Genotoxic stress triggers the activation of IRE1α-dependent RNA decay to modulate the DNA damage response

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The molecular connections between homeostatic systems that maintain both genome integrity and proteostasis are poorly understood. Here we identify the selective activation of the unfolded protein response transducer IRE1α under genotoxic stress to modulate repair programs and sustain cell survival. DNA damage engages IRE1α signaling in the absence of an endoplasmic reticulum (ER) stress signature, leading to the exclusive activation of regulated IRE1α-dependent decay (RIDD) without activating its canonical output mediated by the transcription factor XBP1. IRE1α endoribonuclease activity controls the stability of mRNAs involved in the DNA damage response, impacting DNA repair, cell cycle arrest and apoptosis. The activation of the c-Abl kinase by DNA damage triggers the oligomerization of IRE1α to catalyze RIDD. The protective role of IRE1α under genotoxic stress is conserved in fly and mouse. Altogether, our results uncover an important intersection between the molecular pathways that sustain genome stability and proteostasis.
The integrity of the genome is constantly threatened by endogenously produced toxic metabolites, physical, and chemical insults, resulting in a variety of DNA lesions. Inefficient DNA repair translates into cellular dysfunction and death, but also into the propagation of somatic mutations and malignant transformation. To limit genome instability, cells engage the DNA damage response (DDR) and activate repair mechanisms to reverse or minimize alterations in DNA integrity. The DDR pathway involves the interconnection of complex signaling networks that enforce cell cycle arrest and DNA repair. The failure of this adaptive mechanism is detrimental for the cell, resulting in an irreversible cell cycle arrest (senescence) or the activation of different types of regulated death programs. Accordingly, perturbations in the DDR largely contribute to oncogenesis, tumor progression, and the resistance to irradiation and chemotherapy with genotoxic drugs. The accumulation of synonymous mutations, aneuploidy, as well as the activation of oncogenes, deregulate proteostasis. The endoplasmic reticulum (ER) is the main subcellular compartment involved in protein folding and quality control, representing a central node of the splicing, impacting genome stability, cell survival and cell cycle regulation networks that enforce cell cycle arrest and DNA repair. The selective activation of RIDD in the absence of XBP1 mRNA splicing is a specialized mechanism to cope with ER stress, that also influences most hallmarks of cancer. Nevertheless, the possible involvement of the UPR in the surveillance and maintenance of genome integrity remains elusive.

Inositol requiring enzyme 1 alpha (known as ERN1, referred to as IRE1α hereafter) controls the most evolutionary conserved UPR signaling branch, regulating ER proteostasis and cell survival through distinct functional outputs. IRE1α is a serine/threonine protein kinase and endoribonuclease that catalyzes the unconventional splicing of the mRNA encoding X-Box binding protein 1 (Xbp1), generating an active transcription factor that enforces adaptive programs. IRE1α also degrades a subset of mRNAs and microRNAs through a process known as regulated IRE1α-dependent decay of RNA (RIDD), impacting various biological processes, including cell death and inflammation. A screen aiming to define the universe of XBP1-target genes under ER stress identified a cluster of DDR-related components, and suboptimal DNA repair may trigger ER stress. Together, these observations suggest a link between DNA damage and ER proteostasis. Here we investigate the possible contribution of IRE1α to the DDR. Surprisingly, we observed that genotoxic stress engages IRE1α signaling in the absence of ER stress markers. In fibroblasts undergoing DNA damage, IRE1α activation results in the selective activation of RIDD in the absence of XBP1 mRNA splicing, impacting genome stability, cell survival and cell cycle control. At the molecular level, we identify specific RIDD mRNA substrates as possible effectors of the phenotypes triggered by IRE1α deficiency. We also validated the significance of IRE1α signaling to the DDR in vivo using genetic manipulation in mouse and fly models. Our results suggest that IRE1α has an alternative function in cells undergoing genotoxic stress, where it serves to amplify and sustain an efficient DDR to maintain genome stability and cell survival.

Results

DNA damage selectively induces IRE1α signaling toward RIDD. Upon ER stress, IRE1α dimerization leads to its auto-phosphorylation and the formation of large clusters that are needed for optimal signaling. Exposure of mouse embryonic fibroblasts (MEF) to the DNA damaging agent etoposide, a topoisomerase II inhibitor, triggers mild IRE1α phosphorylation and formation of IRE1α clusters, as revealed using an IRE1α-GFP reporter. Similar results were obtained in cells exposed to γ-irradiation. Unexpectedly, MEF cells stimulated with etoposide or γ-irradiation failed to engage Xbp1 mRNA splicing, as determined by two independent PCR-based assays and western blot analysis. Moreover, no signs of ER stress were observed in cells undergoing DNA damage when we assessed canonical markers of UPR activation, including the expression of CHOP, ATF4, BIP, as well as ATF6 processing and the phosphorylation of both PERK and eIF2α. As positive controls of DNA damage, we monitored the levels of phosphorylation of the histone H2AX (γ-H2AX) or the upregulation of the cyclin-dependent kinase inhibitor CDKN1A (also known as p21) (Supplementary Fig. 1b, d). Unexpectedly, classical RIDD mRNAs substrates such as Bloc1s1 and Sparc decayed upon exposure to DNA damaging agents. Importantly, this decrease in Bloc1s1 and Sparc mRNAs did not occur in IRE1α-deficient cells (Fig. 1e), nor upon pharmacological inhibition of the RNase activity of IRE1α with MKC-8866 (Supplementary Fig. 1e, f), confirming the occurrence of RIDD. These results suggest that DNA damage selectively stimulates IRE1α activity toward RIDD and not Xbp1 mRNA splicing in the absence of global ER stress markers.

IRE1α regulates DDR signaling under genotoxic stress. To evaluate the significance of IRE1α expression to the adaptive capacity of cells undergoing DNA damage, we compared the viability of IRE1α-deficient and control (wild type, WT) cells after exposure to various agents that induce distinct types of DNA lesions, including etoposide, 5-hydroxyurea, 5-fluorouracil, and γ-irradiation. Remarkably, IRE1α deficiency sensitized cells to all types of genotoxic stress, thus increasing the incidence of cell death (Fig. 1f and Supplementary Fig. 2a). We confirmed these results by measuring caspase-3 activation, a marker of apoptosis. We then stably reconstituted IRE1α null cells with an HA-tagged version of IRE1α (IRE1α-HA) that expresses levels similar to endogenous (described in refs. 14,15). Importantly, the hypersensitivity of IRE1α deficient cells to DNA damage was partially reverted by expressing IRE1α-HA, suggesting that the phenotypes observed in IRE1α-deficient cells under genotoxic stress are a primary phenotype, and are not due to clonal effects or compensatory changes (Supplementary Fig. 2b). In sharp contrast, IRE1α knockout MEFs did not reveal any differential sensitivity to the ER stress inducer tunicamycin, in line with prior results based on pharmacological inhibition of IRE1α. Also, the pharmacological inhibition of IRE1α RNase activity with MKC-8866 increases the susceptibility to cell death after prolonged genotoxic treatments (Fig. 1h, i). These findings highlight the importance of IRE1α in the adaptation response to DNA damage.

The downstream effects of the DDR are mediated by the activity of check point kinases CHK1 and CHK2, engaging the tumor suppressor protein P53 to induce cell-cycle arrest and the transcription of DNA damage-responsive genes, or to trigger apoptotic cell death. DDR signaling translates into the phosphorylation of histone H2AX (γ-H2AX), and the rate of γ-H2AX decay after DNA injury is a sign of DNA repair. Thus, we monitored the kinetics of H2AX (de)phosphorylation after exposing cells to a pulse of etoposide. IRE1α null cells exhibited a lower response and faster attenuation of γ-H2AX phosphorylation compared to cells in which IRE1α was reintroduced (Fig. 2a). Similar results were obtained when cells where exposed to a shorter pulse of a higher concentration of etoposide (Supplementary Fig. 2c). Analysis of nuclear γ-H2AX foci by immunofluorescence and confocal microscopy also indicated reduced γ-H2AX phosphorylation in IRE1α deficient cells (Fig. 2b). To corroborate these results, we performed the comet assay to directly visualize the DNA damage observing that IRE1α knockout cells exposed to etoposide exhibited increased...
DNA damage (Fig. 2c). We also performed a cytokinesis-block micronucleus cyto metric (CBMN) assay as an indirect measure of DNA breaks. Cells were incubated with etoposide for 3 h, followed by the administration of cytochalasin-B (CytB) for an additional 24 h. IRE1α knockout and WT MEF cells showed similar cell viability analyzed using PI staining and FACS (n = 3). All panels data is shown as mean ± s.e.m.; *p < 0.05, **p < 0.01, ***p < 0.001, based on two-tailed unpaired t-Student’s test, (c, d). One-way ANOVA followed Tukey’s test (e–g, i), two-way ANOVA followed Bonferroni’s test. Data is provided as a Source Data file.

Genotoxic stress triggers RIDD to regulate DDR signaling. To obtain mechanistic insights, we attempted to identify RIDD target mRNAs that might connect IRE1α signaling to the DDR. Previously, a global in vitro screening uncovered a cluster of mRNAs containing consensus sequences cleaved by IRE1α that are associated to a stem-loop structurally similar to the Xbp1 mRNA splicing site. Among the 13 top hits, two DDR-related genes were identified as possible RIDD substrates: PPP2CA-scaffolding A subunit (Ppp2r1a) and RuvB like AAA ATPase 1 (Ruvbl1) mRNAs. Ppp2r1a encodes the scaffold A subunit of protein phosphatase 2 catalytic subunit alpha (PPP2CA, also known as Ppp2a), which dephosphorylates check point kinases, reversing the G2/M arrest, and directly catalyzing the decay of γ-H2AX phosphorylation and foci. RUVBL1 (also known as Pontin), participates in chromatin remodeling and modulates the stability of DDR protein complexes, thus influencing the dephosphorylation of γ-H2AX.

Fig. 1 Selective activation of RIDD under DNA damage. a MEF were treated with 10 μM etoposide (Eto) for indicated time points and phosphorylation levels of IRE1α were detected by Phostag assay (p: phosphorylated 0: non-phosphorylated bands). IRE1α levels were analyzed by western blot. Treatment with 500 ng/ml tunicamycin (Tm) as positive control (8 h) (n = 3). b TREX-IRE1-GFP cells were treated with 25 μM Eto (8 h) or 1 μg/ml Tm (4 h). IRE1-GFP foci were quantified by confocal microscopy (>200 cells, n = 3). c MEF were treated with either 100 ng/ml Tm, 10 μM Eto or 25 Gy of ionizing radiation (IR) at indicated time points. Xbp1 mRNA splicing percentage was calculated by RT-PCR using densitometric analysis (left panel) (n = 3). d Xbp1 mRNA levels were quantified by real-time-PCR in samples described in c (n = 3). e WT and IRE1α KO cells were treated with 10 μM Eto (8 h and 16 h), IR (20 or 30 Gy, 16 h) and the decay of mRNA levels of Blocl1 and Sparc was measured by real-time-PCR. Treatment with 500 ng/ml Tm as positive control (n = 3). f WT and IRE1α KO cells were treated with 25 μM Eto, 1 μM 5-fluorouracil (5-FU), 1 μM hydroxyurea (HU) or 1 μg/ml Tm by 24 h and viability analyzed using propidium iodide (PI) staining and FACS (n = 3). g IRE1α KO (Mock) and IRE1α-HA reconstituted cells were treated with 10 μM Eto for 12 h and apoptosis monitored by caspase-3 positive cells (green). Nucleus (Red) was stained to visualize cells number (n = 3). h MEF cells treated 36 h with 0.5 μM Eto in combination with the IRE1α inhibitor 25 μM MKC-8866. Representative images. i WT cells were treated with 0.5 and 1 μM Eto or in combination with 25 μM MKC-8866 (36 h). Cell viability analyzed using PI staining and FACS (n = 3). All panels data is shown as mean ± s.e.m.; *p < 0.05, **p < 0.01, ***p < 0.001, based on two-tailed unpaired t-Student’s test, (c, d). One-way ANOVA followed Tukey’s test (e–g, i), two-way ANOVA followed Bonferroni’s test. Data is provided as a Source Data file.
Quantification of Ppp2r1a and Ruvbl1 mRNA levels in cells treated with etoposide demonstrated a decay that was dependent on IRE1α expression (Fig. 3b). These effects on mRNA levels translated into reduced protein expression of PP2A and RUVBL1 only in wild-type cells exposed to etoposide and the basal upregulation in IRE1α null cells (Fig. 3c). In a cell-free assay, recombinant IRE1α directly cleaves a fragment of the Ppp2r1a mRNA that contains the RIDD consensus site (spanning nucleotides 1336–1865), but not an adjacent fragment (Fig. 3d). This reaction was suppressed significantly by the IRE1α inhibitor 4µ8C (Fig. 3d). The lack of Xbp1 mRNA splicing under DNA damage conditions might involve inhibitory signals, for example mediated by the downstream regulation of the mRNA ligase RTTCB, the targeting of the Xbp1 mRNA to the ER membrane, or the activity of other regulatory components that are part of IRE1α clusters and component associated with them. Analysis of RTTCB levels revealed no changes in IRE1α knockout cells undergoing DNA damage (Supplementary Fig. 4a). To test if DNA damage inhibits Xbp1 mRNA splicing, we pre-treated cells with tunicamycin for 2 h and then added etoposide at different time points. Remarkably, etoposide failed to interfere with Xbp1 mRNA splicing induced by tunicamycin (Fig. 3e). Virtually identical results were obtained when a pulse of etoposide was performed followed by the stimulation of ER stress (Fig. 3g). In contrast, an additive effect was observed on the decay of Blocl1s1 and Sparc mRNAs when ER stress and DNA damaging agents were combined (Fig. 3f, h). These results indicate that DNA damage selectively engages RIDD yet does not cause active suppression of Xbp1 mRNA splicing.

Considering that PP2A and Pontin are upregulated in IRE1α null cells under genotoxic stress and are involved in the DDR, we attempted to reverse the phenotype of those cells by depleting Ppp2r1a or Ruvbl1 mRNA with suitable short hairpin RNAs (shRNAs) (Supplementary Fig. 4b). Remarkably, knocking down Ppp2r1a or Ruvbl1 in IRE1α null cells augmented the levels of phosphorylated γ-H2AX foci after etoposide treatment during the recovery phase (Fig. 3i). Similar results were obtained when phosphorylated γ-H2AX was monitored by immunoblot (Supplementary Fig. 4c). Moreover, knocking-down Ppp2r1a and Ruvbl1 expression in IRE1α null cells reestablished normal levels of CHK1 phosphorylation (Fig. 3j) and Supplementary Fig. 4d, e) and increased population of cells in S/G2M after etoposide treatment (Supplementary Fig. 4f). Taken together, these experiments suggest that the regulation of PP2A and Pontin by IRE1α contributes to DDR signaling under genotoxic treatment.

C-Abl triggers IRE1α activation under DNA damage. Recent studies suggest that IRE1α activation can occur independently from ER stress, impacting various biological processes including...
cell migration, synaptic plasticity, angiogenesis and energy metabolism. However, until now there are no examples for a selective activation of RIDD in the absence of Xbp1 mRNA splicing. The release of the ER chaperone BiP from the luminal domain of IRE1α correlates with its activation under ER stress. Co-immunoprecipitation experiments indicated that the BiP-IRE1α interaction decrease after etoposide treatment, but in a lesser extent than ER stress (Supplementary Fig. 5a).
We then explored possible signaling events that may link the DDR with the induction of RIDD. Interestingly, a recent report indicated that the non-receptor c-Abl tyrosine kinase physically interacts with IRE1α under metabolic stress, allosterically inducing its oligomerization into a conformation that is more likely to catalyze RIDD than Xbp1 mRNA splicing. Of note, c-Abl has been extensively associated to the DDR, regulating cell-cycle arrest and apoptosis. We confirmed the activation of c-Abl under genotoxic stress (Supplementary Fig. 5b). Treatment of cells with the c-Abl inhibitor imatinib reduced the decay of Ruvbl1 mRNA in cells exposed to tunicamycin or etoposide (Supplementary Fig. 5c, d). Furthermore, blocking the expression of c-Abl using shRNAs (Supplementary Fig. 5e) had no effects on the levels of Xbp1 mRNA splicing (Fig. 4a), but fully prevented the decay of Ruvbl1 and Pprr21α mRNAs under ER stress or DNA damage (Fig. 4b), phenocopying the consequences of IRE1α deficiency. Consistent with these results, imatinib treatment attenuated the generation of IRE1α-GFP positive clusters in HEK293 cells undergoing genotoxic stress (Fig. 4c, d). Moreover, we also generated a set of c-Abl null MEF cells using the CRISPR/CAS9 technology (Fig. 4e). The deletion of c-Abl prevented the decay of Ruvbl1, Pprr21α (Fig. 4f) and Blocs1 mRNA, without an effect on the levels of Xbp1 mRNA splicing (Supplementary Fig. 5f-g). These observations correlated with the formation of a protein complex between IRE1α and c-Abl as monitored using co-immunoprecipitation in 293T HEK cells overexpressing the proteins (Fig. 4g). We confirmed these results using Proximity Ligation Assay of endogenous c-Abl and IRE1-HA at basal conditions (Fig. 4h) or after exposure to etoposide (Supplementary Fig. 5h, i). Furthermore, using a cell free system, we assessed the effects of recombinant c-Abl on the oligomerization status of purified cytosolic domain of IRE1α. Incubation of purified IRE1α at 37 °C induced its spontaneous oligomerization, which was further enhanced when c-Abl was present in the reaction (Fig. 4i). Taken together, these results suggest that the activation of c-Abl in cells undergoing DNA damage contributes to the selective engagement of RIDD, possibly through a direct interaction with IRE1α.

**IRE1α protect flies against genotoxic stress.** To test the possible role of IRE1α in the maintenance of genome integrity in vivo, we took advantage of *D. melanogaster* as a model organism. The GAL4/UAS system was employed to knockdown the fly orthologue of IRE1α (dIRE1) using RNAi transgenic animals (Supplementary Fig. 6a). Etoposide treatment failed to trigger an increase in the levels of *dXbp1s* mRNA in larval tissue, as monitored by real time PCR (Fig. 5a). However, larvae fed with etoposide or tunicamycin exhibited a similar reduction in the mRNA levels of *dsparc* and *dMys*, two well-known RIDD targets in flies, in addition to dPontin, fly orthologue of Pontin (Fig. 5b). dIRE1 depletion ablated the downregulation of *dsparc*, *dMys* and *Pprr21a*, confirming the occurrence of RIDD (Fig. 5b). Moreover, we then determined the impact of dIRE1 on the survival of animals under genotoxic conditions and quantified the number of larvae reaching adulthood. Knock-down of dIRE1 generated a hypersensitivity phenotype, meaning that most etoposide-treated animals died before reaching maturity (Fig. 5c). Next, we determined the participation of dIRE1 in the maintenance of genome integrity. To this end, we performed the somatic mutation and recombination test (SMART). This assay is based on the induction of mutant spots (clones) that arise from loss of heterozygosity in cells of developing animals, which are heterozygous for a recessive wing cell marker mutation (Supplementary Fig. 6b) generating a multiple wing hair (mwh) phenotype (Fig. 5d, left panel). We expressed a dIRE1 RNAi construct in the wing imaginal disc using a Nubbin-Gal4 driver (Nub-Gal4). Exposure to doxorubicin increased the number of mutant spots in the fly wing, and this phenotype was exacerbated upon depletion of dIRE1 (Fig. 5d, right panel), suggesting compromised DNA repair. Doxorubicin also caused higher rates of apoptosis-associated caspase-3 activation upon dIRE1 knockdown (Fig. 5e). Next, we developed a mosaic analysis to ablate dIRE1 expression with a repressible cell marker (MARCM), a strategy that allows the comparison of wild-type and mutant cells in the same tissue by assessing GFP expression (Supplementary Fig. 6c). Using this mosaic technology, we generated mutant clones for dIRE1 in the eye-antenna imaginal disc and determined the frequency of GFP-positive (dIRE1 null cells) and negative cells (WT cells) that persist in the tissue after etoposide treatment. While dIRE1 expressing cells maintained their viability after exposure to etoposide (Fig. 5f, left panels), dIRE1 null cells proved highly susceptible to this genotoxic agent (Fig. 5f, right panels). Taken together, these results indicate that the fly orthologue of IRE1α protects against genotoxic stress in vivo.

**IRE1α deficiency impairs the DDR in mice.** We then moved forward and investigated the significance of IRE1α expression to the DDR in vivo and deleted the Rnase domain of IRE1α in the liver and bone marrow using a conditional knockout (cKO) system controlled by the Mx-Cre system. Poly[I:C] was injected to induce Cre expression, and three weeks later animals were treated with a single dose of either etoposide or tunicamycin, followed by the analysis of liver tissue. A well-established mammalian model of ER stress consists in the intraperitoneal injection...
of tunicamycin, which elicits a rapid stress response in the liver. Although evident signs of DNA damage were observed in both control and IRE1αcKO animals (indicated by a rise in p21 mRNA) (Fig. 6a upper panel and Supplementary Fig. 7a), no Xbp1 mRNA splicing was detected in the etoposide treated group (Fig. 6a, bottom panel). In sharp contrast, a clear down-regulation of Ppp2r1a and Bloc1s1 mRNA levels occurred in the livers (Fig. 6b) and bone marrows (Supplementary Fig. 7b) from control (but not hiRuvbl1 mRNA log2(treated/untreated)) Fig. 4 c-Abl contributes to the RIDD activation under DNA damage. a c-Abl was knocked down through the stable delivery of an shRNA. Then cells were treated with 10 μM Etoposide (Eto) or 500 ng/mL tunicamycin (Tm) for 8 h, and the decay of Ppp2r1a and Ruvbl1 was measured by real-time-PCR (n = 3). c TREX-IRE1-GFP cells were pre-treated with 10 μM imatinib by 1 h, and then treated with 10 μM Eto, or 500 ng/mL Tm for 8 h and IRE1-GFP foci visualized by confocal microscopy. d Quantification of the percentage of cells positive IRE1-GFP clusters is shown (>200 cells, n = 3). e c-Abl expression in CRISPR control and c-Abl KO cells was monitored by western blot (n = 3). f CRISPR control and c-Abl KO cells were treated with 10 μM Eto or 500 ng/mL Tm for 8 h, and the decay of Ruvbl1 and Ppp2r1a was measured by Real-Time-PCR (n = 3). g HEK-293T cells reconstituted with IRE1α-HA and c-Abl-GFP were exposed to 10 μM Eto for 8 h. Immunoprecipitation (IP) was performed using the HA epitope (IRE1α-HA) and GFP (c-Abl) to assess the possible interaction with c-Abl. h IRE1α KO (Mock) and reconstituted cells with an IRE1α-HA were treated 8 h with 10 μM Eto and stained with a proximity ligation assay (PLA) using an anti-HA or anti-c-Abl antibodies and analyzed by confocal microscopy. Right panel: Number of dots per cell analyzed and percentage of PLA positive cells were quantified (n = 6). i Recombinant IRE1α and c-Abl proteins were incubated at indicated time points and assess its possible interaction by western blot. All data represents the mean ± s.e.m. of three independent experiments, except for co-IP that were performed twice. *p < 0.05, **p < 0.01, and ***p < 0.001, based on (b, f) two-way ANOVA followed Bonferroni’s test, (d, h) One-way ANOVA followed Tukey’s test. Data is provided as a Source Data file.
IRE1α-deficient) animals injected with etoposide. Again, no Xbp1 mRNA splicing was detected in the etoposide treated group, whereas exposure of animals to tunicamycin triggered a very mild response in bone marrow tissue (Supplementary Fig. 7c). In addition, IRE1α deficiency in the liver altered the DDR, reflected in reduced phosphorylation of CHK1 in animals injected with etoposide (Fig. 6c). Importantly, ablation of IRE1α resulted in enhanced susceptibility of liver cells to apoptosis measured as enhanced caspase-3 activation (Fig. 6d).

Finally, to assess the significance of IRE1α to the DDR on an unbiased manner, we performed a gene expression profile analysis of liver tissue derived from mice exposed to etoposide or tunicamycin. Pathway enrichment analysis indicated that IRE1α deficiency attenuated the establishment of a global DDR, delayed the expression of cell cycle arrest genes and activated pro-apoptotic pathways (Fig. 6e and Supplementary Fig. 8a). As expected, under ER stress induced by tunicamycin, IRE1α deficiency in the liver significantly impacted the expression of genes involved in proteostasis control in the secretory pathway (trafficking, folding and quality control) (Supplementary Fig. 8b, c). Taken together, these findings demonstrate that IRE1α signaling contributes to maintaining the stability of the genome when cells face DNA damage.

Discussion

The current study supports a conserved function for IRE1α as a signaling module of the DDR that differs from its canonical role as an UPR mediator. We propose that IRE1α is part of a key decision-making node in a complex interplay between cell survival and DNA repair upon genotoxic stress. In this context, IRE1α regulates the levels of PP2A and RUVBL1 through the selective engagement of RIDD, controlling the kinetics and amplitude of γ-H2AX phosphorylation. The contribution of IRE1α to genome stability is conserved in evolution from insects to mammals and impacts whole animal survival as demonstrated using flies. Our results suggest a regulatory mechanism in which the RNase domain of IRE1α is selectively regulated to specifically engage RIDD, presumably upon interaction with c-AbI (Fig. 4g, h). This view is consistent with recent studies that connected using unbiased approaches the pathways involved in maintenance of genome integrity and proteostasis, showing that dysregulation of the DDR resulted in protein aggregation and autophagy induction. Moreover, previous work demonstrated that the function and structure of the ER is drastically affected by DNA damaging agents used in chemotherapy. Other recent reports suggested that chronic ER stress suppresses DNA repair and sensitizes cancer cells to ionizing radiation and chemotherapy, in addition to enhancing oxidative damage to the DNA. Interestingly, a recent study also reported that XBP1s, the protein encoded by the unspliced version of Xbp1 mRNA, regulates the stability of TP53, suggesting alternative connections between the UPR and the DDR under resting conditions. Our results suggest that IRE1α specifically affects signaling events regulating the DDR, and not the DNA damage sensing process. IRE1α operates as an amplification loop, impacting the sustained activation of CHK1/2 and the phosphorylation of γ-H2X through the control of the RIDD targets Ppp2r1a and Ruvb1l, leading to cell cycle arrest and improved DNA repair and as a consequence maintenance of cell survival (see working model in Fig. 6f).

Although RIDD is proposed to be necessary for the maintenance of ER homeostasis and to contribute to the pathogenesis of diabetes, cancer, and inflammatory conditions, most of the available evidence is difficult to interpret due to the concomitant existence of Xbp1 mRNA splicing. Our study supports a fundamental biological function for RIDD in the maintenance of...
genotoxic stress activates IRE1 response encoding for involvement of the c-Abl kinase that is activated by DNA damage response kinases as ATM. The expression and function of IRE1 to promote survival under DNA damage conditions by controlling cell cycle arrest and DNA repair programs.

**Fig. 6 IRE1α deletion in liver alters the DDR under genotoxic stress.** a IRE1α was conditionally deleted the liver using the MxCre and LoxP system (IRE1αKo). Mice were intraperitoneally injected with 50 mg/Kg etoposide (Eto) or 1 mg/Kg tunicamycin (Tm) and sacrificed 6 h and 16 h later. Total mRNA levels of the deleted IRE1, and p21 were measured 6 h later in the liver by real-time-PCR (n = 3–4 mice per group). Xbp1 mRNA splicing (bottom panel) was monitored in the same samples by RT-PCR. b Liver extracts of animals described in a, Ppp2r1a and Blob1 mRNA expression levels were measured 6 h later of Eto treatment by real-time-PCR (n = 3). c Protein liver extracts were obtained from mice treated described in a and the expression levels of indicated proteins were monitored 6 h later of Eto treatment by western blot. Quantification of the levels of p-CHK1 is shown (Right panel). d Mice from a were intraperitoneally injected with 50 mg/Kg Eto and sacrificed 48 h later. Then, livers active-caspase 3 detected by immunohistochemistry (n = 2–3). e Gene expression profile analysis was performed in mRNA from liver extracts of animals described in a. Most significant pathways altered by Eto administration in WT and IRE1α null livers are shown. Three independent biological samples were used. In all panels, data is shown as mean ± s.e.m.; *p < 0.05, **p < 0.01, and ***p < 0.001, based on a-d two-way ANOVA followed Bonferroni’s test. Data is provided as a Source Data file. f Working model: genotoxic stress activates IRE1α in the absence of ER stress markers, selectively engaging RIDD. IRE1α degrades mRNAs involved in the DNA damage response encoding for Ppp2r1a and Ruvbl1, regulating the (de)phosphorylation of the histone H2AX and CHK1/2. The non-canonical activation of IRE1α involves the participation of the c-Abl kinase that is activated by DNA damage response kinases as ATM. The expression and function of IRE1α is essential to promote survival under DNA damage conditions by controlling cell cycle arrest and DNA repair programs.
genome integrity, representing a unique example for a selective and specific activation of RIDD with clear physiological implications. IRE1α is frequently affected by loss-of-function mutations in cancer, contrasting with the notion that cancer cells require IRE1α to survive in hypoxic conditions. Our present results support the idea that the genetic alterations of IRE1α observed in cancer may synergize with oncogenes to promote genomic instability due to inefficient DNA repair. Altogether, we uncovered a previously unanticipated function of a major UPR signal transducer as an integral component of the DDR, revealing an intimate connection between the pathways that assure the integrity of the proteome and the genome.

**Methods**

Reagents. Etoposide, doxorubicin, 5-fluouracil, hydroxyurea, imatinib and 4μC were purchased from Sigma Aldrich. Tunicamycin was obtained from Calbiochem EMB Bioscience Inc. IRE1α inhibitor MKC-8866 was provided by Dr. John Patterson (Fossun Orinove). Cell culture media, fetal calf serum, and antibiotics were obtained from Life Technologies and Sigma Aldrich. Fluorescent probes and secondary antibodies coupled to fluorescent markers were purchased from Molecular Probes, Invitrogen. All other reagents were obtained from Sigma or the highest grade available.

Cell culture and DNA constructs. All MEF and HEK cells used here were maintained in Dulbecco’s modified Eagles medium supplemented with 5% fetal bovine serum, non-essential amino acids and grown at 37 °C and 5% CO2. TREX-IRE1α was maintained in Dulbecco’s modified Eagles medium supplemented with 5% fetal calf serum, and antibiotics were obtained from Life Technologies and Sigma Aldrich. Fluorescent probes and secondary antibodies coupled to fluorescent markers were purchased from Molecular Probes, Invitrogen. All other reagents were obtained from Sigma or the highest grade available.

**RNA isolation, RT-PCR and real time PCR.** Total RNA was prepared from cells and tissues using Trizol (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized with SuperScript III (Invitrogen) using random primers p(dN)6 (Roche). Quantitative real-time PCR reactions were performed using SYBRgreen fluorescent reagent and/or EvaGreenTM using a Stratagene Mx3000P system (Agilent Technologies). Quantitative real-time PCR reactions were performed using SYBRgreen fluorescent reagent and/or EvaGreenTM using a Stratagene Mx3000P system (Agilent Technologies). Real-time polymerase chain reactions were run in a Stratagene Mx3000P system (Agilent Technologies) using standard SYBRgreen reaction conditions (2 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C).

**Immunoprecipitations.** HEK-293T cells reconstituted with IRE1α-HA and c-Abl-GFP and IRE1α deficient MEF cells stably transduced with retroviral expression vectors for IRE1α-HA or empty vector were incubated in presence or absence of tunicamycin (500 ng/mL for 4 h) or etoposide (10 μM for 16 h). Cell lysates were prepared for immunoprecipitations in lysis buffer (0.5% NP-40, 50 mM NaCl, 30 mM Tris pH 7.6, 30 mM NaF, 1 mM Na3VO4 and protease inhibitors). Immune precipitations (IP) were performed as described. Briefly, to IP HA-tagged IRE1α, protein extracts were incubated with anti-HA antibody-agarose complex (Roche), for 4 h at 4 °C, and then washed three times with 1 mL of lysis buffer and then one time in lysis buffer with 500 mM NaCl. Beads were dried and resuspended in Sample Buffer 2X. Samples were heated for 5 min at 95 °C and resolved by SDS-PAGE 8% followed by western blot analysis.

**Western blot analysis.** Cells were collected and homogenized in RIPA buffer (20 mM Tris pH 8.0, 150 mM NaCl, 0.1% SDS, 0.5% Triton X-100) containing 5% BSA, cells were incubated with the indicated antibodies overnight at 4 °C following by Duolink manufacturer’s instructions (Duolink®). Membranes were blocked using PBS, 1% Tween-20 (PBST) containing 5% milk (Roche) prior to transfer onto PVDF membranes. To evaluate IRE1α phosphorylation, SDS-PAGE minigel were made in presence of the 5 M of Phostag reagent and 10 μM M MnCl2. In vitro oligomerization assay was previously described. Briefly, 0.5 μM of Phostag reagent and/or EvaGreenTM using a Stratagene Mx3000P system (Agilent Technologies) using standard SYBRgreen reaction conditions (2 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C).

**In vitro oligomerization assay.** In all, 0.5 μg of the cytoplasmic domain of GST-tagged IRE1α (Sino Biologicals) and 0.1 μg of His tagged c-Abl (Carna Biosciences) were incubating and mixing for indicated time points, at 37 °C in a heat block. The mixture was resolved by SDS-PAGE 8% followed by western blot analysis.

**Immunofluorescence.** IRE1α-HA, and γ-H2AX proteins were visualized by immunofluorescence. Cells were fixed for 30 min with 4% paraformaldehyde and then permeabilized 0.5% NP-40 in PBS containing 0.5% BSA and incubated with Alexa-conjugated secondary antibodies (Molecular Probes) for 1 h at 37 °C. Nuclei were stained with Hoechst dye. Coverslips were mounted with Fluoromount G onto slides and visualized by confocal microscopy (Fluoview FV1000). The number and size of IRE1α foci was quantified using segmentation and particle analysis of Image J software.

**Automated microscopy.** Cells were seeded in 96-well imaging plates (BD Falcon, Sparks, USA) 24 h before stimulation. Cells were treated with the indicated agents. Subsequently, cells were fixed with 4% paraformaldehyde and stained with 0.5% PFA and counterstained with 10 μM Hoechst 33342. After fixing for 1 h with 10% FBS in PBS containing 0.5% BSA, cells were subsequently incubated with anti-HA 1:1000 (Invitrogen, Cat. 715500), anti-phosphorylated-Chk1, Ser348 1:1000 (Cell Signaling, Cat. 2341), anti-cleaved caspase 3, Asp175 1: (Cell Signalling, Cat. 9661) or anti–Phosphorylated S139-H2AX 1:503 (Millipore, Cat. 05-636) antibodies fixed with 4% paraformaldehyde for 30 min. Nuclei were stained with Hoechst dye. Coverslips were mounted with Fluoromount G onto slides and visualized by confocal microscopy (Fluoview FV1000).
**Comet assay.** The Comet assay was performed as previously described. Briefly, agarose-slides were prepared with 1% low-gelling-temperature agarose and 2 x 10⁶ cells/mL and submerged in lysis solution (1.2 M NaCl, 100 mM Na₂EDTA, 0.1% sodium lauryl sarcosinate, 0.26 M NaOH (pH > 13)) for 18 h at 4°C in the dark. Then, carefully slides removed and submerged in room temperature (18 – 25°C) in rinse solution (0.03 M NaOH, 2 mM Na₂EDTA (pH ~12.3)) for 20 min. Electrophoresis was conducted in the same solution for 25 min at a voltage of 0.6 V/cm. Finally, slides were stained in a solution containing 10 μg/mL of propidium iodide in distilled water for 20 min and observed in a epifluorescence microscopy. Images were analyzed using Comet Assay IV software.

**Cytokinesis-block micronucleus assay.** Cytokinesis-block micronucleus (CBMN) assay was performed as previously described. In brief, cells were treated with 5 etoposide (5 μM for 3 h). Then, cells were washed three times with PBS and incubated for 24 h in PBS with 2 μM of Cytochalasin-B. Cells were fixed, stained with Hoescht. Binucleated cells (BN) with micronucleus (MN), Nuclear fragments were described as previously.

**In vitro RNA cleavage assay.** Bicis1 (NM_0014873), Pp2r1a (NM_014225) and Ruvb1 (NM_00370) cDNA were obtained from MGC cDNA library (Dharmacon). Sense and anti-sense oligonucleotides containing a minimal T7 RNA polymerase promoter (5’-TAATACGACTCACTATAGG-3’) fused upstream of the sequence containing different fragments of the genes Pp2r1a, Ruvb1 and Bicis1 harboring 5’-EcoRI and 3’-BanHI overhangs were annealed and ligated into the cognate restriction sites of pUC19 (Invitrogen, Life Technologies). Oligonucleotide sequences to clone Pp2r1a and Ruvb1 fragments were described previously. In vitro transcription reactions were performed with T7 RNA polymerase using the HiScribe T7 high-yield RNA synthesis kit (New England Biolabs) with 150 μM of each nucleotide, 2 mM Mg(OAc)₂, 1 mM TCEP, and 5% glycerol). Stop solution (10 M urea, 0.1% SDS, 1 mM EDTA, 0.05% xylene cyanol, 0.05% bromophenol blue) was added at five-fold excess to stop the reactions followed by heating at 80°C for 3 min. The denatured samples were then loaded on 6% TBE-urea gels (Invitrogen, Life Technologies) and the gels stained with SYBR Gold nucleic acid stain (Invitrogen, Life Technologies). As a negative control we utilized the IRE1a inhibitor 4qBc to a final concentration of 5 μM.

**Viability assay.** In all, 2 x 10⁴ cells were seeded in 48-well plate and the maintained by 24 h in DMEM cell culture media supplemented with 5% bovine fetal serum containing 0.5% FBS and essential amino acids. Genotoxic and ER stress were measured by adding genotoxic and ER stress agents to the cells at the different concentrations, and maintained for 24 h. Then, cell viability was monitored using propidium iodide staining and flow cytometry (BD FACs Canto, Biosciences).

**Mouse model.** Enr1 floxed mice were previously described and crossed with Mxi-cre transgenic mice to generate a conditional KO animal (IRE1a-CreKO). Deletion was induced by injection of polysynthetic-polylysylacyclic acid (Poly (lc) (Ic)) which efficiently delete the floxed gene in the liver and bone marrow. All 5–6-week-old mice were intraperitoneally (i.p.) injected three times with 250 μg of poly(Ic) each time with 2 day intervals to induce the Cre expression. Mice were used for experiments at least 2–3 weeks after the final poly(Ic) injection. DMSO or 50 mg/Kg etoposide or 1 mg/Kg tunicamycin or were i.p. injected and 6 h later mice were sacrificed as reported. The liver and bone marrow were frozen at −80°C for biochemical analysis and the right major lobe of the liver was placed in a petri dish (on ice). Liver tissue was washed in PBS to remove the blood and then, it was fixed in 4% paraformaldehyde (72 h) for histological analyses. The animals’ works and care was in accordance with institutional guidelines. Institutional Committee for Animal Care and Handling, University of Chile (Protocol CICUA-CBA-0833).

**Fly studies.** Flies were kept at 25°C on standard medium with a 12–12 dark-light cycle. Drug administration protocol for all experiments is as follows: Larvae were grown in standard fly medium until day 3 after egg laying (AEL). Then they were transferred to fly instant medium (Carolina Biological Supply 2700 York Burglinton, NC, USA) supplemented with the appropriate drug for the different treatments. Larvae were fed with the corresponding drug-supplemented media until dissection or adulthood.

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Acknowledgements

We thank Dr. Takao Iwasawa for providing IRE1α flox mice. We thank Dr. David Ron for providing IRE1α null MEFs. We thank Dr. Alexis Rivar for assistance with Cellomics array scan (FONDEQUIP EQM120164). We thank Javiera Ponce for assistance with animal care. ANID/FONDAP program 15150012, Millennium Institute P09-015-F, CONICYT-PID 1709186, FONDECYT 1180186 (C.H.), and Ecos-Conicyt n° C17S02 (C.H.). In addition, we thank the support from: FONDECYT 11180186 (C.H.), and FONDECYT 3190738 (A.I.S.); ANID/FONDAP program 15150012, Conicyt P ID 1709186, CONICYT P ID 1709186, and CONICYT P ID 1709186. We thank Javiera Ponce for assistance with animal care. ANID/FONDAP program 15150012, Millennium Institute P09-015-F, CONICYT-PID 1709186, FONDECYT 1180186 (C.H.), and Ecos-Conicyt n° C17S02 (C.H.). In addition, we thank the support from: FONDECYT 11180186 (C.H.), and Ecos-Conicyt n° C17S02 (C.H.). In addition, we thank the support from: FONDECYT 11180186 (C.H.), and Ecos-Conicyt n° C17S02 (C.H.). In addition, we thank the support from: FONDECYT 11180186 (C.H.), and Ecos-Conicyt n° C17S02 (C.H.).
FONDECYT 3180427 (Y.H.), FONDECYT 3150113 (A.C.-S.), and EMBO ASTF 385-2016 (A.C.-S.); CONICYT fellowship (PCHA/Doctorado Nacional/2016-21160232) (M.G.-Q.), MSCA RISE-734749 (INSPIRED) (M.Q.-G.) and FONDAP-GERO-15150012 (A.I.S., P.P.). A.G. is supported by FONDAP-CRG-15090007 and ACT1401. D.A. was supported by an Irvington Postdoctoral Fellowship of the Cancer Research Institute. A.A. is supported by FONDECYT 1161065 and AFBI170005. G.K. is supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR)—Projets blancs; ANR under the frame of E-Rare-2, the ERA-Net for Research on Rare Diseases; Association pour la recherche sur le cancer (ARC); Cancéropôle Ile-de-France; Chancellerie des universités de Paris (Legs Poix), Fondation pour la Recherche Médicale (FRM); a donation by Elior; the European Commission (ArtForce); European Research Area Network on Cardiovascular Diseases (ERA-CVD, MINOTAUR); the European Research Council (ERC); Fondation Carrefour; Institut National du Cancer (INCa); Inserm (HTS); Institut Universitaire de France; LeDucq Foundation; the LabEx Immuno-Oncology; the RHU Torino Lumière; the Seerave Foundation; the SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); the SIRIC Cancer Research and Personalized Medicine (CARPEM); and the Paris Alliance of Cancer Research Institutes (PACRI). P.M.D was supported by FCT LISBOA-01-0145-FEDER-007660, PTDC/NEU-NMC/2459/2014 and IF/00697/2014 (Portugal). R.P. is supported by Fondation pour la Recherche Médicale (FMR, DEQ20180339169) and Institut National de la Santé et de la Recherche Médicale (INSERM). E.C. is supported by Institut National du Cancer (INCa PLBIO), ANR under the frame of ERANET (ERAAT) and EU H2020 MSCA ITN-675448 (TRAINERS) and MSCA RISE-734749 (INSPIRED).

Author contributions
E.D., M.G-Q., and A.I.S.: conceived and designed the project, carried out experimental work with cell culture and mouse models, and wrote the paper; J.M.B., C.E., H.U., D.S., P.P., A.C.-S., Y.H., E.A.S., D.G., C.V., A.P., D.A.-A., G.C., P.G., A.G.: carried out experimental work and interpreted data; P.G. and E.A.S.: performed bioinformatics work, interpreted data and wrote the manuscript; A.A., P.M.D., Contributed to project design and data interpretation; C.H., P.W., G.K., R.P., E.C.: contributed to project design, interpreted data and wrote the paper.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41467-020-15694-y.

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Peer review information Nature Communications thanks Martin Lavin, Hermann Steller and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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