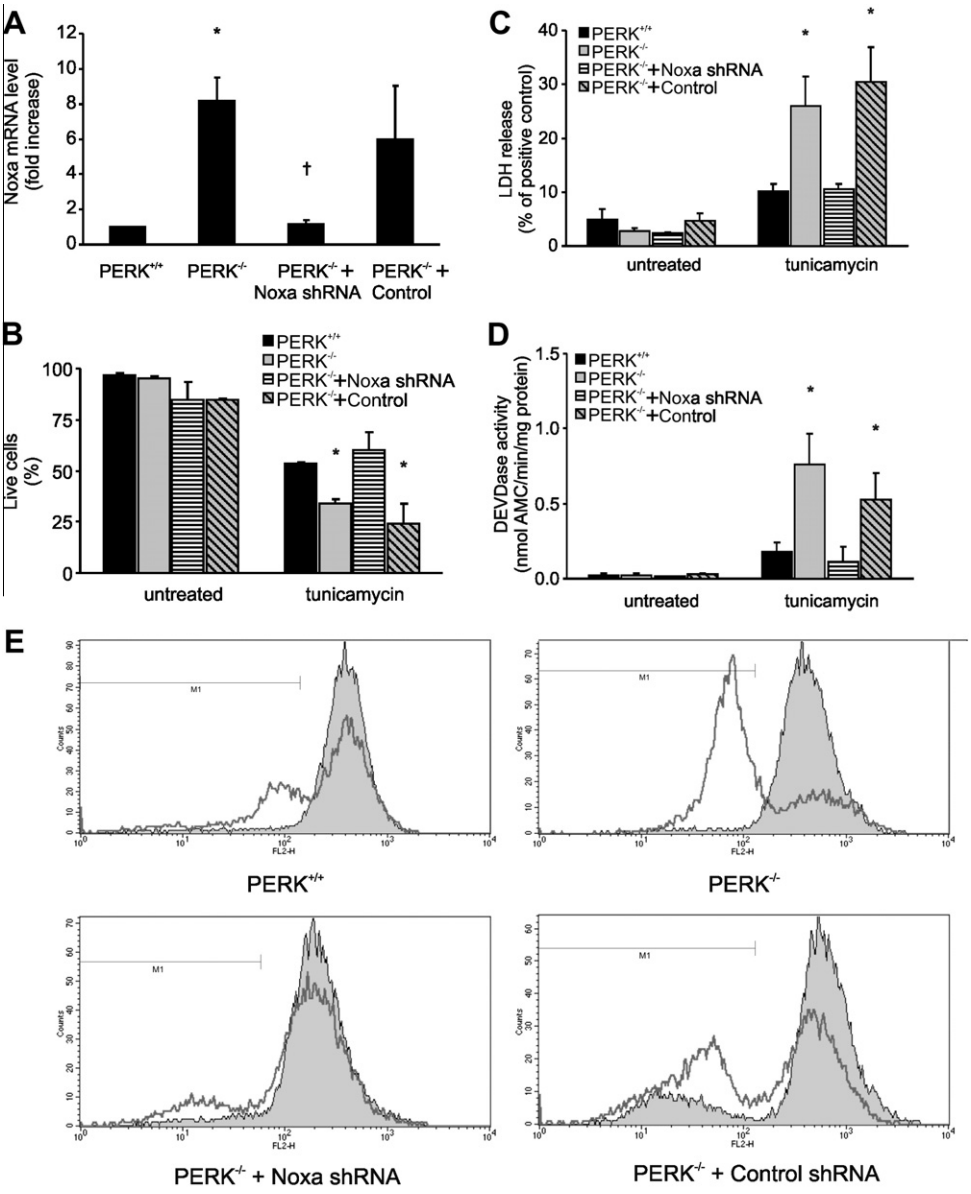

Fig. 5. Knockdown of NOXA expression can rescue PERK$^{-/-}$ MEFs from ER stress-induced apoptosis. (A) Total RNA was isolated from PERK$^{+/+}$, PERK$^{-/-}$, PERK$^{-/-}$ NOXA shRNA and PERK$^{-/-}$ control MEFs and the expression level of NOXA was quantified by real-time RT-PCR, normalizing against GAPDH expression. Ratios of indicated gene to GAPDH are plotted in arbitrary units ± SD with expression level of PERK$^{+/+}$, untreated control set at 1 ($n = 3$). (B) PERK$^{+/+}$, PERK$^{-/-}$, PERK$^{-/-}$ NOXA shRNA and PERK$^{-/-}$ control MEFs were treated with Tm (25 ng/ml) for 48 h and cell death was assessed with annexinV-PE staining ($n = 3$). (C) PERK$^{+/+}$, PERK$^{-/-}$, PERK$^{-/-}$ NOXA shRNA and PERK$^{-/-}$ control MEFs were treated as in B, and plasma membrane integrity was assessed with LDH release assay ($n = 3$). (D) PERK$^{+/+}$, PERK$^{-/-}$, PERK$^{-/-}$ NOXA shRNA and PERK$^{-/-}$ control MEFs were treated as in B, and DEVD-AMC cleavage activity was measured in whole cell extracts. The DEVDase activity was normalized to the amount of protein as determined by Bradford assay. ($n = 3$). (E) PERK$^{+/+}$, PERK$^{-/-}$, PERK$^{-/-}$ NOXA shRNA and PERK$^{-/-}$ empty vector MEFs were either untreated (filled) or treated with tunicamycin (25 ng/mL) for 24 h (open) and mitochondrial membrane potential was assessed using TMRE and flow cytometry. A representative image of three independent experiments is shown; *= vs. PERK$^{+/+}$ control; †= vs. PERK$^{-/-}$ control, $P < 0.05$.

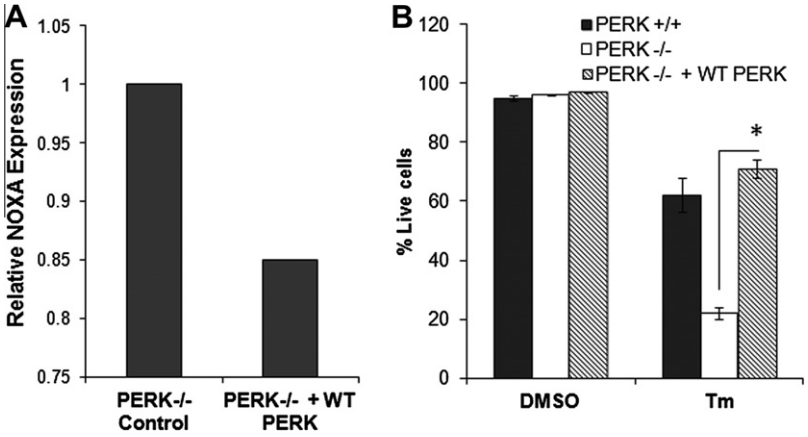

Fig. 6. Expression of wild type PERK can rescue PERK$^{-/-}$ MEFs from ER stress-induced apoptosis. (A) Total RNA was isolated from PERK$^{-/-}$ control and PERK$^{-/-}$ reconstituted with wild type PERK (PERK$^{-/-}$ + WT PERK) MEFs and the expression level of NOXA was quantified by real-time RT-PCR, normalizing against GAPDH expression. Ratios of indicated gene to GAPDH are plotted in arbitrary units. (B) Parental PERK$^{+/+}$ and PERK$^{-/-}$ MEFs and PERK$^{-/-}$ MEFs transduced with wild type PERK were treated with 25 ng/ml Tm for 24 h and apoptosis was quantified using Annexin V staining. Cells negative for annexin V-FITC staining are shown as live cells ($n = 3$); $^{*}P < 0.005$.

4. Discussion

We have reported findings which better characterize the increased sensitivity of PERK$^{-/-}$ MEFs to ER stress-induced apoptosis. We confirm the role of PERK in maintaining redox homeostasis and report a loss of optimal UPR in PERK$^{-/-}$ MEFs. Previous studies have shown that PERK$^{-/-}$ cells are hypersensitive to ER stress [12]. In the present study, we confirm these results and show that PERK contributes to the maintenance of cellular homeostasis and survival through the suppression of ROS-dependent NOXA expression. We show that higher levels of oxidative stress in PERK$^{-/-}$ MEFs lead to elevated expression of NOXA, which contributes, in part, to sensitizing PERK$^{-/-}$ MEFs to ER stress-induced cell death. In support of these observations, NOXA has been show to play important role in oxidative stress mediated apoptosis [19]. In agreement with an important role for NOXA in facilitating apoptosis induced by ER stress, our results show that reduction of NOXA expression restored the sensitivity of PERK$^{-/-}$ MEF cells to ER stress-induced apoptosis to PERK$^{+/+}$ levels.

How might loss of PERK lead to accumulation of NOXA? Oxidative stress is intrinsic to the biosynthesis and posttranslational oxidative processing of secreted proteins and is kept under control by PERK-dependent pathways [6,17,20]. Our results suggest that increased levels of ROS in PERK$^{-/-}$ MEFs contribute to increased expression of NOXA. Although NOXA was first characterized as a p53 target gene [21], FOXO3a has recently been implicated as a transcriptional activator of NOXA expression [22]. It is noteworthy that neither p53 nor FOXO3a appear to play an important role in increased expression of NOXA and hypersensitivity to ER stress-induced apoptosis in PERK$^{-/-}$ MEFs (Supplementary Figs. 1 and 2). Recently, ROS-mediated upregulation of NOXA in chronic lymphocytic leukemia (CLL) was found to be mediated by p38 MAPK and independent of p53 [23]. Furthermore, in keratinocytes NOXA upregulation following UVB irradiation was reported to be mediated by p38 MAPK /HIF-1, in a p53-independent manner [24]. An additional mechanism which has been described to promote expression of NOXA mRNA is down regulation of ubiquitinated histone H2A, which was shown to directly block transcription of the NOXA gene [25].

Since NOXA is a key mediator of apoptosis, it is rather surprising that PERK$^{-/-}$ MEFs are specifically hypersensitive to ER stress. While NOXA is well known for its role as a pro-apoptotic member of the BCL-2 family, it has become increasingly evident that NOXA plays important roles in other cellular processes. NOXA expression is upregulated upon estrogen stimulation and is required for cell cycle progression in estrogen receptor-positive breast cancer cells [26]. NOXA has also been shown to promote cell growth by stimulating glucose consumption via the pentose phosphate pathway [27]. These reports suggest multiple roles of NOXA, as a context-dependent regulator of many different physiological processes. We envisage that there are two key phenomena responsible for the increased sensitivity of PERK$^{-/-}$ MEFs to ER stress, (i) the reduced induction of several key UPR target genes, and (ii) the increased expression of NOXA. Our data suggest that a more detailed understanding of NOXA's regulation by PERK is required in order to understand the hypersensitivity of PERK$^{-/-}$ cells to ER stress-induced cell death.

Acknowledgements

Our research is supported by Science Foundation Ireland (09/RFP/BIC2371; /RFP/BMT2153) and the Health Research Board (HRA/2009/59).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.10.002.

References

[1] Malhotra, J.D. and Kaufman, R.J. (2007) The endoplasmic reticulum and the unfolded protein response. Semin. Cell Dev. Biol. 18, 716–731.
[2] Szegezdi, E., Logue, S.E., Gorman, A.M. and Samali, A. (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep. 7, 880–885.
[3] Gorman, A.M., Healy, S.J., Jager, R. and Samali, A. (2012) Stress management at the ER: regulators of ER stress-induced apoptosis. Pharmacol. Ther. 134, 306–316.
[4] Ron, D. and Walter, P. (2007) Signal integration in the endoplasmic reticulum unfolded protein response. Nat. Rev. Mol. Cell Biol. 8, 519–529.
[5] Harding, H.P., Zhang, Y. and Ron, D. (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397, 271–274.
[6] Cullinan, S.B. and Diehl, J.A. (2004) PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. J. Biol. Chem. 279, 20108–20117.
[7] Niture, S.K., Kaspar, J.W., Shen, J. and Jaiswal, A.K. (2010) Nrf2 signaling and cell survival. Toxicol. Appl. Pharmacol. 244, 37–42.
[8] Yan, W., Frank, C.L., Korth, M.J., Sopher, B.L., Novoa, I., Ron, D. and Katze, M.G. (2002) Control of PERK eIF2alpha kinase activity by the endoplasmic reticulum stress-induced molecular chaperone P58IPK. Proc. Natl. Acad. Sci. USA 99, 15920–15925.

[9] Novoa, I., Zeng, H., Harding, H.P. and Ron, D. (2001) Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2alpha. J. Cell Biol. 153, 1011–1022.
[10] Lin, J.H., Li, H., Zhang, Y., Ron, D. and Walter, P. (2009) Divergent effects of PERK and IRE1 signaling on cell viability. PLoS ONE 4, e4170.
[11] Zinszner, H., Kuroda, M., Wang, X., Batchvarova, N., Lightfoot, R.T., Remotti, H., Stevens, J.L. and Ron, D. (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev. 12, 982–995.
[12] Harding, H.P., Zhang, Y., Bertolotti, A., Zeng, H. and Ron, D. (2000) Perk is essential for translational regulation and cell survival during the unfolded protein response. Mol. Cell 5, 897–904.
[13] Gupta, S., Cuffe, L., Szegezdi, E., Logue, S.E., Neary, C., Healy, S. and Samali, A. (2010) Mechanisms of ER stress-mediated mitochondrial membrane permeabilization. Int. J. Cell Biol. 2010, 170215.
[14] Gupta, S., Deepti, A., Deegan, S., Lisbona, F., Hetz, C. and Samali, A. (2010) HSP72 protects cells from ER stress-induced apoptosis via enhancement of IRE1alpha-XBP1 signaling through a physical interaction. PLoS Biol. 8, e1000410.
[15] Samali, A., Fitzgerald, U., Deegan, S. and Gupta, S. (2010) Methods for monitoring endoplasmic reticulum stress and the unfolded protein response. Int. J. Cell Biol. 2010, 830307.
[16] Samali, A., Cai, J., Zhivotovsky, B., Jones, D.P. and Orrenius, S. (1999) Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. EMBO J. 18, 2040–2048.
[17] Harding, H.P. et al. (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol. Cell 11, 619–633.
[18] Wang, H.Q., Imai, Y., Kataoka, A. and Takahashi, R. (2007) Cell type-specific upregulation of Parkin in response to ER stress. Antioxid. Redox Signal. 9, 533–542.
[19] Eno, C.O., Zhao, G., Olberding, K.E. and Li, C. (2012) The Bcl-2 proteins Noxa and Bcl-xL co-ordinately regulate oxidative stress-induced apoptosis. Biochem. J. 444, 69–78.
[20] Cullinan, S.B. and Diehl, J.A. (2006) Coordination of ER and oxidative stress signaling: the PERK/Nrf2 signaling pathway. Int. J. Biochem. Cell Biol. 38, 317–332.
[21] Oda, E. et al. (2000) Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 288, 1053–1058.
[22] Obexer, P., Geiger, K., Ambros, P.F., Meister, B. and Ausserlechner, M.J. (2007) FKHRL1-mediated expression of Noxa and Bim induces apoptosis via the mitochondria in neuroblastoma cells. Cell Death Differ. 14, 534–547.
[23] Tonino, S.H., van Laar, J., van Oers, M.H., Wang, J.Y., Eldering, E. and Kater, A.P. (2011) ROS-mediated upregulation of Noxa overcomes chemoresistance in chronic lymphocytic leukemia. Oncogene 30, 701–713.
[24] Nys, K., Van Laethem, A., Michiels, C., Rubio, N., Piette, J.G., Garmyn, M. and Agostinis, P. (2010) A p38(MAPK)/HIF-1 pathway initiated by UVB irradiation is required to induce Noxa and apoptosis of human keratinocytes. J. Invest. Dermatol. 130, 2269–2276.
[25] Wang, Q. et al. (2009) ERAD inhibitors integrate ER stress with an epigenetic mechanism to activate BH3-only protein NOXA in cancer cells. Proc. Natl. Acad. Sci. USA 106, 2200–2205.
[26] Liu, W., Swetzig, W.M., Medisetty, R. and Das, G.M. (2011) Estrogen-mediated upregulation of noxa is associated with cell cycle progression in estrogen receptor-positive breast cancer cells. PLoS ONE 6, e29466.
[27] Gimenez-Cassina, A. and Danial, N.N. (2010) Noxa: a sweet twist to survival and more. Mol. Cell 40, 687–688.