BAP1 inhibits the ER stress gene regulatory network and modulates metabolic stress response

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The endoplasmic reticulum (ER) is classically linked to metabolic homeostasis via the activation of unfolded protein response (UPR), which is instructed by multiple transcriptional regulatory cascades. BRCA1 associated protein 1 (BAP1) is a tumor suppressor with de-ubiquitinating enzyme activity and has been implicated in chromatin regulation of gene expression. Here we show that BAP1 inhibits cell death induced by unresolved metabolic stress. This prosurvival role of BAP1 depends on its de-ubiquitinating activity and correlates with its ability to dampen the metabolic stress-induced UPR transcriptional network. BAP1 inhibits glucose deprivation-induced reactive oxygen species and ATP depletion, two cellular events contributing to the ER stress-induced cell death. In line with this, *Bap1* KO mice are more sensitive to tunicamycin-induced renal damage. Mechanically, we show that BAP1 represses metabolic stress-induced UPR and cell death through activating transcription factor 3 (ATF3) and C/EBP homologous protein (CHOP), and reveal that BAP1 binds to *ATF3* and *CHOP* promoters and inhibits their transcription. Taken together, our results establish a previously unappreciated role of BAP1 in modulating the cellular adaptability to metabolic stress and uncover a pivotal function of BAP1 in the regulation of the ER stress gene-regulatory network. Our study may also provide new conceptual framework for further understanding BAP1 function in cancer.

BAP1 | ER stress | unfolded protein response | energy stress | glucose starvation

Animal cells rely on nutrient supplies (e.g., glucose, and oxygen) to generate energy and biomaterials and to maintain cellular homeostasis under both physiological and pathological conditions. The metabolic stress response, defined as how cells respond to the lack of nutrient supplies in an adaptive or suicidal manner, is therefore essential to cellular functions and survival. Cells use multiple signaling cascades to adapt cellular functions and control cell fate in a manner dependent on the duration and strength of stress (1). Elucidating the molecular mechanisms of metabolic stress response is thus important for more in-depth understanding of organism development and human disease.

The evolutionarily conserved unfolded protein response (UPR) protects cells against the stress of misfolded proteins in the endoplasmic reticulum (ER) for continued survival, and will initiate regulated cell death if the ER stress cannot be resolved (2). The key to UPR-mediated cell fate decision is the gene-expression network driven by the ER stress-activated transcriptional factors (TFs) (3). The canonical UPR TFs include X-box binding protein 1 (XBP1), activating transcription factor 6 (ATF6), ATF4, and C/EBP homologous protein (CHOP), which function downstream of three ER-localized stress sensors: inositol-requiring enzyme 1α (IRE1α), ATF6, and double-stranded RNA-dependent protein kinase (PKR)-like ER kinase (PERK), respectively. Of the UPR gene regulatory network, the ATF4/CHOP arm mediates expression of genes that promote the ER stress-induced cell death by causing ATP depletion and inducing reactive oxidative stress (ROS) (4). Although the three parallel arms of UPR use different signaling cascades and TFs to independently transduce the ER stress signals into the nucleus, their transcriptional effects significantly overlap because of the feed-forward regulations of the expression of these UPR TFs (5). However, little is known as how the expression of these UPR TFs is coregulated.

BAP1 (BRCA1-associated protein 1) functions as a nuclear de-ubiquitinating (DUB) enzyme, and regulates cellular processes, including transcription, DNA replication fork progression, and DNA double-strand break repair in a DUB-dependent manner (6). BAP1 interacts with several chromatin-modifying factors and TFs (6), underscoring the important role of BAP1 in the regulation of gene transcription. *BAP1* is a tumor-suppressor gene located on chromosome 3p21, a genomic locus frequently deleted in human cancers. Both somatic and germ-line inactivating mutations of *BAP1* occur in a variety of cancers, including uveal melanomas, mesotheliomas, and renal cell carcinoma (6). Paradoxically, in certain cancers, low expressions of WT *BAP1* or *BAP1* mutations correlate with longer patient survival (7, 8), suggesting that BAP1 may play complex and context-dependent roles in the regulation of cancer cell survival and death, a question that remains largely unexplored. The direct transcriptional targets of BAP1 in the mammalian system, particularly through which BAP1 controls cell death, also remains unknown currently. Because cancer cells consistently experience metabolic stress during tumor development and therapeutic prevention, and

Significance

BRCA1 associated protein 1 (BAP1) is a tumor suppressor and its inactivating mutations frequently occur in a subset of human cancers. This study reveals an unexpected finding that loss of BAP1 compromises the cellular adaptability to metabolic stress, and links BAP1 to unfolded protein response to regulate cell survival under metabolic stress. We also report the first line of in vivo evidence that *Bap1* KO mice experienced unresolved endoplasmic reticulum stress in the kidney. Our study not only provides mechanical insights for BAP1 functions in cell survival upon metabolic stress through endoplasmic reticulum stress signaling, but also may provide a conceptual framework for further understanding BAP1 function in cancer.

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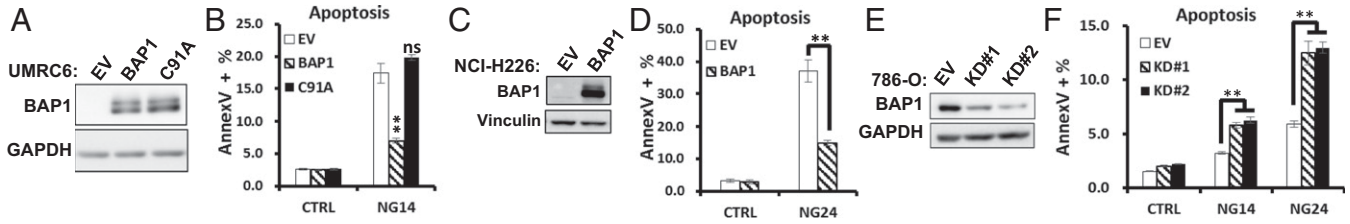

Fig. 1. BAP1 inhibits cell apoptosis induced by glucose deprivation. (*A–D*) The effects of reexpression of BAP1-WT or BAP1-C91A mutant on glucose deprivation-induced cell death in *BAP1*-deficient UMRC6 (*A* and *B*) or NCI-H226 cells (*C* and *D*). (*E* and *F*) The effect of *BAP1* knockdown on glucose deprivation-induced apoptosis in 786-O cells. $^{**}P < 0.01$; ns, nonsignificant. CTRL, with glucose; EV, empty vector (*A–D*) or control shRNA (*E* and *F*). NG14/NG24, no glucose for 14 or 24 h.

compromised adaptability to cellular metabolic stress may influence tumor incidence as well as patient survival (9), in this study we have investigated the potential role of BAP1 in metabolic stress response.

Results

BAP1 Inhibits Glucose Deprivation-Induced Apoptosis. To investigate the potential role of BAP1 in energy stress response, we established cell lines stably expressing *BAP1* WT, *BAP1* C91A mutant (which abolishes BAP1 DUB activity), and the empty vector (EV) control in UMRC6 cells, a *BAP1*-deficient renal cancer cell line (10) (Fig. 1*A*). We found that, under normal culture conditions (with 25 mM glucose), BAP1 reexpression in these cells did not significantly affect basal cell death, and only inhibited cell proliferation very moderately (Fig. S1). We then examined whether glucose deprivation provoked any differential cytotoxic effect in these cells. Examination of cell morphology and further analysis by Annexin V/Propidium Iodide (PI) staining revealed that restoring the expression of BAP1 WT, but not BAP1-C91A mutant, in UMRC6 cells attenuated glucose deprivation-induced cell death (Fig. 1*B* and Fig. S2*A*), suggesting that BAP1 promotes cell survival in UMRC6 cells under glucose starvation, which is dependent on its DUB activity.

We next compared glucose starvation-induced cell death in a few cancer cell lines with *BAP1*-deficient or -proficient status. Such analysis revealed that two *BAP1*-deficient cancer cells, UMRC6 and NCI-H226 cells, are more sensitive to glucose starvation-induced cell death than other *BAP1*-proficient cancer cells (Fig. S2*B*). Importantly, similar to UMRC6 cells, restoration of *BAP1* expression in NCI-H226 cells protected cells from glucose starvation-induced cell death (Fig. 1 *C* and *D*), whereas *BAP1* knockdown by two independent shRNAs in *BAP1* proficient 786-O cells sensitized cells to glucose starvation-induced cell death (Fig. 1 *E* and *F*). Finally, *BAP1* knockdown in HK2 cells (Fig. S2*C*), an immortalized kidney epithelial cell line isolated from normal human kidney, or *Bap1* deletion in primary *Bap1*$^{F/F}$*; Rosa26-CreERT2* MEFs (Fig. S2*D*), also promoted glucose starvation-induced cell death, suggesting that BAP1 also regulates glucose deprivation-induced cell death in nontransformed cellular contexts. Collectively, these results clearly demonstrated a prosurvival role of BAP1 in glucose deprivation-induced cell death and suggested an uncharacterized role of BAP1 in mediating metabolic stress response.

BAP1 Targets the ER Stress Gene Network Under Glucose Deprivation. Because glucose deprivation did not affect the nuclear localization of BAP1 in UMRC6-BAP1 or 786-O cells (Fig. S3), we reasoned that nuclear BAP1 likely regulates this metabolic regulatory network at the level of gene transcription. To test this theory, we performed RNA sequencing (RNA-Seq) analysis in UMRC6-EV and UMRC6-*BAP1* WT stable cell lines at 0, 4, and 8 h upon glucose starvation (Fig. S4*A*). Computational analysis identified many genes differentially regulated by BAP1 (either up- or down-regulated) at each time point (Fig. S4 *B* and *C*). Ingenuity pathway analysis (IPA) revealed that, although some pathways were enriched at all three time points, other pathways—most notably UPR and oxidative stress response-related pathways—were not enriched under basal condition (at the 0-h time point), but specifically enriched upon glucose starvation (Fig. S4 *D* and *E*). Consistent with this finding, IPA comparison analysis for the enriched upstream regulators revealed that thapsigargin (a known ER stress inducer) -induced UPR and hydrogen peroxide-induced oxidative stress response were most significantly up-regulated upon glucose starvation (Fig. 2*A*). Together, these computational analyses suggested that BAP1 may regulate UPR and oxidative stress response under glucose starvation.

We then integrated the RNA-Seq data with the pathways of protein processing in ER [Kyoto Encyclopedia of Genes and Genomes (KEGG): ko04141] and visualized the expression fold-changes of genes involved in UPR. Consistent with the notion that glucose deprivation induces UPR (11), 8-h glucose deprivation transcriptionally induced a large set of genes related to UPR in UMRC6-EV cells (Fig. S5*A*). Notably, reexpression of BAP1 in UMRC6 cells significantly inhibited the induction of these UPR effectors under glucose starvation (Fig. S5*B*). Among these UPR effectors, *CHOP* and *ATF3* were rapidly induced even at 4 h upon glucose deprivation in UMRC6-EV cells, and their induction was dramatically inhibited by BAP1 (Fig. S6).

Consistent with our RNA-Seq data, real-time quantitative PCR (qPCR) and Western blot analyses confirmed that *BAP1*-WT, but not *BAP1*-C91A, inhibited glucose starvation-induced expression of these UPR effectors (except ATF4, which is mainly modulated at translational level in UPR) at both mRNA and protein levels in UMRC6 cells (Fig. 2 *B–F*). BAP1 also repressed glucose starvation-induced IRE1α and PERK phosphorylation of eukarotic initiation factor 2-α (eIF2α) (Fig. 2*F*). Finally, we showed that *Bap1* deletion in mouse embryonic fibroblasts (MEFs) or *BAP1* knockdown in 876-O cells further increased glucose starvation-induced UPR effectors (Fig. S7). Taken together, these results clearly demonstrate that BAP1 regulates the ER stress gene network under glucose deprivation.

BAP1 Inhibits UPR-Mediated ROS Induction and ATP Depletion. Because unresolved ER stress leads to cell death, we next sought to determine whether BAP1 inhibits glucose deprivation-induced apoptosis through repressing UPR. It has been consistently documented that PERK-mediated phosphorylation of eIF2α and up-regulation of ATF4 and CHOP lead to ATP depletion or ROS induction through the ATF4/CHOP-driven gene regulatory network, eventually resulting in unresolved ER stress-induced cell death (4, 12). Indeed, PERK kinase inhibitor (PERKi) treatment normalized glucose deprivation-induced apoptosis in UMRC6-EV and UMRC6-*BAP1* C91A cells (Fig. 3*A*) or 786-O cells with *BAP1* knockdown (Fig. S8*A*). PERKi also inhibited the induction of *ATF3*/*CHOP* mRNAs upon glucose withdrawal (Fig. 3*B*). These results suggested that BAP1 regulates glucose deprivation-induced apoptosis at least partly through PERK-mediated ER stress signaling.

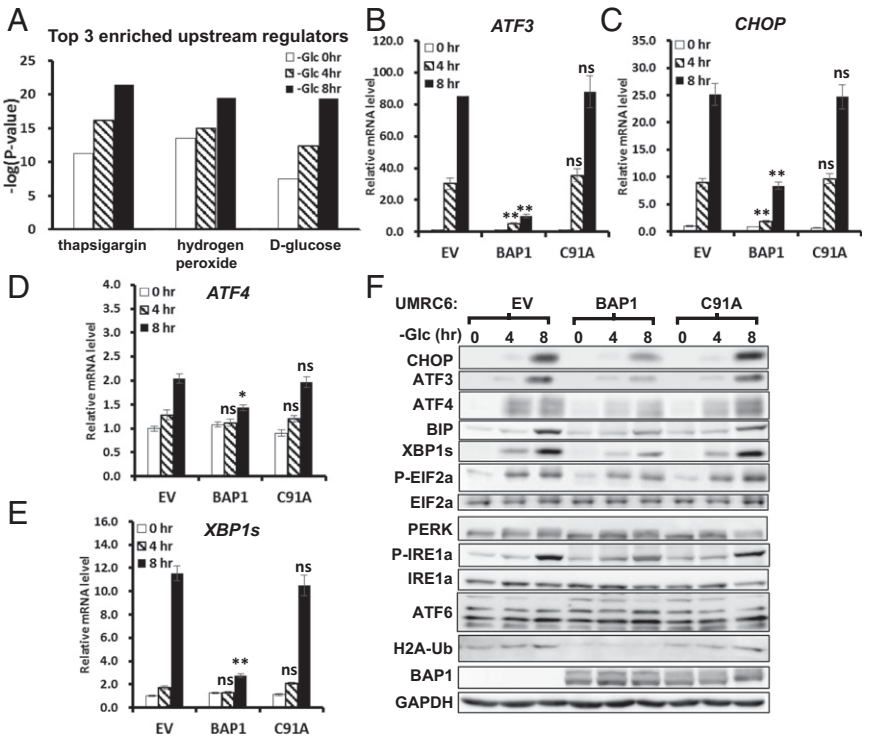

Fig. 2. BAP1 targets the ER stress gene network under glucose deprivation. (*A*) Top three enriched upstream regulators (as indicated on *x* axes) from IPA comparison analysis. (*B–F*) The effect of BAP1 or BAP1-C91A reexpression on glucose deprivation-induced UPR effectors in UMRC6 cells analyzed by real-time qPCR (*B–E*) and Western blotting (*F*). $*P < 0.05$; $**P < 0.01$; ns, nonsignificant.

Next we examined whether BAP1 also regulates UPR-mediated ROS induction and ATP depletion. Reexpression of BAP1-WT, but not BAP1-C69A mutant, in UMRC6 cells inhibited the increase of ROS under glucose starvation, and glucose starvation-induced ROS can be normalized by PERKi treatment (Fig. 3*C*). Relieving ROS by using *N*-acetyl cysteine (NAC), a known antioxidant, blocked glucose deprivation-induced cell death in UMRC6 cell lines, confirming the contribution of ROS induction on glucose starvation-induced cell death (Fig. S8*B*). Glucose deprivation led to more decrease of cellular ATP levels in *Bap1* KO MEFs than in WT MEFs, and ATP depletion in *Bap1* KO MEFs upon glucose starvation can be largely rescued by PERKi treatment (Fig. 3*D*), suggesting that loss of *Bap1* compromised the cellular adaptability to main ATP levels. Correspondingly, glucose deprivation combined with the treatment of ATP synthase inhibitor oligomycin induced substantially more cell death in *Bap1* KO MEFs than in *Bap1* WT MEFs (Fig. S9*A*). Similar observation was also made by the treatment of 2 deoxy-glucose (2DG), a glycolysis inhibitor, in combination with oligomycin (Fig. S9*B*). Collectively, these results suggested that BAP1 protects cells from apoptosis by preventing ATP depletion or ROS induction through inhibiting PERK-mediated ER stress signaling.

BAP1 Directly Represses *ATF3* and *CHOP* Transcription. The rapid down-regulation of ATF3 and CHOP, among other UPR effectors, by BAP1 (Fig. 2) raised the possibility that they may be BAP1 direct targets. Indeed, the ChIP assay demonstrated that glucose deprivation induced the binding of BAP1 to the promoter of *ATF3* or *CHOP*, and interestingly, BAP1-C91A mutant bound to the promoters with the comparable level to that of BAP1 WT (Fig. 4*A*), suggesting that the DUB activity of BAP1 likely is not required for BAP1 association with these promoters. BAP1-mediated transcriptional repression has been associated with BAP1 function to remove the mono-ubiquitination of histone 2A (H2A) through its DUB activity (13). ChIP analysis revealed that 4-h glucose deprivation promoted H2A-Ub association with the promoter of *ATF3* or *CHOP* in UMRC6-EV cells; furthermore, BAP1 did not significantly affect the level of H2A-Ub binding to *ATF3* or *CHOP* promoter (Fig. 4*B*).

BMI1-containing Polycomb repressive complex 1 (PRC1), a ubiquitin ligase complex, mediates the monoubiquitination of H2A. It has been suggested that an appropriate balance between H2A ubiquitination and DUB is important for the maintenance of target gene repression (14). We thus examined the binding of BMI1 to *ATF3* or *CHOP* promoter under glucose deprivation. ChIP analysis revealed that glucose deprivation induced the binding of BMI1 to these promoters (Fig. 4*C*). Interestingly, there was significantly more promoter-associated BMI1 in UMRC6-*BAP1* WT cells than that in UMRC6-EV cells or UMRC6-*BAP1* C91A cells under glucose starvation (Fig. 4*C*), suggesting that BAP1 promotes glucose deprivation-induced BMI1 binding to *ATF3*/*CHOP* promoters in a DUB-dependent manner. Given the important role of RING1B in BMI1-containing PRC1 complex (15), we also examined the binding of RING1B to *ATF3* or *CHOP* promoter by ChIP analysis, which showed that the enrichment of RING1B to *ATF3* or *CHOP* promoter mirrored that of BMI1 (Fig. S10).

We then examined whether BAP1-mediated transcriptional repression of *ATF3* and *CHOP* requires BMI1, by using small chemical inhibitor that specifically targets BMI1 (BMI1i). Real-time qPCR analysis revealed that, under glucose starvation condition, BMI1i treatment increased *ATF3* and *CHOP* expression in either UMRC6-EV or UMRC6-*BAP1* WT cells (Fig. 4*D*), suggesting that, similar to BAP1, BMI1 also represses *ATF3* and *CHOP* expression in response to glucose deprivation. In addition, in the presence of BMI1i, BAP1 failed to repress *ATF3* or *CHOP* expression under glucose deprivation (Fig. 4*D*), suggesting that BMI1 is required for BAP1-mediated transcriptional repression of *ATF3* and *CHOP*. Similar observation was made in 786-O cells with *BAP1* knockdown (Fig. S11). We have also

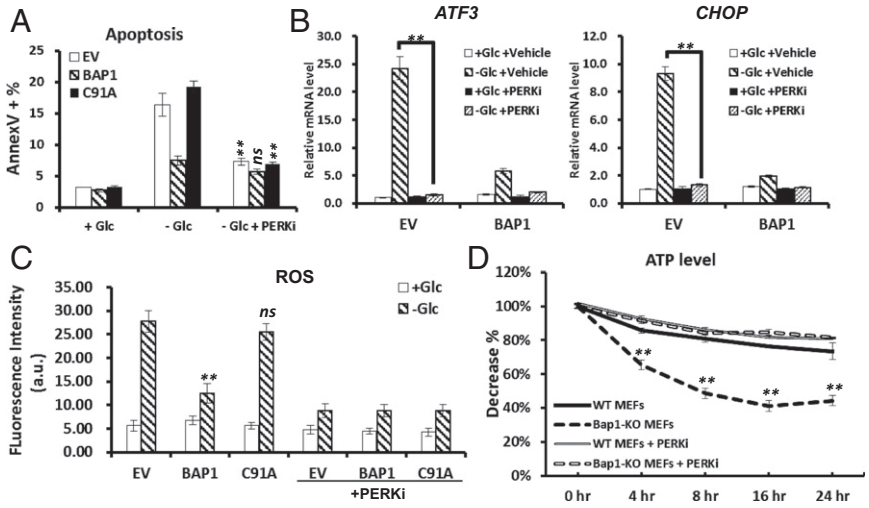

Fig. 3. BAP1 inhibits UPR-mediated ROS induction and ATP depletion. (*A*) The effect of PERK inhibitor on glucose deprivation-induced apoptosis in UMRC6 stable cells. (*B*) The effect of PERK inhibitor on glucose deprivation-induced UPR effectors in UMRC6 stable cells. (*C*) The effect of reexpression of BAP1 and BAP1-C91A mutant on glucose deprivation-induced ROS in UMRC6 stable cells. (*D*) The effect of *Bap1* deletion on glucose deprivation-induced ATP depletion in MEFs. $^{**}P < 0.01$; ns, nonsignificant.

analyzed ATF4 in all of the experiments described above, which revealed negative results for ATF4 (Fig. S12). Collectively, these results provided evidence that BAP1 directly targets *ATF3* and *CHOP* transcription upon glucose deprivation, and suggested a profound interplay between BAP1 and BMI1 (*Discussion*).

BAP1 Modulates Metabolic Stress-Induced UPR and Apoptosis Through ATF3 and CHOP. We next sought to determine the extent to which BAP1 inhibits glucose starvation-induced apoptosis through its target gene *ATF3* or *CHOP*. To this end, we established *ATF3* KO or *CHOP* KO UMRC6 cells via CRISPR/Cas9 technology. Our analyses revealed that deficiency of either *ATF3* or *CHOP* in UMRC6 cells inhibited glucose deprivation-induced apoptosis to the level comparable to that in UMRC6 *BAP1*-WT cells (Fig. 5*A*), suggesting that both ATF3 and CHOP are important downstream effectors of BAP1 to regulate glucose starvation-induced apoptosis. We then examined glucose starvation-induced UPR effectors that were repressed by BAP1 in *ATF3* KO or *CHOP* KO UMRC6 cells. We observed that *ATF3* deficiency significantly attenuated glucose starvation-induced UPR effectors; on the other hand, *CHOP* deficiency had minimal or very moderate effect on glucose starvation-induced UPR effectors (Fig. 5 *B–E* and Fig. S13). As expected, the deficiency of *ATF3* or *CHOP* did not significantly alter *ATF4* mRNA levels (Fig. 5*D*). Taken together, these results suggested that BAP1 modulates UPR gene regulatory network and glucose starvation-induced apoptosis through ATF3 and CHOP.

***Bap1* KO Mice Are More Sensitive to Tunicamycin Treatment.** To investigate the potential role of BAP1 in the regulation of the ER stress in vivo, we generated conditional KO mice carrying floxed alleles of *Bap1* ($Bap1^{F/F}$), which were then crossed with *Rosa26-CreERT2* mice to generate $Bap1^{F/F}$*; Rosa26-CreERT2* mice (Fig. S14*A*). $Bap1^{F/F}$*; Rosa26-CreERT2* and control littermates $Bap1^{F/F}$ (or $Bap1^{+/+}$*; Rosa26-CreERT2*) were injected with tamoxifen for 5 consecutive days at 4 wk of age, resulting in *Bap1* WT and KO mice. The deletion of *Bap1* in the kidney was confirmed by Western blotting (Fig. S14*B*). Tunicamycin-induced renal lesions

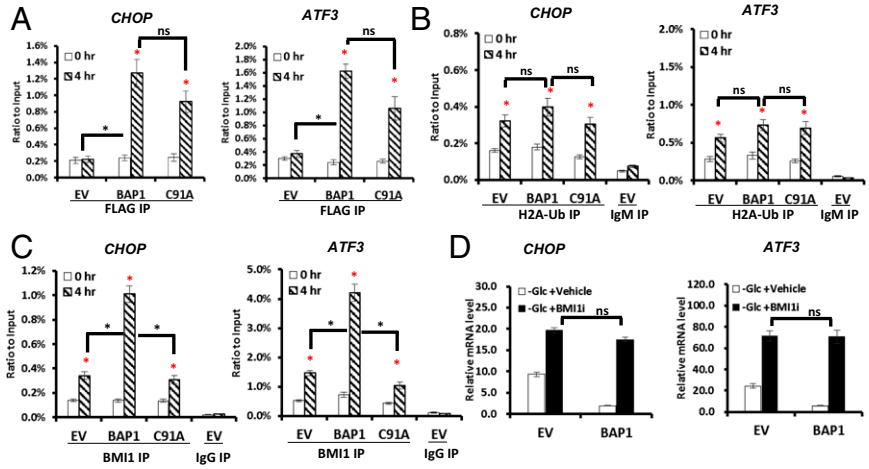

Fig. 4. BAP1 directly represses *ATF3* and *CHOP* transcription in a manner dependent on BMI1. (*A–C*) Glucose deprivation-induced binding of BAP1 (*A*), H2A-Ub (*B*), or BMI1 (*C*) to the promoters of *CHOP* and *ATF3* in UMRC6 cells. (*D*) The effect of BMI1 inhibitor on glucose deprivation-induced UPR TFs in UMRC6 stable cells. $^{*}P < 0.05$; ns, nonsignificant. Red asterisks indicate the comparison between glucose deprivation for 0 and 4 h in each cell line.

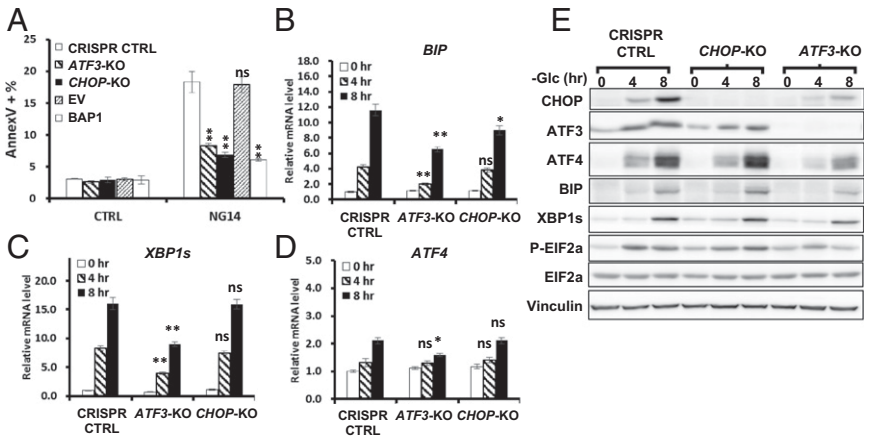

Fig. 5. BAP1 modulates metabolic stress-induced UPR and apoptosis through ATF3 and CHOP. (*A*) The effects of *ATF3* KO, *CHOP* KO and BAP1 reexpression on the glucose deprivation-induced apoptosis in UMRC6 cells. (*B–E*) The effect of *ATF3* KO or *CHOP* KO on glucose deprivation-induced UPR effectors in UMRC6 cells analyzed by real-time qPCR (*B–D*) or Western blot (*E*). $*P < 0.05$; $**P < 0.01$; ns, nonsignificant.

have been well characterized to study the physiological relevance of ER stress in vivo (4). To examine whether *Bap1* KO mice were more sensitive to tunicamycin-induced kidney damage, we treated *Bap1* WT or KO mice with tunicamycin or vehicle at 4 d post-completing tamoxifen injection (DPI), and then analyzed kidneys from these mice at 9 DPI (Fig. S14*C*). We found that, whereas *Bap1* KO mice without tunicamycin treatment did not exhibit any obvious kidney damage phenotype at 9 DPI, *Bap1* KO mice exhibited more severe kidney damage, characterized by larger vacuolization at the cortico–meduallary junction region in the kidney, than *Bap1* WT mice treated with the same dosage of tunicamycin (100 ng/g body weight) (Fig. 6 *A* and *B*). Consistent with this, there were increased Atf3/Chop/Atf4 levels and enhanced apoptosis (as evidenced by increased Parp cleavage) in *Bap1* KO kidneys than in WT kidneys upon tunicamycin treatment (Fig. 6*C*). Collectively, these results suggest that *Bap1* KO mice experienced increased ER stress in vivo and were sensitive to tunicamycin treatment, which is consistent with the data from our in vitro analyses.

Discussion

In this study, we show that BAP1 serves to repress the UPR gene regulatory network under glucose starvation, and uncover a prosurvival role of BAP1 in unresolved metabolic stress-induced cell death via BAP1-mediated UPR repression. Our data suggest a model that glucose starvation induces BAP1 binding to the *ATF3* or *CHOP* promoter, either directly or indirectly through other associated proteins, and BAP1 binding to *ATF3*/*CHOP* promoters does not require its DUB activity. Once binding on *ATF3*/*CHOP* promoters, BAP1 represses *ATF3*/*CHOP* expression in a DUB-dependent manner. This model is consistent with our data that *BAP1* C91A mutation does not affect BAP1 binding to *ATF3*/*CHOP* promoters, but leads to loss-of-function phenotypes in other biological assays. It seems that glucose starvation or tharpsgagin treatment does not affect the global level of H2A-Ub (Fig. 2*F* and Fig. S15), suggesting that these metabolic stresses do not regulate BAP1 DUB activity toward H2A-Ub. How BAP1 is recruited to *ATF3* or *CHOP* promoter in response to metabolic stress remains less clear. BAP1 forms a complex with several other nuclear proteins involved in transcriptional or chromatin regulation, including HCF1, OGT, FoxK1/2, ASXL1/2, and KDM1b (6). However, it seems that glucose starvation did not significantly affect the interaction between BAP1 and its known associated proteins (Fig. S16). It is possible that glucose starvation-induced post-translational modifications on BAP1 may play a role in recruiting BAP1 to *ATF3* or *CHOP* promoter, a hypothesis that remains to be tested in the future studies.

The exact mechanisms by which BAP1 represses ATF3 and CHOP transcription under glucose starvation through its DUB activity remain less understood. BAP1 forms the Polycomb repressive deubiquitylase (PR-DUB) complex that removes monoubiquitin from H2A at lys 119 (13), whereas the BMI1-containing PRC1 functions as an ubiquitin ligase complex to mediate the monoubiquitination of H2A at lys 119, which was initially proposed to mediate gene repression (16, 17). This model would predict that BAP1, through DUB H2A, antagonizes PRC1-mediated gene

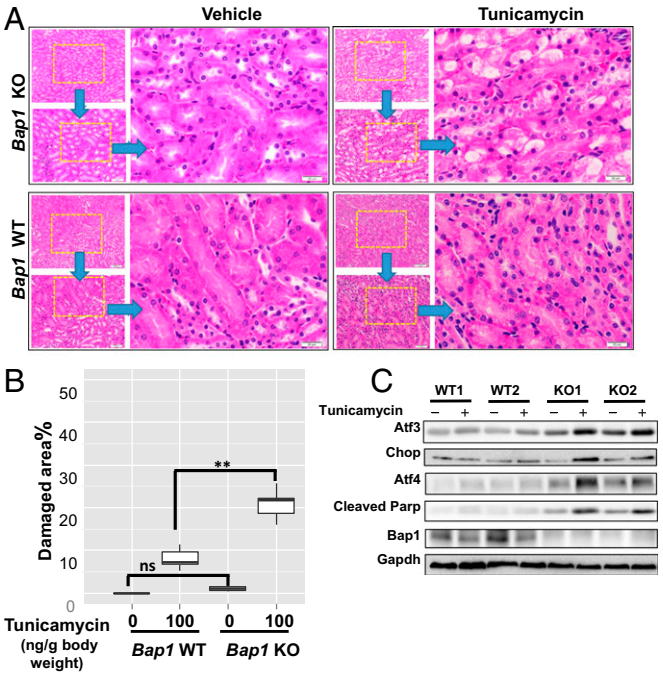

Fig. 6. *Bap1* KO mice are more sensitive to tunicamycin treatment. (*A*) Histology analysis of kidneys from *Bap1* WT and KO mice with or without tunicamycin treatment. (Scale bars: *Upper Insets*, 200 μm; *Lower Insets*, 50 μm; *Right*, 20 μm.) (*B*) Box plot of relative quantification of tunicamycin-induced kidney damages in *Bap1* WT and KO mice. $**P < 0.01$; ns, nonsignificant. (*C*) Western blot analysis of kidneys from *Bap1* WT and KO mice with or without tunicamycin treatment.

repression. However, whether H2A-ubiquitination per se functions as a repression marker is still an open question in the field (18, 19). Indeed, other studies showed that the repression of certain target genes requires not only the PRC1-mediated H2A ubiquitination, but also PR-DUB–mediated H2A DUB, which suggests that, at least in some contexts, an appropriate balance between H2A ubiquitination and DUB, rather than H2A ubiquitination per se, is important for the maintenance of target gene repression (20). Our data showed that glucose starvation promotes the binding of not only BAP1, but also BMI1 and RING1B, two integral and critical components of PRC1, on *ATF3/CHOP* promoters, and that inactivation of either BAP1 or BMI1 similarly enhances *ATF3* or *CHOP* expression in response to glucose deprivation. Thus, our data are in line with the second model described above, and suggest a hypothesis that glucose starvation may promote the binding of both BMI1 and BAP1 on *ATF3/CHOP* promoters to ensure a dynamic balance between BMI1/RING1B-containing PRC1-mediated H2A ubiquitination and BAP1-containing PR-DUB–mediated H2A DUB at *ATF3/CHOP* promoters, and to maintain *ATF3/CHOP* transcription repression.

Because the somatic and germ-line *BAP1* mutations frequently occur in several forms of human cancers, it is of particular importance and interest to study BAP1 function in tumor biology. The prosurvival function of BAP1 revealed from this study may seem counterintuitive with its well-documented role as a tumor suppressor. It is important to note that several other tumor suppressors, such as LKB1, TSC1, and TSC2, also have prosurvival functions, and deficiency of these tumor suppressors renders tumor cells sensitive to metabolic stresses, including the ER stress and glucose starvation-induced energy stress (21–24). As nutrient supplies are generally more limited in tumors than in normal tissues, and tumor cells often encounter enormous metabolic stress (9), it is conceivable that, although *BAP1* deficiency promotes tumor initiation, in established tumors *BAP1*-deficient tumor cells may be hypersensitive to metabolic stress-induced cell death, resulting in limited tumor progression and improved patient survival. Notably, whereas *BAP1* is a bona fide tumor suppressor in mesothelioma, its mutation or loss of expression frequently predicts longer patient survival in mesothelioma (7, 8). Our study may also provide a mechanistic rationale for exploring the therapeutic use of drugs targeting metabolism (such as metformin) in *BAP1*-deficient cancers.

Materials and Methods

See *SI Materials and Methods* for detailed description.

Mice. All animal manipulations were performed under MD Anderson Institutional Animal Care and Use Committee-approved protocols. The $Bap1^{F}$ allele was generated by flanking the exon 4 of mouse *Bap1* gene with two loxP sites. C57BL/6J $Bap1^{F/F}$ mice were crossed with tamoxifen-inducible Cre line *Rosa26-CreERT2* to generate $Bap1^{F/F}$; *Rosa26-CreERT2* mice. Tamoxifen treatment in mice to induce target gene deletion was performed as previously described (25–28).

Cell Culture Studies. Primary MEFs were prepared from embryonic day 13.5 embryos, as previously described (29). The generation of *Bap1* WT and KO MEFs from $Bap1^{F/F}$; *Rosa26-CreERT2* MEFs was conducted as previous described (30, 31). Briefly, MEFs (passage 0 or 1) were treated with either vehicle (ethanol) or 200 μM 4-hydroxytamoxifen (4-OHT; Sigma, H7904) for 5 d, resulting in *Bap1* WT and KO MEFs. Other parental cell lines used in this study were purchased from American Type Culture Collection. For glucose-starvation experiments, cells were cultured in DMEM with different concentrations of glucose (0 or 25 mM) + 10% (vol/vol) dialyzed FBS, as described in our previous publications (32).

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