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GIV/Girdin Promotes Cell Survival During Endoplasmic Reticulum Stress

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Abstract

Endoplasmic reticulum (ER) stress is a form of cellular stress that is experienced by cells both under normal physiological conditions such as in professional secretory cells and disease states such as cancer, diabetes and neurodegeneration. Upon facing ER stress, cells activate a conserved signaling pathway called the Unfolded Protein Response (UPR) to restore normal function by halting general protein translation, upregulating expression of chaperones and promoting ER associated degradation. However, if the stress is overwhelming and cells are not able to recover within a reasonable time frame, the UPR ultimately commits cells to programmed cell death. How cells make this life-or-death decision remains an exciting yet poorly understood phenomenon. Here we show that Gα-interacting vesicle associated protein (GIV) aka Girdin plays an important role in promoting cell survival during ER stress. Cells lacking GIV are impaired in activating the pro-survival Akt pathway upon induction of ER stress. These cells also show enhanced levels of the pro-apoptotic transcription factor, CCAAT/enhancer binding protein homologous protein (CHOP) as compared to control cells. Due to decreased pro-survival signals and a concomitant increase in proapoptotic signals, GIV depleted cells show a significant reduction in cell survival upon prolonged ER stress which can be rescued by re-expression of GIV or by directly activating Akt in these cells. Together, this study shows a novel, cytoprotective role for GIV in ER stressed cells and furthers our understanding of the mechanisms that contribute to cell survival during ER stress.

Keywords

ER stress; Unfolded protein response; GIV/Girdin; Akt; Cell survival

INTRODUCTION:

The ER is a site of many important cellular functions including proper protein folding, post-translational modifications and maintaining Ca²⁺ homeostasis in virtually every mammalian

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ETHICAL STATEMENT:

This article does not contain any studies with human participants or animals performed by any of the authors.

cell [1]. Perturbations in any of these functions generate ER stress and lead to activation of an evolutionarily conserved signaling program known as the “Unfolded protein response (UPR)” [1–3]. By triggering UPR, cells initially try to restore normal function by halting general protein translation, selectively enhancing production of molecular chaperones involved in protein folding and up-regulating cytoprotective responses [4]. Alternatively, if the damage is beyond repair and homeostasis is not achieved within a reasonable time frame, the UPR commits cells to apoptosis [5]. How cells make the life-or-death decision remains an exciting yet poorly understood phenomenon.

The UPR is mediated by three main sensors embedded in the ER membrane –Activating transcription factor 6 (ATF6), Inositol-requiring enzyme 1 (IRE1) and Protein Kinase R-like ER kinase (PERK) [3]. Under normal conditions, these sensors are kept in an inactive state primarily by binding of the ER chaperone Glucose regulated protein of 78 kDa (GRP78) on the luminal side. Upon induction of ER stress, GRP78 dissociation triggers the activation of these sensors leading to a collective response aimed at resolving the stress [6]. ATF6 is a type II membrane bound transcription factor which translocates to the Golgi upon induction of ER stress where it undergoes proteolytic cleavage by Golgi-resident proteases to release the active N-terminal fragment [7–9]. The active fragment then enters the nucleus to upregulate transcription of several downstream targets including chaperones and the ER associated protein degradation (ERAD) machinery. IRE1 is a type I transmembrane protein that possesses dual enzymatic activity as a kinase and an endoribonuclease [10]. While its kinase activity is involved in autoregulation, the main target of the RNase domain is the transcription factor X-box binding protein 1 (XBP1) [11]. Activated IRE1 splices an intron from XBP1 mRNA allowing it to be translated into a functional transcription factor (XBP1s), which has several downstream targets involved in the UPR [11,12]. PERK, a type I membrane protein, is a Serine/Threonine kinase which gets activated upon oligomerization and autophosphorylation [13]. The main effector of PERK is the eukaryotic translation initiation factor 2 α (eIF2 α). PERK phosphorylates eIF2 α at S51, inactivating it and thus halting general protein translation to reduce further protein load in the ER while allowing selective translation of the transcription factor Activating transcription factor 4 (ATF4) [14–15]. Like XBP1s and ATF6, ATF4 also promotes transcription of chaperones and other effectors involved in alleviation of the stress [16]. In addition to mounting an adaptive response to ER stress, these sensors also regulate the apoptotic signals to determine the fate of the cell depending on the duration and severity of the stress. One of the key pro-apoptotic targets for the UPR is the CCAAT/enhancer binding protein homologous protein (CHOP) [17]. The CHOP gene promoter contains binding sites for the three major effectors of the UPR - ATF4, XBP1s and ATF6 [18–20]. CHOP enhances cell death by inducing expression of proteins involved in apoptosis [21–23].

Apart from normal physiological conditions [24], ER stress is also experienced during many pathological conditions such as diabetes, neurodegeneration, cardiovascular disease and cancer [25–28]. Cancer cells exhibit ER stress due to their high rates of glucose metabolism and hypoxic conditions resulting in the accumulation of underglycosylated misfolded proteins in the ER [29]. While cells under normal and other pathophysiological conditions succumb to high levels of ER stress, cancer cells manage to alleviate the stress and continue to survive [30,31]. The ability to overcome ER stress has been correlated to the invasiveness/

malignancy and chemo-resistance of cancer cells [32–34]. Thus, to be able to design effective therapeutic strategies, it is crucial to delineate the signaling mechanisms that endow cells with a cytoprotective phenotype.

The pro-survival phosphatidylinositol 3-kinase (PI3K)-Akt pathway has been implicated in cell survival during ER stress [35–39]; however, the mechanism(s) underlying its activation remains poorly understood. The **Gα-Interacting Vesicle associated protein (GIV** aka Girdin), is a large multi-domain protein that acts as a non-receptor guanine nucleotide exchange factor (GEF) for Gi family of heterotrimeric G proteins [40–41]. GIV mediates activation of heterotrimeric Gi downstream of multiple receptor tyrosine kinases resulting in enhanced PI3K-Akt signals [42–44]. GIV mediated signals regulate a multitude of cellular processes including motility, autophagy, survival, intracellular trafficking, mitosis and are required for normal physiological responses such as wound healing and epithelial morphogenesis [42–44]. On the other hand, dysregulation of GIV expression and activity promote motility and invasiveness of cancer cells [45–47]. Since Akt signaling has been shown to promote cancer cell survival during ER stress [35,36,38,39] and GIV is frequently over-expressed and hyperactivated in cancer cells [42–44,48,49], here, we asked if GIV plays a role in enhancing cancer cell viability during ER stress. We found that GIV promotes cell survival during ER stress by activating the Akt pathway. GIV also suppresses expression of CHOP, a pro-apoptotic transcription factor whose expression is induced by the unfolded protein response sensors. Our study shows a previously unidentified role for GIV in endowing cells with enhanced viability during ER stress.

MATERIALS AND METHODS:

Materials and Reagents:

All reagents used were of analytical grade. Tunicamycin, Dithiothreitol (DTT), Puromycin and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma. Thapsigargin was bought from Cayman Chemical Company. SC79, the small molecule activator of Akt, was purchased from Tocris Bioscience (catalog #4635). Antibodies against GIV (catalog #133371) and GRP78 (catalog #13968) were obtained from Santa Cruz Biotechnology. Antibodies against total Akt (tAkt; catalog #4691), phospho-Akt (pAkt-S473; catalog #4060), IRE1α (catalog #3294), XBP1s (catalog #12782), PERK (catalog #3192), eIF2α (catalog #5324), phospho-eIF2α (peIF2α; catalog #3398), ATF4 (catalog #11815), CHOP (catalog # 2895) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH; catalog #5174) were from Cell Signaling. Anti-ATF6 (catalog #ab122897) was from Abcam and anti-FLAG M2 antibody was from Sigma (catalog #F1804). Goat anti-rabbit and goat anti-mouse IRDye 680RD and IRDye 800CW secondary antibodies were obtained from LI-COR Biosciences.

Cell culture and transfection:

HeLa cells (American type culture collection) were maintained in Dulbecco's modified eagle medium (DMEM; Hyclone, Logan, UT) supplemented with 10% Fetal bovine serum (FBS; Hyclone) and 1X Penicillin, Streptomycin and Glutamine (PSG; Corning, Manassas, VA). HeLa Scr and GIV-Sh1 cell lines were a kind gift from Dr. Mikel Garcia-Marcos,

Boston University [50]. These cell lines were maintained in DMEM supplemented with 10% FBS, 1X PSG, and 1µg/mL Puromycin (Gibco, Grand Island, NY). Cells were maintained in a humidified atmosphere containing 5% CO₂ at 37°C. Dulbecco's Phosphate Buffered Saline (DPBS) and Trypsin (0.25%) containing 2.21mM EDTA were from Hyclone and Corning, respectively. Transfections were performed using *TransIT*-LT1 reagent (Mirus Bio) following the manufacturer's instructions. ER stress was triggered by incubating cells with medium containing Tunicamycin (10µM), Thapsigargin (1µM), or DTT (3mM) for various time-points followed by cell lysis/western blot analysis or the cell viability MTT assay. The control cells for Tunicamycin and Thapsigargin treatments were incubated with medium containing DMSO. The FLAG-ATF6 construct was a gift from Dr. Ron Prywes (Addgene plasmid # 11975). The FLAG-HA-GIV CT construct was generated by amplifying the cDNA corresponding to residues 1660–1870 by polymerase chain reaction. The amplified product was cloned into the HindIII-BamHI sites of the pcDNA3 FLAG HA vector (a gift from Dr. William Sellers, Addgene plasmid # 10792). The fidelity of the construct was confirmed by sequencing.

Immunoblotting:

Samples were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred to Immobilon™ FL PVDF membranes (EMD Millipore). The recipes for buffers and transfer conditions for GIV were as previously described [51]. After the transfer was complete, membranes were blocked with PBS supplemented with 5% nonfat milk and then incubated sequentially with primary and secondary antibodies. Infrared imaging with two-color detection and quantification of blots were performed according to the manufacturer's protocols using Odyssey Fc imaging system (Li-COR Biosciences). All western blotting images were processed and assembled using Image Studio Lite (Li-COR Biosciences), Adobe Photoshop and Adobe Illustrator software.

MTT Cell Viability Assay:

Cells were plated in triplicates at a density of 10,000 cells per well in a flat bottom tissue culture treated 96-well plate (Olympus, San Diego, CA) and incubated at 37°C in the CO₂ incubator. Cells were treated with Tunicamycin or DMSO (control) for the desired amount of time. For the rescue experiment with SC79, the HeLa-Sh1 cells were incubated with SC79 (final concentration 22µM) 15 minutes prior to addition of Tunicamycin. After the desired incubation, the medium was replaced with fresh medium containing 1mM MTT, and cells were incubated for 3h. DMSO was then added and cells were incubated for an additional 10 minutes before reading absorbance at 540nm using a Varioskan Flash Microplate Reader (Thermo Electron Corporation, Waltham, MA) with the SkanIt Software 2.2 (Thermo Electron Corporation, Waltham, MA).

Statistical Analysis:

Each experiment was performed at least three independent times. The densitometric analysis was performed using the Image Studio software (Li-COR Biosciences). All graphical data were prepared and statistical analyses were performed using GraphPad Prism. Statistical significance between the difference of means was calculated by paired student's t-test. **p* <0.05; ***p* <0.01, ****p* <0.001.

RESULTS:

ER stress leads to activation of Akt in HeLa Cells:

Cancer cells are susceptible to ER stress due to intrinsic and extrinsic factors such as elevated glucose metabolism and hypoxia, respectively and often display upregulated pro-survival signals [29,30]. Although the PI3K-Akt signaling pathway has been implicated in cancer cell survival during ER stress [35,36,38,39], the exact mechanism of activation of this pathway during ER stress remains poorly understood. Since GIV is a known activator of the PI3K-Akt pathway [41–47], we hypothesized that GIV may play a critical role in cancer cell survival during ER stress. In order to test our hypothesis, we first investigated if the Akt pathway was activated in cervical cancer cell line, HeLa, upon induction of ER stress using three different stressors - Tunicamycin, DTT, and Thapsigargin [52,53].

Tunicamycin, a structural mimetic of UDP-N-acetylglucosamine, induces ER stress by inhibiting N-linked glycosylation thus interfering with processing and maturation of newly synthesized proteins in the ER. DTT perturbs the oxidizing environment of the ER resulting in misfolding of proteins containing disulfide bonds. Thapsigargin is a noncompetitive inhibitor of the sacro/endoplasmic reticulum Ca^{2+} ATPase. The inhibition of Ca^{2+} influx into the ER compromises the activity of Ca^{2+} dependent proteins, including several ER chaperones. Thus, treatment with Tunicamycin, DTT or Thapsigargin can trigger misfolding of proteins in the ER lumen resulting in ER stress [52,53]. Lysates of HeLa cells treated with Tunicamycin, DTT and Thapsigargin for 0, 0.5, 1 and 3 h were analyzed by immunoblotting for phosphorylated Akt (pAkt-S473, referred to as pAkt henceforth) (Figure 1). Total Akt (tAkt) and GAPDH were blotted as loading controls. As shown in Figure 1A-C, all three stressors led to increased phosphorylation of Akt, albeit showing peak activation at different time-points. This is not surprising considering these stressors induce ER stress by different mechanisms and the dynamics of Akt activation may vary depending on the type of stressor used. Tunicamycin showed a steady increase in pAkt levels with increasing duration of treatment (Figure 1A). Thapsigargin showed the earliest Akt activation peaking within 30 min of treatment (Figure 1B) whereas DTT showed a robust activation of Akt peaking at 1h (Figure 1C). Nevertheless, our results showed that the Akt pathway is activated upon induction of ER stress regardless of the stressor used. For all our subsequent experiments, we chose Tunicamycin as the ER stressor as it induces stress by inhibiting N-linked glycosylation – an ER specific protein modification. The other two stressors, Thapsigargin and DTT, are not as specific and can also have non-ER related effects on the cell.

shRNA-mediated GIV depletion reduces Akt activation during ER stress:

Next we asked if GIV plays a role in Akt activation during ER stress. To do so, we used a HeLa cell line stably knocked down for GIV expression (GIV-Sh1) along with a control cell line harboring scrambled shRNA (Scr) [50]. Since we saw a steady increase in pAkt levels up to 3h of Tunicamycin treatment (Figure 1A), we added two additional time-points (6 and 10 h) to establish a more extended temporal profile of Akt activation. Cells were treated with Tunicamycin for 0, 3, 6 and 10 h followed by lysing and immunoblotting. As shown in Figure 2A (lanes 1–4), pAkt levels peaked at 3h of tunicamycin treatment in Scr cells and showed a significant increase over basal levels (Figure 2B). In comparison, GIV-Sh1 cells

failed to show a substantial increase in pAkt levels upon treatment with Tunicamycin (Figure 2A, lanes 5–8; Figure 2B). Induction of ER stress was confirmed by monitoring expression of the ER chaperone GRP78 which was enhanced in both cell lines with increased duration of Tunicamycin treatment (Figures 2A and S1). These results suggested that ER-stress dependent activation of Akt is mediated by GIV.

GIV depletion also affects the UPR:

Next we asked if GIV knock-down affects the UPR in any way. We determined the expression levels of the three master sensors – IRE1, PERK and ATF6 and their downstream effectors [52,53] in ER stressed Scr and GIV-Sh1 cells by immunoblotting. As shown in Figure 3A, while the expression level of IRE1 α was similar in Scr and GIV-Sh1 cells, the latter showed significantly higher levels of XBP1s (the downstream effector of IRE1) at all time-points as compared to Scr cells (Figure 3B). For the PERK sensor, the p $\text{eIF2}\alpha$ levels were similar indicating that PERK was activated similarly in both cell lines (Figures 4, S2). ATF4 expression, on the other hand, was significantly higher in GIV-Sh1 cells as compared to Scr cells (Figure 4A, B). To analyze ATF6 cleavage, we initially used the antibody recognizing the N terminus of endogenous ATF6. As shown in Figure S3, we observed that the GIV-Sh1 cells expressed ~1.9X higher amount of ATF6 as compared to Scr cells even in the absence of any ER stress (Figure S3A - Lanes 1 and 5; Figure S3B). Treatment with Tunicamycin inhibited glycosylation of ATF6 resulting in a smaller, faster migrating unglycosylated ATF6 (Figure S3A, band marked by asterisk) in both cell lines. Unfortunately, the antibody recognized a non-specific band at ~60 kDa where the cleaved N-terminal fragment of ATF6 is expected to run making it difficult for us to draw any conclusions. To circumvent this issue, we performed the experiment on cells transfected with N-terminally tagged FLAG-ATF6 construct, which has been used in previous studies on ATF6 [7,54,55]. Also, since DTT is a stronger activator for ATF6 than Tunicamycin [56], we utilized DTT as the ER stress inducer (Figure 5). As shown in Figure 5A, both cell lines showed cleavage of ATF6 at 3h post DTT treatment, however, with a significantly higher level in GIV-Sh1 cells (Figure 5B). Together, these results indicated that GIV depletion affects UPR by enhancing levels of the three transcription factors - ATF4, XBP1s and ATF6.

We next looked at the expression of CHOP - a pro-apoptotic transcription factor which is one of the common downstream targets of ATF4, XBP1s and ATF6 [18]. As shown in Figure 6A, both Scr and Sh1 cells expressed CHOP upon induction of ER stress with its levels increasing with the duration of Tunicamycin treatment. GIV-Sh1 cells, however, showed significantly higher expression of CHOP at 10h of Tunicamycin treatment as compared to Scr cells (Figure 6B).

GIV depletion decreases cell survival during ER stress:

Because GIV depleted cells displayed a decrease in pro-survival (Figure 2) and an increase in pro-apoptotic (Figure 6) signals when responding to ER stress, we reasoned that depletion of GIV may cause enhanced cell death during prolonged ER stress. To directly assess the effect of GIV knock-down on cell survival, we carried out the cell viability assays on Scr and GIV-Sh1 cells after exposing them to prolonged ER stress. Cells treated with Tunicamycin for 0, 24 and 36 h were analyzed for cell survival using the MTT assay.

Metabolically active, live cells reduce MTT (yellow) into formazan (purple), which can be read colorimetrically thus allowing to quantitatively determine cell viability. As shown in Figure 7A, HeLa Scr cells showed greater cell viability than GIV-Sh1 cells both at 24h and 36h of ER stress. Statistical analysis revealed that cell death was significantly increased in GIV-Sh1 cells as compared to Scr cells (Figure 7A).

We next asked if the increased vulnerability of GIV-depleted cells to ER stress could be rescued by reconstituting GIV in these cells. The carboxyl-terminal domain of GIV (GIVCT, residues 1660–1870) has been shown to function independently from being recruited to activated RTKs to enhancing downstream signaling [57]. Taking advantage of GIV-CT's functionality independent of the rest of the protein and because the shRNA targets a region outside the C-terminus of GIV, we carried out the MTT assay on GIV Sh1 cells expressing a FLAG-HA tagged GIV-CT construct. As shown in Figure 7B (Gray bar *vs.* White bar), we were able to rescue cell viability upon expression of FLAGHA-GIV CT in GIV-Sh1 cells. In fact, these cells seemed to survive ER stress slightly better than the control Scr cells expressing vector alone (Black bar *vs.* White bar) further supporting our conclusion that GIV may play a role in promoting cell survival during ER stress.

Finally, we asked if the decreased cell survival in GIV depleted cells was a result of reduced Akt activation or if GIV had a direct, Akt independent role in promoting cell survival in these cells. To do so, we treated GIV-Sh1 cells with SC79, a small molecule activator of Akt [58]. As shown in Figure 8A, GIV-Sh1 cells treated with SC79 showed a higher level of pAkt upon ER stress induction as compared to the control GIV-Sh1 cells (lanes 2 and 3). The SC79 treated GIV-Sh1 cells also displayed significantly higher level of survival than the control GIV-Sh1 cells as determined by the MTT assay (Figure 8B; Gray bar *vs.* White bar). The GIV Scr cells showed a slightly higher (albeit statistically non-significant) viability than the SC79 treated GIV-Sh1 cells. Together, these results indicate that the effect of GIV on cell viability during ER stress is mostly mediated through activation of Akt.

DISCUSSION

Summary and Working Model:

Collectively, our findings presented in this manuscript indicate a novel function for GIV in cell survival during ER stress. Based on our data, we propose the following working model (Figure 9). Upon induction of ER stress, HeLa cells activate the Akt pathway in a GIV-dependent manner. GIV also affects the unfolded protein response where it suppresses the expression of XBP1s, ATF4 and ATF6, leading to a decreased expression of pro-apoptotic transcription factor CHOP. The integrated effect of the upregulation of pro-survival Akt signals and downregulation of pro-apoptotic marker CHOP results in enhanced cell survival during prolonged ER stress (Figure 9, Left). On the other hand, in cells lacking GIV, the opposite is true and these cells show higher cell death upon long-term ER stress (Figure 9, Right). Together, our model proposes that GIV provides cells with a cytoprotective advantage and that its knock-down negatively influences cell survival in the face of ER stress.

Akt activation precedes activation of UPR signaling.

We observed that pAkt levels peak within 3h of Tunicamycin treatment and start declining thereafter (Figure 2A). A similar profile was also seen in another study using primary glial cells [59]. We also observed that activation of Akt precedes the expression/activation of UPR effectors XBP1s, ATF4 and ATF6. Our time-line of UPR activation is consistent with what has been previously reported in literature [52]. It is not surprising that Akt is activated earlier on, considering activation of Akt is a post-translational modification event whereas XBP1s involves a splicing event followed by translation of the spliced transcript [11]. ATF4 also requires translation of its mRNA at the correct open reading frame [15] and ATF6 involves its trafficking to the Golgi and proteolytic processing before releasing the active fragment [7,8]. It is possible that Akt activation is a distinct and parallel pathway with the sole aim to provide cytoprotection while cells prepare to deal with the unfolded protein load in the ER via the UPR. On the other hand, Akt can also potentially be involved in regulation of the UPR as discussed below.

GIV downregulates the proapoptotic signals originating downstream of the UPR:

Once activated, the UPR has a dual role: (1) to refold/degrade the unfolded proteins and restore cellular homeostasis and (2) to determine the cell's fate based on the extent/duration of the stress [60,61]. The decision of life and death hangs on the balance between cytoprotective and apoptotic signals. The longer and more potent the ER stress is, the more the level of propapoptotic signals rises and tilts the balance towards cell death [60,61]. One of the key proapoptotic signals that accumulate during prolonged ER stress is CHOP whose expression is upregulated by XBP1s, ATF4 and ATF6 [18]. We observed a significant increase in levels of these three UPR effectors in GIV depleted cells undergoing ER stress (Figures 3–5). Consequently, these cells also showed a significant increase in CHOP levels (Figure 6) and cell death (Figure 7) upon being ER stressed. These GIV mediated effects on the UPR effectors may be a consequence of these cells not being able to activate Akt or these may be the result of an Akt-independent function of GIV. However, the former scenario is more plausible because it has been previously shown that inactivation of the PI3K-Akt pathway using chemical inhibitors can induce CHOP expression and cause death in ER-stressed cells [62–64]. The restoration of cell viability we observed by directly activating Akt in GIV-Sh1 cells (Figure 8) further supports this hypothesis. Nevertheless, it is still possible for GIV to have Akt-independent roles in regulation of the UPR, which will require further investigation.

Cross-talk between GIV-Akt-UPR during ER stress:

The antagonistic relationship between the PI3K-Akt pathway and the UPR has been previously reported. Inhibition of the PI3K-Akt pathway using chemical inhibitors has been shown to lead to higher apoptosis whereas its activation has been correlated to higher survival and proliferation [62–64]. The results presented here are in line with these studies where decreased Akt activation during ER stress correlates to increased response through UPR and consequently higher cell death. In addition, our findings further the current understanding of the Akt-UPR cross-talk by showing GIV as an upstream regulator of the cell's response to ER stress through the Akt pathway. Interestingly, GIV not only acts

upstream of Akt to activate the latter, it is also a known substrate for Akt [65–67]. Phosphorylation of GIV by Akt has been shown to be required for GIV-mediated cellular functions including migration, repair and proliferation [65–67]. Thus, it is reasonable to envision a positive feedback regulation between GIV and Akt to mitigate the signaling response through the UPR sensors in order to keep the pro-apoptotic signals suppressed and enhance cell survival during ER stress. Multiple studies have also shown GRP78, the ER chaperone, to be a positive regulator of as well as a downstream effector of Akt especially in cancer cells where they have been shown to promote cell survival and chemoresistance [35–39]. It remains to be seen if the GRP78-Akt cross-talk is mediated through GIV.

In summary, our study has identified a previously unknown role of GIV in cell survival during ER stress. By upregulating the pro-survival signals and downregulating the proapoptotic signals, GIV helps tilt the life-or-death balance more toward life by providing cells with a cytoprotective advantage. Our current and future studies are aimed at further delineating the specific mechanism(s) by which GIV-Akt influence the outcome of the UPR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: ER stress activates the Akt pathway in HeLa cells.

HeLa cells were ER stressed using (A) Tunicamycin, (B) Thapsigargin, or (C) DTT for the indicated timepoints before lysis. Cell lysates were analyzed for pAkt and tAkt by immunoblotting. GAPDH was blotted as a loading control.

Figure 2: GIV depletion hinders Akt activation during ER stress.

HeLa Scr and GIVSh1 cells were ER stressed using Tunicamycin for the indicated time-points before lysis. **(A)** Cell lysates were analyzed for GIV, pAkt, and tAkt by immunoblotting. GAPDH was blotted as a loading control. **(B)** pAkt levels at 0 and 3h were quantified and normalized to the amounts of tAkt and GAPDH at those time points by densitometric analysis. Data are presented as mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$.

Figure 3: GIV depletion enhances XBP1s expression during ER stress. (A) HeLa Scr and GIV-Sh1 cells were ER stressed using Tunicamycin for the indicated timepoints before lysis. The cell lysates were analyzed by immunoblotting for GIV, IRE1α, XBP1s and GAPDH. (B) XBP1s levels were quantified and normalized to GAPDH levels by densitometric analysis. Data are presented as mean ± S.E.M. * $p < 0.05$.

Figure 4: GIV depletion enhances ATF4 expression during ER stress.

(A) HeLa Scr and GIV-Sh1 cells were ER stressed using Tunicamycin for the indicated time-points before lysis. The cell lysates were analyzed by immunoblotting for GIV, PERK, peIF2α, eIF2α, ATF4 and GAPDH. (B) ATF4 levels were quantified and normalized to GAPDH levels by densitometric analysis. Data are presented as mean ± S.E.M. * $p < 0.05$, *** $p < 0.001$.

Figure 5: GIV depletion enhances expression and cleavage of ATF6 during ER stress. HeLa Scr and GIV-Sh1 cells were ER stressed using DTT for the indicated timepoints before lysis. The cell lysates were analyzed by immunoblotting for GIV, ATF6 and GAPDH. **(B)** The levels of the N-terminal fragment of ATF6 were quantified and normalized to GAPDH levels by densitometric analysis. Data are presented as mean $\pm$ S.E.M. ** $p < 0.01$.

Figure 6: GIV depletion enhances level of CHOP expression during ER stress. HeLa Scr and GIV-Sh1 cells were ER stressed using Tunicamycin for various timepoints before lysis. **(A)** Cell lysates were analyzed by immunoblotting for GIV and CHOP. GAPDH was blotted as a loading control. **(B)** CHOP levels at 0 and 10h were quantified and normalized to the levels of GAPDH at those time points by densitometric analysis. Data are presented as mean $\pm$ S.E.M. * $p < 0.05$.

Figure 7: GIV enhances cell viability during prolonged ER stress.

A. HeLa Scr and GIV-Sh1 cells were treated with either DMSO or Tunicamycin for the indicated timepoints before performing the MTT assay. **B.** Scr cells expressing empty vector and GIVSh1 cells expressing either the empty vector or GIV-CT were treated with either DMSO or Tunicamycin for 36h before performing the MTT assay. **A and B.** Cell viability was determined by recording the changes in absorbance at 540nm. Viability of control (DMSO) cells was set as 100% and the decline in cell survival after Tunicamycin treatment was plotted relative to the control cells. Data are presented as mean $\pm$ S.E.M. * $p < 0.05$, ** $p < 0.01$.

Figure 8: Akt activation can rescue viability in ER stressed GIV-Sh1 cells.

A. HeLa Scr and GIV-Sh1 cells and GIV-Sh1 cells treated with SC79 were stressed with Tunicamycin for 3h. GIV-Sh1 cells treated with SC79 were incubated with SC79 (22 μ M) 15 min prior to addition of Tunicamycin. The cell lysates were analyzed by immunoblotting for GIV, pAkt, tAkt and GAPDH. **B.** Scr, GIV-Sh1 and GIV-Sh1+SC79 cells were treated with either DMSO or Tunicamycin for 36h before performing the MTT assay. Cell viability was determined by recording the changes in absorbance at 540nm. Viability of control (DMSO) cells was set as 100% and the decline in cell survival after Tunicamycin treatment was plotted relative to the control cells. Data are presented as mean $\pm$ S.E.M. * p < 0.05, ** p < 0.01.

Figure 9: Proposed model for GIV's role in cell survival during ER stress.

Left - GIV promotes cell survival during ER stress by enhancing Akt signaling and by suppressing expression of CHOP. **Right** – Cells lacking GIV fail to activate Akt upon induction of ER stress and show upregulation of CHOP expression resulting in increased cell death.