




# The integrated stress response in pulmonary disease

Giulia Emanuelli<sup>1,2,3</sup>, Nikou Nassehzadeh-Tabriz<sup>1,3</sup>, Nick W. Morrell<sup>2</sup> and Stefan J. Marciniak<sup>1,2</sup>

**Affiliations:** <sup>1</sup>Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge, UK. <sup>2</sup>Division of Respiratory Medicine, Dept of Medicine, University of Cambridge, Cambridge, UK. <sup>3</sup>Equal first authors.

**Correspondence:** Stefan J. Marciniak, Cambridge Institute for Medical Research (CIMR), University of Cambridge, Cambridge CB2 0XY, UK. E-mail: [sjm20@cam.ac.uk](mailto:sjm20@cam.ac.uk)

 @ERSpublications  
**By integrating signals from multiple stressors, the integrated stress response plays key roles in the pathophysiology of lung disease. Understanding the mechanisms involved will identify novel means of therapy.** <https://bit.ly/2Bg2kj4>

**Cite this article as:** Emanuelli G, Nassehzadeh-Tabriz N, Morrell NW, *et al.* The integrated stress response in pulmonary disease. *Eur Respir Rev* 2020; 0: 200184 [<https://doi.org/10.1183/16000617.0184-2020>].

**ABSTRACT** The respiratory tract and its resident immune cells face daily exposure to stress, both from without and from within. Inhaled pathogens, including severe acute respiratory syndrome coronavirus 2, and toxins from pollution trigger a cellular defence system that reduces protein synthesis to minimise viral replication or the accumulation of misfolded proteins. Simultaneously, a gene expression programme enhances antioxidant and protein folding machineries in the lung. Four kinases (PERK, PKR, GCN2 and HRI) sense a diverse range of stresses to trigger this “integrated stress response”. Here we review recent advances identifying the integrated stress response as a critical pathway in the pathogenesis of pulmonary diseases, including pneumonias, thoracic malignancy, pulmonary fibrosis and pulmonary hypertension. Understanding the integrated stress response provides novel targets for the development of therapies.

---

Provenance: Commissioned article, peer reviewed

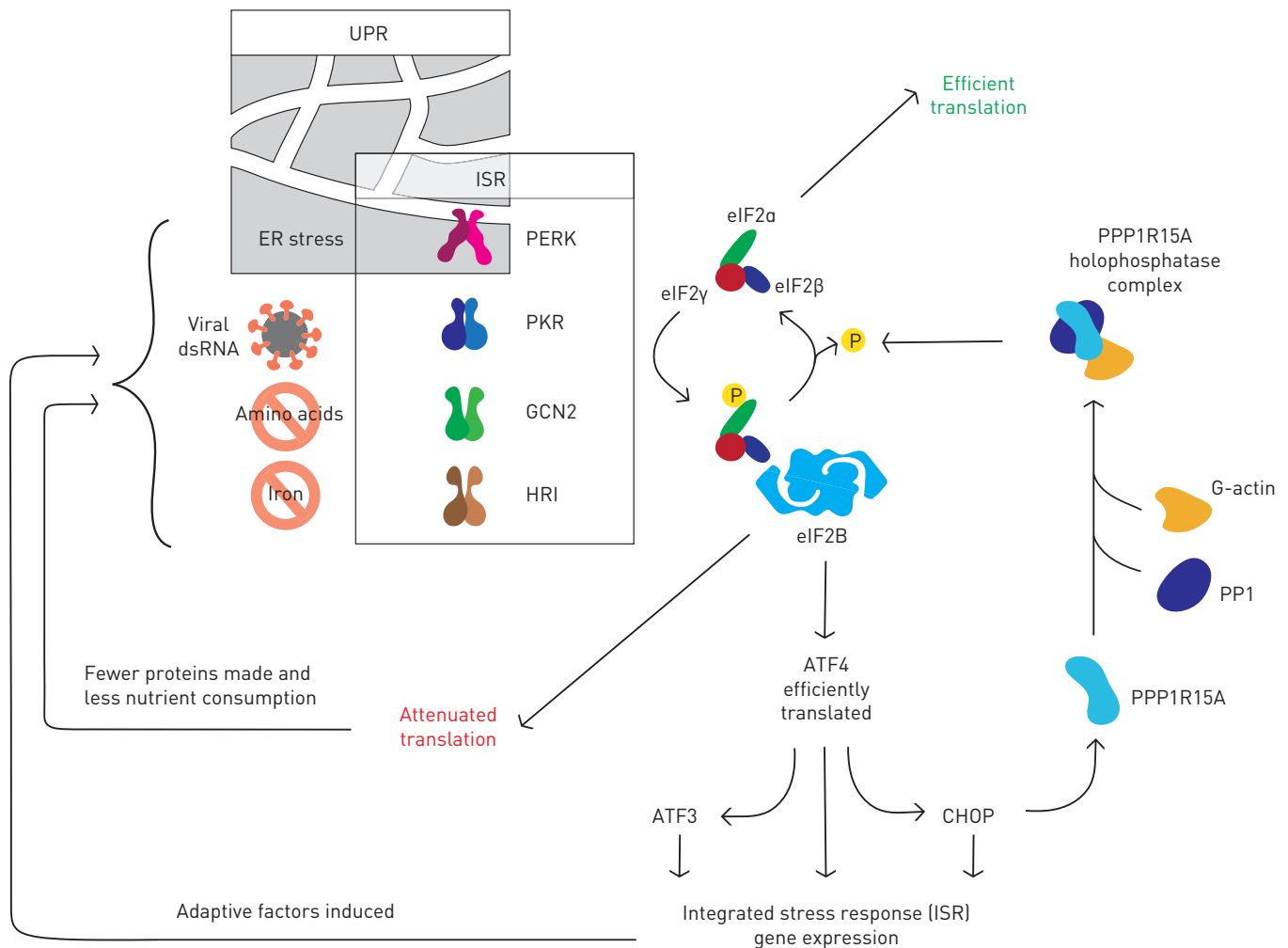
Received: 8 June 2020 | Accepted after revision: 15 July 2020

Copyright ©ERS 2020. This article is open access and distributed under the terms of the Creative Commons Attribution Non-Commercial Licence 4.0.

### The integrated stress response

The airway epithelium faces many stresses, both extrinsic, *e.g.* inhaled toxins, and intrinsic, *e.g.* protein misfolding [1, 2]. Stress-responsive pathways determine whether these stresses are withstood or if they cause disease. A variety of insults trigger the phosphorylation of eukaryotic initiation factor (eIF)2 $\alpha$  [3]. Since eIF2 $\alpha$  phosphorylation integrates signals from diverse sources, its downstream pathway has been named the “integrated stress response” (ISR) (figure 1). Four stress-sensing kinases trigger the ISR [4]: protein kinase R (PKR) responds to cytosolic double-stranded (ds)RNA [5]; PKR-like endoplasmic reticulum kinase (PERK) detects endoplasmic reticulum (ER) stress [6, 7]; heme-regulated inhibitor (HRI) responds to iron deficiency and oxidative stress [8]; while general control non-depressible (GCN2) is activated during amino acid starvation [9, 10].

The eIF2 complex recruits methionyl-transfer (t)RNA to the ribosome at the beginning of protein synthesis. During cycles of translation initiation, eIF2 hydrolyses its bound GTP, which is replenished by the guanine nucleotide exchange factor eIF2 $\beta$ . However, when eIF2 $\alpha$  is phosphorylated it binds avidly to eIF2 $\beta$  inhibiting further GTP exchange. As a result, protein synthesis is attenuated, although a subset of transcripts is translated more efficiently thanks to regulatory elements within their 5'-untranslated regions [11, 12]. The transcription factors ATF4 and CHOP are induced in this way by the ISR and upregulate



**FIGURE 1** The integrated stress response (ISR) is triggered by stress-sensing kinases that phosphorylate eukaryotic initiation factor (eIF)2 $\alpha$ , a component of the eIF2 translation initiation complex. Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) responds to endoplasmic reticulum (ER) stress, and so the ISR overlaps with the unfolded protein response (UPR). PKR detects viral double-stranded (ds)RNA. General control non-depressible [GCN2] is activated by amino acid deficiency. Heme-regulated inhibitor (HRI) responds to iron depletion. Phosphorylated eIF2 $\alpha$  binds avidly to eIF2 $\beta$  to inhibit most translation, but some mRNAs including those encoding the transcription factors ATF4 and CHOP are translated more efficiently. The resulting gene expression restores homeostasis by enhancing oxidative protein folding in the ER; promoting amino-acyl transfer (t)RNA synthesis; and inducing antioxidant genes. PPP1R15A (also known as GADD34) is eventually induced and in complex with PP1 and G-actin dephosphorylates eIF2 $\alpha$  to terminate the ISR.

hundreds of ISR target genes. One of these encodes PPP1R15A (also known as GADD34), which forms a complex with protein phosphatase (PP)1 and G-actin to generate an eIF2 $\alpha$ -specific phosphatase that terminates the ISR [13]. This negative feedback mechanism allows the ISR to be activated transiently, but premature inactivation of the ISR can cause cell death [14, 15].

### Infection

The ISR can be activated by viral infection. During replication of RNA viruses and complex DNA viruses, dsRNA can be detected by PKR [16]. In addition, enveloped viruses require high levels of membrane protein synthesis, which can trigger PERK [17]. Phosphorylation of eIF2 $\alpha$  then antagonises viral protein synthesis while triggering ISR gene expression [1, 17]. Innate antiviral pathways induce interferon (IFN)-dependent genes including PKR, thus enhancing ISR activation [16]. Simultaneously, phosphorylation of eIF2 $\alpha$  causes formation of stress granules [18], membraneless organelles formed by a liquid-liquid phase separation of ribonuclear proteins, which sequester viral factors, block viral gene expression and recruit viral sensors such as PKR [19]. The importance of the ISR in defending against viral infection is illustrated by the wide variety of evasion mechanisms that viruses have evolved to overcome its effects, including decoy dsRNAs, PKR degradation, viral RNA sequestration, blockade of PKR dimerisation, pseudosubstrates that compete for PKR's activity and even expression of a viral eIF2 $\alpha$  phosphatase [5, 20–44] (table 1). In addition, some viruses inhibit IFN signalling to prevent induction of PKR [46].

### Influenza

Influenza A causes half a million deaths annually [47]. Two decades ago, it was discovered that influenza circumvents PKR by means of a nonstructural protein called NS1 [26] (table 1). NS1-deleted strains of influenza are enfeebled, but their virulence is restored in PKR-deficient cells [45]. NS1 was initially thought to enable influenza to evade PKR through activation of the co-chaperone p58<sup>IPK</sup> [48]. Indeed, p58<sup>IPK</sup> was named because of its putative role as a 58-kDa inhibitor of PKR [49]. However, subsequent work revealed that p58<sup>IPK</sup> resides exclusively in the ER lumen [50], while PKR is cytosolic, making a direct

TABLE 1 Viral-mediated inhibition of protein kinase R (PKR) activity

	Virus(es)	Viral protein	Mode of PKR inhibition	References
<b>ssRNA</b>				
Orthomyxoviridae	Influenza A Influenza B	NS1	Direct interaction/dsRNA-mediated interaction	[26, 45]
Coronaviridae	Infectious bronchitis virus hCoV-229a MERS-CoV	NSp2	Inhibition of phosphorylation	[21]
		NSp15	dsRNA sequestration	[22]
		NS4a	dsRNA sequestration	[23, 27]
Bunyaviridae	Rift Valley fever virus	NSs	Proteasome-mediated degradation	[28]
Filoviridae	Ebolavirus	VP35	Unknown/possible dsRNA sequestration	[29]
Retroviridae	HIV	Tat	Pseudosubstrate/direct interaction	[9]
		TAR RNA	Decoy dsRNA	[10]
Flaviviridae	Hepatitis C virus	NS5a	Blocking of dimerisation/direct interaction	[32]
		E2	Pseudosubstrate/direct interaction	[33]
Reoviridae	Reoviruses	$\sigma$ 3/ $\sigma$ 4	dsRNA sequestration	[34]
<b>dsRNA</b>				
Herpesviridae	Human cytomegalovirus	pTRS1/pIRS1	Interaction and relocalisation/dsRNA sequestration	[35]
<b>dsDNA</b>				
Herpesviridae	Herpes simplex virus	$\gamma$ 134.5	Dephosphorylation of eIF2 $\alpha$ substrate	[24, 36, 37]
		US11	dsRNA sequestration/direct interaction	
		US3/UL13	Inhibition of activation	
		Kaposi's sarcoma herpes virus	vIRF2	Direct interaction and inhibition of phosphorylation
Epstein-Barr virus	Epstein-Barr virus	vIRF3 (LANA2)	Inhibition of PKR-induced apoptosis	[39]
		EBER RNAs	Decoy dsRNA	[40]
		SM	dsRNA sequestration/direct interaction	[41]
Poxviridae	Vaccinia virus	E3L [p25/p20]	dsRNA sequestration/direct interaction	[42, 43]
		K3L	Pseudosubstrate/direct interaction	[44]
Adenoviridae	Adenovirus	VAI RNA	Decoy dsRNA/pseudoactivator	[40]

Summary table of viral-encoded proteins that mediate evasion of PKR-mediated innate immune response. Viruses are classified by family and type of genome. ss: single-stranded; ds: double-stranded; NSp: nonstructural protein; VP: viral protein; TAR: transactivation responsive; vIRF3: viral IRF3-like protein; EBER: Epstein-Barr virus encoded RNAs; VAI: adenovirus-associated RNA-I.

interaction impossible. By contrast, through its putative RNA-binding domain, cytosolic NS1 can bind to PKR, probably mediating its inhibition directly [45]. Supporting this, mutations of this NS1 domain restore PKR activation and stress granule formation, impairing influenza replication [51]. In addition, NS1 can inhibit PKR indirectly by promoting the expression of vtRNA [52], a form of long noncoding RNA found in ribonucleoprotein particles called vaults (vt) [53]. For example, vtRNA2-1 directly inhibits PKR activity [54]. Influenza A infection or isolated expression of NS1 enhances the production of vtRNAs in human and mouse cell lines [52]. By contrast, NS1-deleted viruses cause the production of little vtRNA, and depletion of host vtRNAs leads to PKR activation, which reduces influenza replication [52].

PKR activation may not always be beneficial. Different strains of influenza vary in their ability to activate or evade the ISR [55, 56]. Some highly pathogenic avian influenza viruses (*e.g.* H5N1, H7N7 and H7N9) induce severe pulmonary inflammation owing to excessive production of IFN- $\beta$ , interleukin (IL)-6 and IL-8 [55]. This response appears to be mediated by PKR triggering a cascade that leads to phosphorylation of TRIM28, a regulator of many immunomodulatory genes. This raises the possibility that PKR-directed therapies might modify the immune response to highly pathogenic strains of influenza. The complex roles of PKR during inflammation are discussed in more detail later.

### Coronaviruses

Coronaviruses primarily cause respiratory and enteric disease. Many human coronaviruses including HCoV-229E, OC43, NL63 and HKU-1 cause only mild respiratory symptoms [57], but the zoonotic coronaviruses severe acute respiratory syndrome (SARS)-CoV-1, SARS-CoV-2 and Middle East respiratory syndrome (MERS)-CoV cause severe damage to the respiratory epithelium, which can be fatal [58]. The coronavirus genome encodes a number of structural proteins including spike, envelope, membrane, nucleoprotein and hemagglutinin-esterase [59]. The expression of virus-specific accessory proteins modulates activation of the ISR during infection [6], and being enveloped, coronaviruses impose significant stress on the ER, triggering PERK [21, 60–62]. Of note, although PERK is activated by expression of SARS-CoV-1 spike protein and accessory protein 3a [61, 62], rather than promoting an antiviral ISR, it has been suggested that the accompanying unfolded protein response (UPR) favours viral protein synthesis through upregulation of ER chaperones.

It was reported that during infection with SARS-CoV-1, both PKR and PERK are phosphorylated [63]. In a kinome-wide short interfering (si)RNA screen of 293T cells expressing the SARS-CoV receptor angiotensin-converting enzyme-2, both PKR and PERK were necessary for cells to restrict SARS-CoV-1 replication. However, others have found that, although activated, PKR was unable to reduce viral titres, suggesting that SARS-CoV-1 can evade PKR's antiviral activity [64]. It is unclear whether the net effect of PKR is to limit infection or to exacerbate deleterious inflammation *in vivo*. Recent reports describe how the  $\beta$ -coronavirus endoribonuclease nonstructural protein (nsp)15 interferes with host RNA sensing [22, 65, 66] (table 1). Mutations of nsp15 in mouse hepatitis virus and HCoV-229E enhance the phosphorylation of PKR and eIF2 $\alpha$  in infected macrophages [22, 65]. This increases the induction of type I IFN and reduces viral titres. Consistent with this, deletion of nsp15 renders the virus unable to replicate in cells except those lacking PKR. Conversely, MERS-CoV inhibits the formation of stress granules in a manner dependent upon another viral protein ns4a [23] (table 1). Ns4a contains a dsRNA-binding motif and can antagonise the activation of PKR, perhaps by preventing dsRNA sensing [23, 27].

Infectious bronchitis virus (IBV), a  $\gamma$ -coronavirus causing respiratory disease in birds, suppresses the ISR to enable viral protein synthesis [21]. *In vitro*, IBV infection of ISR-deficient eIF2 $\alpha^{AA}$  cells (in which a serine-51 to alanine mutation blocks phosphorylation) results in elevated viral replication confirming the importance of eIF2 $\alpha$  phosphorylation for antiviral activity. During IBV infection, activation of PKR is suppressed by viral protein nsp2 (table 1). However, nsp2 is only a partial inhibitor of PKR and so, although it prevents a marked reduction of protein synthesis, there is sufficient residual ISR signalling to permit PPP1R15A expression, which then supports protein synthesis and therefore IBV replication. Similarly, depletion of CHOP, which induces PPP1R15A, suppresses viral replication [60]. Disappointingly, the putative PPP1R15A inhibitor, salubrinal, failed to block IBV replication. However, we have shown that salubrinal has little effect on PPP1R15A and most likely antagonises the UPR through other mechanisms [67] (table 2).

### Other respiratory viruses

Infection with respiratory syncytial virus (RSV) triggers formation of stress granules [100]. These are maintained throughout the infection *via* PKR-mediated eIF2 $\alpha$  phosphorylation, and contain viral RNA. Similarly, human enterovirus (EV)-D68, which causes severe epidemic respiratory disease, triggers stress granules to form, although these are only transient and wane within 14 h [101]. This disaggregation of

TABLE 2 Integrated stress response (ISR)-modifying drugs

	Drug	Putative mode of action	Cautions	References
<b>ISR inhibitors</b>				
PERK	GSK2656157	Targets ATP binding site of PERK	Inhibits RIPK1	[68, 69]
	GSK2606414	Targets ATP binding site of PERK	Inhibits RIPK1 and PKR Weakly activates GCN2	[69, 70]
	4-PBA	Reduces ER stress by unclear mechanism	Affects all arms of the UPR	[71]
	TUDCA	Reduces ER stress by unclear mechanism	Affects all arms of the UPR	[71]
HRI	Aminopyrazolindane		Not commercially available	[72]
PKR	C16	Targets ATP binding site of PKR		[73]
	C22	Targets ATP binding site of PKR		[73]
GCN2	2-Aminopurine	Targets ATP binding site of PKR		[74]
	6D	Targets ATP binding site of GCN2	Not commercially available	[75]
eIF2β	6E (aka GCN2iA)	Targets ATP binding site of GCN2	Not commercially available	[75, 76]
	ISRIB	Stabilises eIF2β dimers	Cell lines can acquire ISRIB resistance mutations	[77–79]
	Dibenzoylmethane	Cells insensitive to p-eIF2α	Mechanism of action unclear	[80]
	Trazodone	Cells insensitive to p-eIF2α	Mechanism of action unclear	[80]
<b>ISR activators</b>				
PERK	CCT020312	Enhances PERK activation	Mechanism of action unclear	[81]
	Tunicamycin	Induces ER stress: inhibits N-glycosylation	Activates all arms of the UPR	[82]
	Bortezomib	Induces ER stress: inhibits the proteasome	Pleotropic effects of proteasome inhibition	[83]
	Montelukast	Enhances PERK signalling Mechanism unclear	Leukotriene receptor antagonist	[84]
HRI	BTdCPU			[85]
	cHAUs			[86]
PKR	Interferon poly I:C	Increases expression of PKR RNA mimetic	Pleotropic effects of interferon signalling Requires transfection to enter cell	[87] [88]
	BEPP	Mechanism of action unclear		[89]
GCN2	Histidinol	Inhibits histidyl-tRNA synthetase		[90]
	Tryptophanol	Inhibits tryptophan-tRNA synthetase		[91]
	Halofuginone	Inhibits prolyl-tRNA synthetase		[92]
PPP1R15A	L-asparaginase	Depletes extracellular asparagine		[93]
	Salubrinol	Putative PPP1R15 inhibitor	Concerns that effects may be PPP1R15 independent	[67, 94]
	Guanabenz	Putative PPP1R15 inhibitor	Concerns that effects may be PPP1R15 independent	[67, 95, 96]
	Sephrin1	Putative PPP1R15 inhibitor	Concerns that effects may be PPP1R15 independent	[67, 97, 98]
PPP1R15A and B	Jasplakinolide	Depletes G-actin required for PPP1R15 function	Pleotropic effects of actin stabilisation	[99]

PERK: protein kinase R (PKR)-like endoplasmic reticulum kinase; HRI: heme-regulated inhibitor; GCN: general control nondepressible; eIF: eukaryotic initiation factor; 4-PBA: 4-phenylbutyric acid; ER: endoplasmic reticulum; UPR: unfolded protein response; TUDCA: tauroursodeoxycholic acid; C16: CAS 608512-97-6 [6,8-dihydro-8-(1H-imidazol-5-ylmethylene)-7H-pyrrolo[2,3-g]benzothiazol-7-one]; C22: CAS 852547-30-9 [5-chloro-3-[[3,5-dichloro-4-hydroxyphenyl)methylidene]-2,3-dihydro-1H-indol-2-one]; ISRIB: integrated stress response inhibitor [trans-2-(4-chlorophenoxy)-N-(4-[2-(4-chlorophenoxy)acetamino]cyclohexyl)acetamide]; CCT020312 [6-bromo-3-[5-(4-bromo-phenyl)-1-(3-diethylamino-propionyl)-4,5-dihydro-1H-pyrazol-3-yl]-4-phenyl-1H-quinolin-2-one]; cHAUs [1-((1,4-trans)-4-aryloxycyclohexyl)-3-arylureas]; polyI:C: polyinosinic-polycytidylic acid; BEPP [1H-benzimidazole-1-ethanol, 2,3-dihydro-2-imino-α-(phenoxymethyl)-3-(phenylmethyl)-monohydrochloride].

stress granules correlates with increased virus production and, since PKR remains active, appears to be mediated by cleavage of the stress granule protein G3BP1 by virally encoded factors.

Porcine reproductive and respiratory syndrome virus (PRRSV, also known as beta-arterivirus suid 1) infects pigs and inflicts a great economic strain on the farming industry, but frequent antigenic variations have hampered the development of a vaccine [102–105]. PRRSV primarily infects alveolar macrophages where it inhibits PKR to enable its own replication [102]. However, early pharmacological activation of PKR with polyinosinic-polycytidylic acid can block viral replication (table 2). Interestingly, UPR signalling

by PERK and IRE1 $\alpha$  appears to be more important than PKR in promoting stress granule formation during PRRSV infection [104, 105], but PERK may also play a role in severe pneumonias caused by co-infection with PRRSV and bacteria, since the resulting eIF2 $\alpha$  phosphorylation inhibits IFN and tumour necrosis factor (TNF) production. Curiously, ISR activation during PRRSV infection might increase viral titres [103]. ATF4 is held in the cytosol by the viral proteins nsp2 and nsp3, preventing its migration to the nucleus. While impairing normal ISR signalling, this mislocalisation of ATF4 to sites of viral replication also benefits the virus through a poorly understood protein–RNA interaction.

### **Bacterial infection**

We identified a role for the ISR during infections with *Pseudomonas aeruginosa* [106]. This opportunistic bacterium, which can cause morbidity in patients with bronchiectasis, secretes several virulence factors, among which pyocyanin and AprA can trigger the UPR and ISR [106]. Exposure of human bronchial epithelial cells to such factors caused a rapid induction of PPP1R15A, which protected against *Pseudomonas*-induced cytotoxicity. Surprisingly, the upstream eIF2 $\alpha$  kinase involved proved to be the iron sensor, HRI. Iron availability is necessary for bacterial growth and its availability is limited by the host as a means to protect against bacterial infection [107]. It appears that in the airway, further depletion of iron from the microenvironment by *P. aeruginosa* is sensed by HRI to trigger a cytoprotective PPP1R15A response. Other studies have confirmed the phosphorylation of eIF2 $\alpha$  during the exposure of human lung epithelial cells to pyocyanin [108, 109]. It has also been suggested that this *P. aeruginosa*-mediated activation of the ISR triggers a cytoprotective autophagic response [108].

### **Interventions against infection**

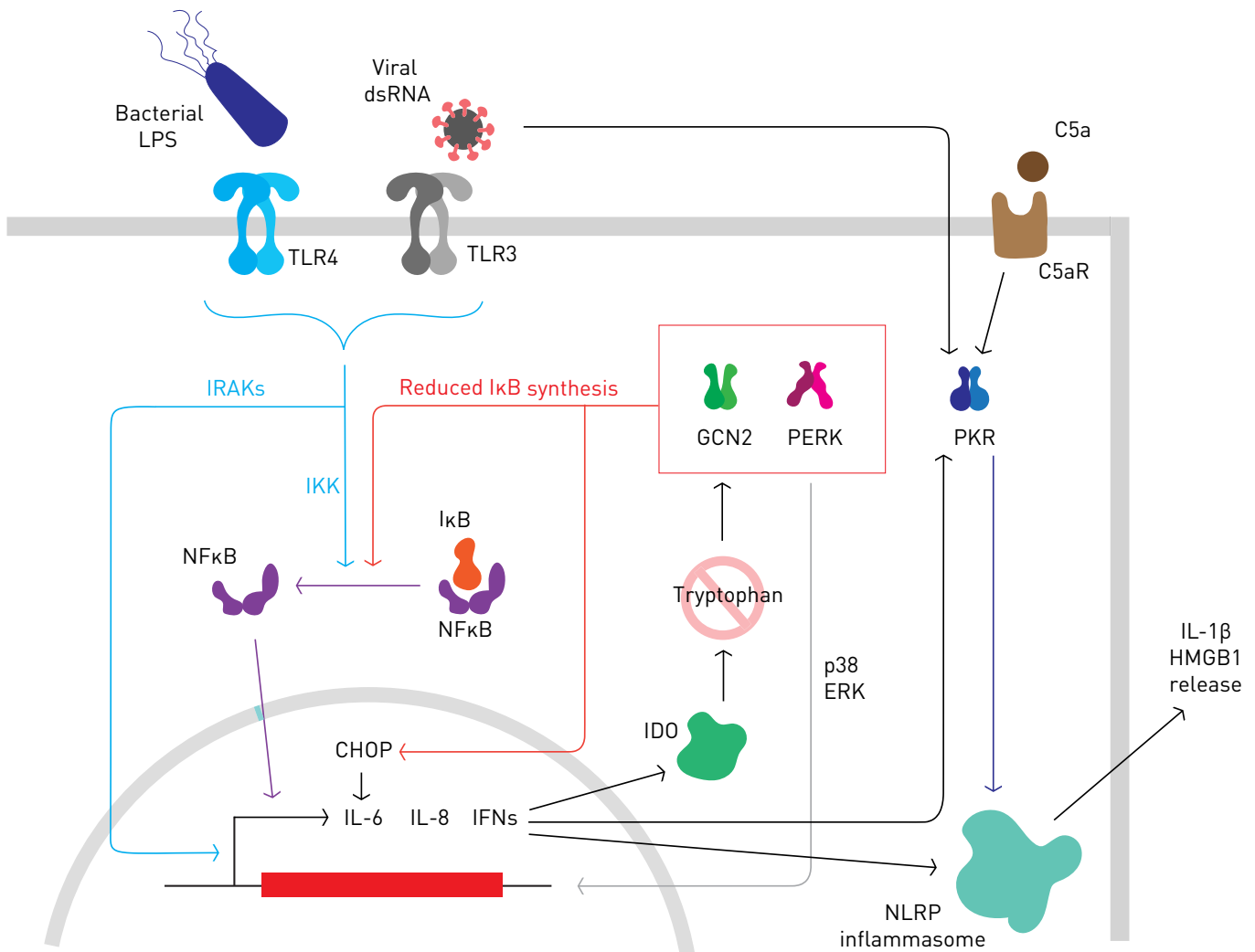
The innate antiviral role of the ISR makes it an attractive target for the development of antiviral therapies. In a repurposing-screen of drugs approved for human use, an anti-asthma medication, montelukast, was found to inhibit influenza A replication (table 2) [84]. It appeared to exert a mild antiviral effect by enhancing PERK signalling. Accordingly, high concentrations of guanabenz, a putative inhibitor of eIF2 $\alpha$  phosphatases, impaired viral protein synthesis [84] and a derivative of guanabenz called sephin1 blocked replication of RSV and EV-D68, but not influenza A in human cells [110]. However, the results could not be replicated *in vivo* owing to the toxicity of the high concentrations of sephin1 required, and there remains doubt as to whether the effects of guanabenz or sephin1 can be attributed to inhibition of PPP1R15A (table 2) [67, 97].

A challenge to the development of vaccines that impart cell-mediated immunity in the lung is the short lifespan of airway-resident memory T (T<sub>RM</sub>)-cells [111]. Unlike the long-lived T<sub>RM</sub> cells of other tissues, airway T<sub>RM</sub> cells are almost completely lost through apoptosis by 180 days following exposure to influenza or Sendai viruses [111]. For durable immunity, it is therefore important to understand what accounts for this short survival. Transcriptomic analysis revealed GCN2-dependent activation of the ISR as a unique signature in airway-resident T<sub>RM</sub> [111]. This appears to be a function of the nutrient-poor microenvironment of the airway but is reversible, since airway T<sub>RM</sub> cells cultured *ex vivo* in amino acid sufficient conditions are rescued. In theory, antagonism of GCN2 might prolong airway T<sub>RM</sub> cells, but the consequences of this for immunopathology and inflammation are unclear. The abnormal pulmonary vascular phenotypes of GCN2-deficient individuals, may serve as a warning in this regard (discussed later).

### **Inflammation**

The airway epithelium has a role in regulating the immune microenvironment and the ISR seems to be important in this. Toll-like receptors (TLRs) enable bronchial epithelial cells to sense pathogen associated molecular patterns (PAMPS) such lipopolysaccharide (LPS), a component of bacterial cell walls (figure 2). TLR activation induces PKR *via* IFNs as part of the inflammatory response, but other ISR factors including ATF3 and PPP1R15A are upregulated through less well understood mechanisms [112].

Early work showed that intense PERK activation can activate NF $\kappa$ B *in vitro* by inhibiting the synthesis of I $\kappa$ B, a short-lived negative-regulator of NF $\kappa$ B [113]. However, it is unclear if sufficiently high levels of phosphorylated eIF2 $\alpha$  are achieved *in vivo* to induce this mechanism in response to ER stress. Nevertheless, PERK activation seems to enhance inflammatory signalling *in vivo* in response to PAMPS. For example, in bronchial epithelial cells treated LPS, PERK activation increases proinflammatory IL-6 and IL-8 production *via* increased p38 and ERK signalling [114]. Furthermore, CHOP can dimerise with other members of the C/EBP transcription factor family to regulate the transcription of cytokines including IL-6 [115, 116]. Translational regulation of I $\kappa$ B $\alpha$  might nonetheless be important in activated macrophages and dendritic cells, where induction of indoleamine 2,3-dioxygenase, an enzyme that metabolises tryptophan, can trigger GCN2 and the ISR *via* amino acid sensing [117]. This enhances the secretion of IL-6 in response to LPS and is mediated, at least in part, by inhibition of I $\kappa$ B synthesis (figure 2).



**FIGURE 2** The integrated stress response and inflammation. Pathogen-associated molecular patterns (PAMPs) recognised by Toll-like receptors (TLRs) can trigger the innate immune response. Phosphorylation of I $\kappa$ B by IKK promotes its destruction and releases NF $\kappa$ B to transactivate pro-inflammatory genes including interleukin (IL)-6, IL-8 and interferons (IFNs). Induction of indoleamine 2,3-dioxygenase (IDO) depletes cells of tryptophan (at least activated macrophages and dendritic cells) to activate general control nondepressible (GCN)2. Activation of GCN2 or protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) can trigger NF $\kappa$ B signalling by reducing the synthesis of I $\kappa$ B, while CHOP increases IL-6 expression directly. Some evidence suggests PERK-mediated p38/ERK signalling might also contribute to inflammatory gene expression. PKR is induced by IFN and further activated if viral double-stranded (ds)RNA is present in the cytoplasm. Inflammatory mediators including C5a fragment can activate PKR, e.g. via C5a receptor (C5aR) signalling. PKR contributes to inflammation by stimulating the NLRP3 inflammasome to generate pro-inflammatory mediators including IL-1 $\beta$  and high mobility group box (HMGB)1.

LPS administration increases PKR phosphorylation in mouse lungs [118, 119]. In murine alveolar macrophages, PKR is critical in TLR-stimulated cytokine secretion and appears to contribute to lung damage in the context of sepsis [120]. A variety of PAMPs transactivate NOD-like receptor (NLR) inflammasomes [121]. During bacterial infection, complement activation generates C5a fragment, which is chemotactic for neutrophils and enhances their capacity to phagocyte and kill bacteria [122]. One function of C5a appears to be the stimulation of PKR, which activates the NLRP3 inflammasome through direct interaction, promoting the release of HMGB1 (high mobility group box 1) (figure 2) [123, 124]. Conversely, HMGB1, which is a DNA binding protein with inflammatory cytokine activity, can itself activate PKR during macrophage M1 polarisation in acute lung injury, suggesting the possibility of a positive feedback loop [125]. Inhibition of PKR with C16 opposes both NLRP3 activation and HMGB1 release from LPS-challenged macrophages (table 2). Consequently, administration of C16 to mice prior to LPS challenge, inhibits PKR activation and so reduces the concentration of pro-inflammatory cytokines in lung lavage, limiting pulmonary damage [118, 119].

Barotrauma caused by mechanical ventilation can also induce pulmonary inflammation and acute lung injury [126]. In cell models of alveolar epithelial stretch, or if the lung is hyperinflated mechanically *in*

*in vivo*, PERK-dependent ISR signalling is triggered, possibly *via* stretch-induced release of calcium from the ER. This may mediate some of the toxicity of barotrauma, since pharmacological inhibition of PERK limits alveolar permeability and production of IL-8 during acute inflammation induced by cyclic strain [126, 127].

In the bronchoalveolar lavage fluid of asthmatic patients the levels of CHOP and the ER chaperone BiP are higher than healthy controls [128]. Ovalbumin (OVA) and LPS administration produces an asthma-like state in murine models in which lungs show evidence of ER stress with induction of the same proteins as seen in patients. OVA/LPS-sensitised mice, treated with 4-PBA, which ameliorates ER stress *via* poorly understood mechanisms, show less I $\kappa$ B degradation therefore reduced NF $\kappa$ B signalling (table 2). This also results in attenuated inflammatory signals such as IL-10 expression and reduces infiltration of neutrophils and dendritic cells into the lung [128, 129].

In other situations, the ISR and pro-inflammatory signalling pathways appear to be antagonistic, suggesting some of these effects might be tissue specific. For example, in the gut, eIF2 $\alpha$  phosphorylation caused by GCN2 during acute amino acid starvation suppresses intestinal inflammation [130]. Conversely, TLR4 activation appears able to stimulate eIF2 $\beta$  to antagonise the inhibition of translation caused by eIF2 $\alpha$  phosphorylation, thus suppressing the translation of ATF4 and CHOP [131, 132].

### Smoke and exhaust fumes

Inhaled pollutants can trigger the UPR and ISR [133–138]. The resulting cytotoxicity is thought to impair epithelial integrity and lead to COPD [139]. The particulate matter found in diesel exhaust induces ER stress in mouse lung tissue [140] and increases the expression of CHOP and PPP1R15A in human airway epithelium [137]. This response appears to be accentuated in patients with COPD [138] and is even more marked if cells are co-exposed to bacterial components of nontypeable *Haemophilus influenzae* [141].

Lungs from patients with COPD have elevated levels of ISR markers [142]. Rats and mice chronically exposed to cigarette smoke also express increased levels of ISR markers, although there appears to be species-dependent differences with few genes modulated in a concordant manner between mice exposed to smoke and the lungs of humans with COPD [140, 143, 144]. It has long been known that primary bronchial epithelial cells exposed to cigarette smoke display a transient PERK-dependent phosphorylation of eIF2 $\alpha$  followed by induction of ATF4 and PPP1R15A [134]. Indeed, PERK-dependent eIF2 $\alpha$  phosphorylation is a consistent feature of cells exposed to cigarette smoke or smoke extract and at least some of the cytotoxicity is mediated by ER stress-induced cell death [135, 145]. For example, the aldehyde acrolein found in smoke induces ER stress-mediated cell death in A549 cells through multiple pathways including perturbed ER calcium homeostasis [146]. Acrolein is highly oxidising, as are numerous components of smoke, and this impairs the function of many macromolecules in cultured airway epithelial cells [147, 148] and in the lungs of humans and mice [149, 150]. Oxidative stress appears to be a key trigger for ISR activation by smoke, since treatment with the antioxidant N-acetylcysteine attenuates ISR induction [133, 135, 136, 151]. Cultured cells or mice treated with acrolein induce PPP1R15A, which appears to be a toxic phenomenon since *Ppp1r15a*<sup>-/-</sup> mice are more resistant to cell death and have better preserved lung architectures [152]. This is reminiscent of the increased tolerance of *Ppp1r15a*<sup>-/-</sup> mice to ER stress [14]. Interestingly, salubrinal, which increases eIF2 $\alpha$  phosphorylation through poorly understood mechanisms also protects cells from smoke (table 2) [153].

As cigarette smoke is a potent oxidative stress, induction of the ISR is likely to be a protective response because ATF4 induces many antioxidant genes [1, 9]. It has been suggested that NRF2, another regulator of the antioxidant response, and ATF4 heterodimerise to regulate the expression of target genes [154, 155]. NRF2 can also induce ATF4 to amplify this response [156, 157]. It should be noted that although early reports suggested that PERK could activate NRF2 directly [158, 159], it was subsequently shown that PERK-dependent antioxidant gene expression is entirely determined by phosphorylation of eIF2 $\alpha$  [160]. Interestingly, differences in the vigour of these antioxidant responses might contribute to individual differences in the susceptibility to smoke, since an ATF4 target gene signature distinguishes the airway of smokers with COPD compared to smokers without COPD [161].

### Pulmonary hypertension

Pulmonary arterial hypertension (PAH) is a rare, fatal condition primarily affecting young adults. It involves vascular remodelling causing increased pulmonary vascular resistance and right-sided heart failure [162]. In 70% of familial cases and 20% of sporadic cases, heterozygous germ line mutations are identified in the gene encoding the bone morphogenetic protein type 2 receptor (*BMPR2*). However, these mutations are variably penetrant, suggesting that additional modifying factors exist. Two subtypes of PAH, pulmonary veno-occlusive disease (PVOD) and pulmonary capillary haemangiomatosis (PCH) were recently shown to be caused by biallelic mutations of *EIF2AK4*, which encodes GCN2 (figure 3) [163–165].



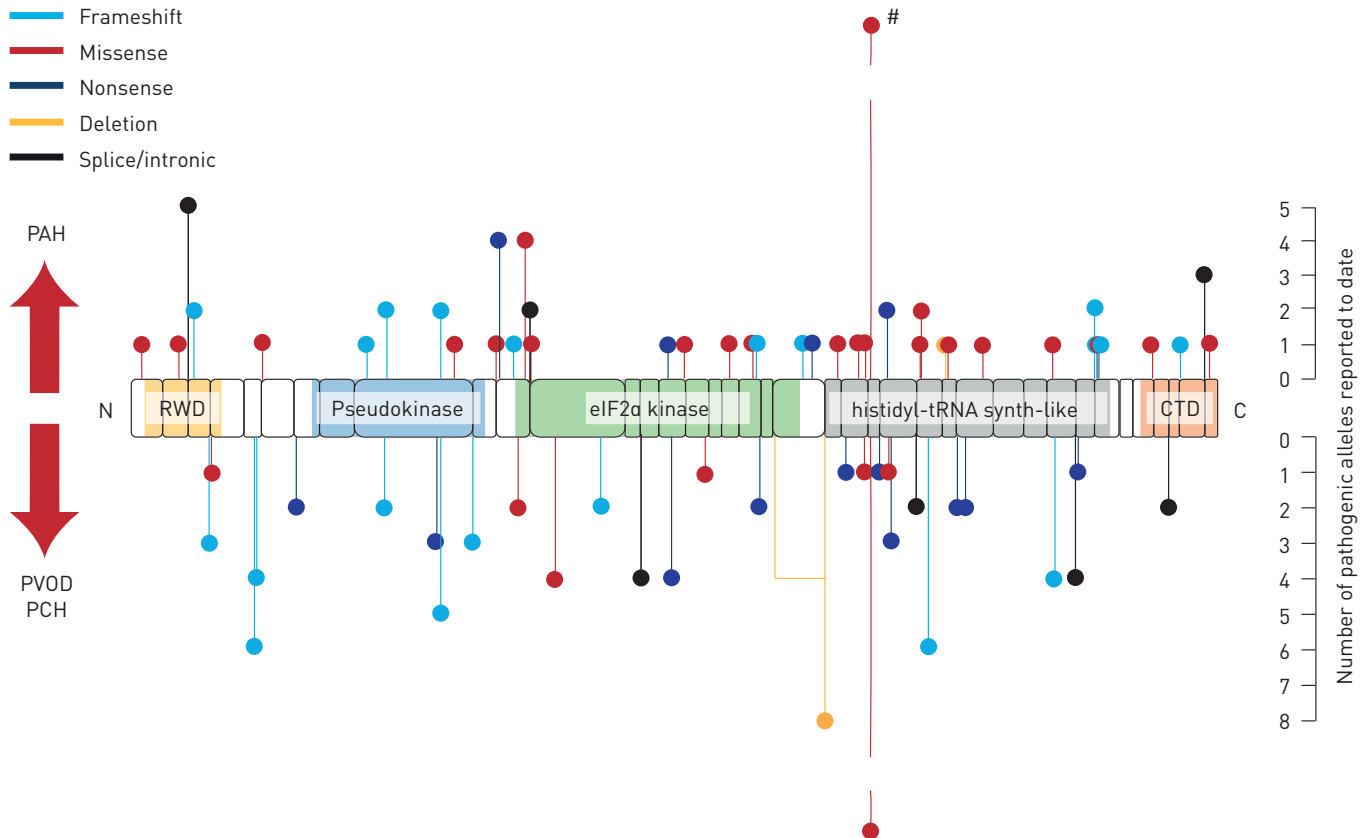


FIGURE 3 Variants of the *EIF2AK4* associated with pulmonary vascular disease. Cartoon representation of the general control nondepressible (GCN)2 protein and its domains; boxes correspond to the 39 exons in *EIF2AK4*. Domains are highlighted: RWD (RING-finger proteins, WD repeat-containing proteins, yeast DEAD-like helicase), pseudokinase, eukaryotic initiation factor (eIF)2 $\alpha$  kinase, histidyl-tRNA synthetase-like and CTD (carboxy-terminal domain). Predicted pathogenic variants are shown as lollipops: above the protein are likely pathogenic variants associated with pulmonary arterial hypertension (PAH), below are likely pathogenic variants associated with pulmonary veno-occlusive disease (PVOD) and/or pulmonary capillary haemangiomas (PCH). The lollipop length indicates the approximate number of such alleles reported in the literature allowing for incomplete reporting. Note, c.3344C>T [p.P1115L] in exon 23 in the histidyl-tRNA synthetase-like domain has been reported in five families affected by PAH or PVOD (marked as #). Potentially, 48 alleles have been described, but this may be confounded by overlaps between published reports.

The PVOD/PCH subtypes have an even worse prognosis than classical PAH and currently have no effective treatments apart from lung transplantation [166]. In families affected by PVOD, mutant alleles of *EIF2AK4* segregate with the disease in a recessive manner, while in the general population such null alleles are vanishingly rare [163]. Remarkably, when histologically proven nonfamilial cases of PVOD were examined, 25% were also found to have pathogenic mutations of *EIF2AK4* [163]. Subsequently, biallelic mutations of *EIF2AK4* have been observed in some patients with classical PAH, suggesting that GCN2 may play a broader role in pulmonary vascular disease [167–171]. Individuals with PAH and biallelic mutations of *EIF2AK4* tend to be younger (aged <50 years) with lower transfer coefficients of the lung for carbon monoxide (<50%) and normal spirometry, leading some to suggest that *EIF2AK4* gene testing may be useful in the diagnosis and management of PAH [170, 172].

GCN2 is normally detectable in pulmonary vessel smooth muscle, interstitial tissue and macrophages, but is absent from individuals homozygous for loss-of-function alleles of *EIF2AK4* [163]. Interestingly, despite the loss of this kinase, lung tissue from patients with PVOD showed increased immunostaining for the downstream ISR targets CHOP and heme oxygenase (HO)-1, which are said to be absent from normal lung and the lungs of patients with classical PAH [173]. CHOP was increased in endothelial cells while HO-1 was found in the capillaries of haemangiomas foci. The mechanism by which loss of GCN2 enhances CHOP expression requires elucidation.

There is a suggestion of genotype–phenotype correlation in PVOD [174]. Individuals with PVOD due to mutations of *EIF2AK4* appear to develop more severe intimal fibrosis and less severe medial hypertrophy of the pulmonary arteries. They show greater smooth muscle hyperplasia of interlobular septal veins compared with individuals without the mutation and have more foci of PCH, rather than the more diffuse haemangiomas changes seen in mutation-negative cases.

Remarkably, while GCN2 protein is absent in those with biallelic *EIF2AK4* mutations, even patients with PVOD and wildtype *EIF2AK4* show reduced GCN2 expression. Moreover, patients with classical PAH can have reduced GCN2 protein expression suggesting that GCN2 might be relevant to many forms of pulmonary hypertension. Intriguingly, mitomycin C, which is a DNA alkylating agent that can induce PVOD in humans and rats [175], has been shown to reduce the expression of GCN2 in lung tissue [175]. This is associated with reduced phosphorylation of SMAD1/5/8, downstream mediators of BMP signalling, while depletion of GCN2 in pulmonary arterial endothelial cells also reduces the phosphorylation of SMAD1/5/9 in rats and human pulmonary arterial endothelial cells [173]. However, it has yet to be shown that restoration of GCN2 expression can rescue PVOD caused by mitomycin C.

The mechanistic links between loss of GCN2 and defects of the pulmonary vasculature are unclear, although we demonstrated recently that GCN2 expression can inhibit BMP signalling during development in *Drosophila*, in part by regulating the synthesis of components of the BMP signalling pathway [176]. Conversely, loss of GCN2 appears to impair the more complex BMP signalling found in mammals [173]. This was demonstrated both in *Eif2ak4*<sup>-/-</sup> rats and in pulmonary artery endothelial cells depleted of GCN2 by siRNA. Such an interaction between the ISR and BMP signalling might contribute to the incomplete penetrance of pathogenic *BMPR2* mutations. This is supported by observations in a family with autosomal dominant PAH caused by a *BMPR2* mutation, in which all living family members with diagnosed PAH also carried an *EIF2AK4* mutation [167].

The relationship between the ISR and cell survival may be relevant to its role in pulmonary hypertension. Dysregulated pulmonary artery endothelial cell proliferation and apoptosis are believed to be important factors in the development of PAH [177]. It has been proposed that the initial apoptosis of pulmonary endothelial cells is followed by hyperproliferation of apoptosis-resistant cells with increased expression of anti-apoptotic factors including survivin [178]. Survivin is highly expressed in the pulmonary arteries of patients with PAH and in the monocrotaline-induced PAH rat model [179]. When a dominant negative mutant of survivin was expressed in monocrotaline-treated rats, established PAH was reversed resulting in prolonged survival [179]. In human pulmonary arterial endothelial cells, depletion of GCN2 by siRNA enhanced the proliferation and increased the expression of survivin, while inhibition of GCN2 with compound 2662034 also increased endothelial cell proliferation (table 2) [173]. This effect of GCN2 inhibition could be antagonised by exogenous BMP-9, which is known to reverse PAH in animal models [173, 180].

Inflammation is a recognised feature of PAH with increased circulating levels of IL-6, IL-8, IL-10 and IL-12p70 correlating with worse survival [181]. Inflammatory signalling appears to modify the penetrance of pathogenic alleles of *BMPR2* in humans and the TLR4 ligand LPS worsens PAH in *Bmpr2* mutant mice [182]. The ISR may play a role in the sensitisation of BMP-signalling-deficient animals to inflammation [183]. When pulmonary artery endothelial cells are treated with TNF, they produce the potent pro-inflammatory chemokine granulocyte-macrophage colony-stimulating factor (GM-CSF). Treatment with BMP2 suppresses GM-CSF production and so deficiency of *BMPR2* results in enhanced TNF-mediated GM-CSF secretion. It appears that loss of *BMPR2* activates p38<sup>MAPK</sup> to increase expression of PPP1R15A. The resultant dephosphorylation of eIF2 $\alpha$  enhances translation of *GM-CSF* mRNA [183]. This suggests that ISR signalling normally limits inflammatory cytokine production in the pulmonary vasculature, and might explain why loss of an ISR kinase worsens PAH through enhanced translation of pro-inflammatory cytokines.

It appears that the ISR plays a role both to limit pulmonary endothelial cell proliferation and inflammatory cytokine production, while sharing a mutually regulating relationship with BMP signalling. It is therefore possible that ISR-directed therapies might have value in more common forms of PAH, not only in rarer genetic varieties.

### Pulmonary fibrosis

The aetiology of pulmonary fibrosis is complex and is likely to involve many different triggers, including the ageing process. Prolonged inflammation or the expression of misfolded mutants of surfactant proteins can also lead to pulmonary fibrosis [2]. It has been noted that treating mice with tunicamycin, a toxin that causes ER stress, promotes lung fibrosis and causes mitochondrial dysfunction in primary type II alveolar epithelial cells (table 2) [82]. This is noteworthy because the type II alveolar epithelial cells from patients with idiopathic pulmonary fibrosis (IPF) also accumulate dysmorphic and dysfunctional mitochondria, as do other ageing animals. The integrity of a cell's mitochondria is maintained through the action of PINK1, the expression of which falls intriguingly with age and during ER stress. It is also downregulated in the lungs of patients with pulmonary fibrosis. PINK1-deficient mice are more susceptible to lung fibrosis induced by intratracheal instillation of bleomycin [82]. This drug drives epithelial to mesenchymal transition, fibroblast proliferation and subsequent extracellular matrix deposition in the lungs [184]. The

repression of PINK1 seen during ER stress appears to be dependent upon ATF3, an ISR transcription factor, which binds directly to the PINK1 promoter [185]. Although circumstantial, it is interesting that the lungs of individuals with IPF show higher levels of ATF3 compared to age-matched controls [82]. In some stressful conditions, ATF3 is thought to contribute to CHOP expression [186]. CHOP, is also elevated in the lungs of patients with IPF and bleomycin-exposed mice, especially in areas affected by fibrosis that notably are also markedly hypoxic [187]. CHOP seems to play a crucial role in the development of these lesions since its deletion protects mice from fibrosis. Hypoxia can induce CHOP *via* ER stress and the UPR. Moreover, hypoxia (inspiratory oxygen fraction 14%) can worsen bleomycin-induced fibrosis, yet this is abrogated in *Chop*<sup>-/-</sup> mice. CHOP expression may have broader relevance in fibrosis, since CHOP is induced by silica, probably *via* ER stress, and inhibition of its induction by 4BPA ameliorates silicosis in rats [188, 189].

### Thoracic malignancy

Having evolved from the ancestral General Amino Acid Control pathway in yeast [190], the ISR in mammals plays a central role in regulating the availability of amino acids [9]. Cancers have a high demand for amino acids, both for new protein synthesis and to provide thiols for production of the antioxidant glutathione. As a tumour outgrows its vascular bed, both amino deprivation and ER stress trigger a cytoprotective ISR *via* GCN2 and PERK, respectively, elevating ATF4 and CHOP in its hypoxic core [191]. Consequently, the ability of cancer cells to phosphorylate eIF2 $\alpha$  is critical for the growth of large solid tumours [191]. In hypoxic tumours, the ER generates increased levels of reactive oxygen species in part through CHOP-mediated induction of the ER oxidoreductase ERO1 $\alpha$  [14, 192]. ERO1 $\alpha$  expression enhances the capacity of a cell to generate disulphide bonds and so aids protein synthesis and tumour growth. Indeed, high ERO1 $\alpha$  expression correlates with worse cancer prognosis and increased cancer cell growth [193, 194]. But excess generation of reactive oxygen species can be cytotoxic, and so one of the functions of the ISR is to defend against this.

In the lung, activated phospho-GCN2 is observed in carcinoma tissue, but less so normal tissues [195]. This is necessary for the expression of ATF4-target genes in lung cancer that promote the synthesis, import and mobilisation of amino acids. Increased ATF4-dependent production of glutathione may account for much of the resistance to cisplatin chemotherapy observed in multiple cell lines including those derived from lung cancers [196, 197]. PERK-mediated resistance to oxidative stress is also thought to be important in the resistance to radiotherapy [198, 199]. It has also been shown that drug-resistant KRAS-mutant lung cancers have elevated levels of ATF4 and are more susceptible than nonresistant lung cancer cells to inhibition of PERK [200]. PERK inhibition restores their sensitivity to the MEK inhibitor trametinib and raises to possibility of ISR-directed personalised therapies [200].

Analysis of The Cancer Genome Atlas reveals that advanced lung adenocarcinomas often exhibit an ATF4 target gene signature [201]. Correspondingly, in lung adenocarcinoma cell lines the drug ISRIB (integrated stress response inhibitor [trans-2-(4-chlorophenoxy)-N-(4-(2-(4-chlorophenoxy)acetyl amino)cyclohexyl)acetamide]), which renders cells insensitive to eIF2 $\alpha$  phosphorylation, blocked ATF4 expression and reduced both cancer cell proliferation and migration in amino acid deficient conditions (table 2) [201]. One of the mechanisms by which the ISR adapts cells to nutrient-poor environments is through enhanced import of amino acids. This too may produce specific vulnerabilities, since in nonsmall cell lung cancers the expression of the Large neutral Amino acid Transporter LAT1 *in vivo* correlates with poor survival [202], while *in vitro* silencing of LAT1 in lung adenocarcinoma A549 cells can impair cancer cell growth [203]. The availability of amino acids can also be increased *via* activation of macro-autophagy, which liberates amino acids from long-lived proteins and damaged organelles. Accordingly, in hypoxic areas of tumour xenografts, increased expression of autophagy factors such as LC3 is observed, which is at least partially mediated by the GCN2/PERK-ATF4-CHOP axis [195, 199, 204].

Nutrient and oxygen deficiency both cause tissues to release signals that induce angiogenesis. It is well known that hypoxia leads to the stabilisation of the transcription factor HIF1 $\alpha$  to promote angiogenesis [205]. However, the ISR also contributes to angiogenesis because ATF4 directly upregulates vascular endothelial growth factor (VEGF)A and downregulates inhibitors of angiogenesis [206–208]. Consequently, depletion of PERK prevents VEGFA secretion in response to glucose deprivation independent of HIF1 $\alpha$ , while inhibition of PERK reduces xenograft vascularity and perfusion in mice [208]. The PERK inhibitor GSK2656157 reduces cancer growth *in vivo*, most likely *via* reducing angiogenesis and impairing amino-acid metabolism (table 2) [209].

Cancers are often associated with local immunosuppression [210]. Expression of the inflammatory ISR kinase PKR is reduced in many non-small cell lung cancers with lower expression correlating with poorer prognosis [211]. Conversely, pharmacological activation of PKR or its overexpression kills lung cancer cells [211, 212]. Recently, it was discovered that many lung cancer cell lines are dependent on ADAR1, an RNA

deaminase [213]. Loss of ADAR1 leads to PKR activation and cell death, while loss of PKR rescues the lethality of *ADAR1* deletion. This suggests that PKR detects an RNA signal in lung cancer cells that is suppressed by ADAR1. It is unclear why lung cancer cells behave in this manner, but this raises the possibility of targeted therapies, for example ADAR1 inhibition, to trigger PKR-mediated lung cancer cell killing [213].

It is often stated that CHOP triggers cell death, but the reality is more nuanced. For example, expression of CHOP is an independent predictor of a poor prognosis in malignant pleural mesothelioma, suggesting CHOP expression is associated with increased tumour growth [214]. However, in chemoresistant mesothelioma, activation of the PERK–ATF4–CHOP pathway by bortezomib reverses the chemoresistance (table 2) [215]. Very few target genes of CHOP are prodeath factors, but rather are involved in oxidative protein folding, protein secretion and autophagy [14, 199, 216]. In fact, the recovery of protein synthesis and oxidative protein folding mediated by CHOP, may explain much of CHOP's relationship with ER stress induced cell death [14, 216]. The recovery of translation at later time points during the ISR is mediated by the CHOP target PPP1R15A, and consequently *Ppp1r15a*<sup>-/-</sup> mice are resistant to ER stress induced tissue damage, as they are protected from the toxicity of excessive protein synthesis [14]. In a murine model of medulloblastoma, loss of PPP1R15A increased phospho-eIF2 $\alpha$  and promoted tumour growth, invasiveness and angiogenesis perhaps *via* the increased expression of VEGFA [217]. Although mutations of CHOP or PPP1R15A are uncommon in human cancers, their expression might be suppressed by other means. For example, in breast carcinoma, PERK-induced expression of microRNA mir211 promotes tumour cell survival in part by repressing CHOP expression [118]. Loss of PPP1R15A is also observed in the more aggressive sarcomatoid subtype of malignant pleural mesothelioma, and so low PPP1R15A expression correlates with a worse prognosis [218]. The mechanism by which PPP1R15A expression is suppressed in sarcomatoid mesothelioma remains unclear.

### Concluding remarks

The ISR is central in responding to infection, oxidative stress and nutrient deprivation. Studies of human lung disease have revealed its importance in normal pulmonary physiology, perhaps relating to the uniquely oxidising and nutrient poor environment of the airway. The role of the ISR in regulating cell survival and inflammation make it an attractive target for the development of immunomodulators and anticancer therapies alike. More recent discoveries of the importance of GCN2 in maintaining pulmonary vascular health also raise the possibility that new treatments for pulmonary hypertension might be developed by targeting the ISR.

Conflict of interest: G. Emanuelli has nothing to disclose. N. Nassehzadeh-Tabriz has nothing to disclose. N.W. Morrell has nothing to disclose. S.J. Marciniak has nothing to disclose.

Support statement: Funding was received from the Alpha-1 foundation (pC ID 614939), the BRITISH Lung Foundation, an Research Councils UK Medical Research Council (MCMB MR/R009120/1). Funding information for this article has been deposited with the Crossref Funder Registry.

### References

- 1 van 't Wout EFA, Hiemstra PS, Marciniak SJ. The integrated stress response in lung disease. *Am J Respir Cell Mol Biol* 2014; 50: 1005–1009.
- 2 Dickens JA, Malzer E, Chambers JE, *et al.* Pulmonary endoplasmic reticulum stress – scars, smoke, and suffocation. *FEBS J* 2019; 286: 322–341.
- 3 Pakos-Zebrucka K, Koryga I, Mnich K, *et al.* The integrated stress response. *EMBO Rep* 2016; 17: 1374–1395.
- 4 Dalton LE, Healey E, Irving J, *et al.* Phosphoproteins in stress-induced disease. In: Shenolikar S, ed. *Protein Phosphorylation in Health and Disease*. 1st Edn. Elsevier, 2012. Vol. 106: 109–221.
- 5 Dauber B, Wolff T. Activation of the antiviral kinase PKR and viral countermeasures. *Viruses* 2009; 1: 523–544.
- 6 Chambers JE, Marciniak SJ. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 2. Protein misfolding and ER stress. *Am J Physiol Cell Physiol* 2014; 307: C657–C670.
- 7 Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 1999; 397: 271–274.
- 8 Suragani RNVS, Zachariah RS, Velazquez JG, *et al.* Heme-regulated eIF2 $\alpha$  kinase activated Atf4 signaling pathway in oxidative stress and erythropoiesis. *Blood* 2012; 119: 5276–5284.
- 9 Harding HP, Zhang Y, Zeng H, *et al.* An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell* 2003; 11: 619–633.
- 10 Masson GR. Towards a model of GCN2 activation. *Biochem Soc Trans* 2019; 47: 1481–1488.
- 11 Lu PD, Harding HP, Ron D. Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. *J Cell Biol* 2004; 167: 27–33.
- 12 Young SK, Wek RC. Upstream open reading frames differentially regulate gene-specific translation in the integrated stress response. *J Biol Chem* 2016; 291: 16927–16935.
- 13 Novoa I, Zeng H, Harding HP, *et al.* Feedback inhibition of the unfolded protein response by GADD34-mediated dephosphorylation of eIF2 $\alpha$ . *J Cell Biol* 2001; 153: 1011–1022.

- 14 Marciniak SJ, Yun CY, Oyadomari S, *et al.* CHOP induces death by promoting protein synthesis and oxidation in the stressed endoplasmic reticulum. *Genes Dev* 2004; 18: 3066–3077.
- 15 Scheuner D, Patel R, Wang F, *et al.* Double-stranded RNA-dependent protein kinase phosphorylation of the  $\alpha$ -subunit of eukaryotic translation initiation factor 2 mediates apoptosis. *J Biol Chem* 2006; 281: 21458–21468.
- 16 Samuel CE. Antiviral actions of interferons. *Clin Microbiol Rev* 2001; 14: 778–809.
- 17 He B. Viruses, endoplasmic reticulum stress, and interferon responses. *Cell Death Differ* 2006; 13: 393–403.
- 18 McInerney GM, Kedersha NL, Kaufman RJ, *et al.* Importance of eIF2 $\alpha$  phosphorylation and stress granule assembly in alphavirus translation regulation. *Mol Biol Cell* 2005; 16: 3753–3763.
- 19 McCormick C, Khapersky DA. Translation inhibition and stress granules in the antiviral immune response. *Nat Rev Immunol* 2017; 17: 647–660.
- 20 Garcia MA, Gil J, Ventoso I, *et al.* Impact of protein kinase PKR in cell biology: from antiviral to antiproliferative action. *Microbiol Mol Biol Rev* 2006; 70: 1032–1060.
- 21 Wang X, Liao Y, Yap PL, *et al.* Inhibition of protein kinase R activation and upregulation of GADD34 expression play a synergistic role in facilitating coronavirus replication by maintaining *de novo* protein synthesis in virus-infected cells. *J Virol* 2009; 83: 12462–12472.
- 22 Deng X, Hackbart M, Mettelman RC, *et al.* Coronavirus nonstructural protein 15 mediates evasion of dsRNA sensors and limits apoptosis in macrophages. *Proc Natl Acad Sci USA* 2017; 114: E4251–E4260.
- 23 Rabouw HH, Langereis MA, Knaap RC, *et al.* Middle East Respiratory Coronavirus Accessory Protein 4a Inhibits PKR-Mediated Antiviral Stress Responses. *PLoS Pathog* 2016; 12: E1005982.
- 24 Pennisi R, Musarra-Pizzo M, Lei Z, *et al.* VHS, US3 and UL13 viral tegument proteins are required for herpes simplex virus-induced modification of protein kinase R. *Sci Rep* 2020; 10: 5580.
- 25 Goodman DE, Pretto CD, Krepostman TA, *et al.* Enhanced replication of mouse adenovirus type 1 following virus-induced degradation of protein kinase R (PKR). *mBio* 2019; 10: e00668-19.
- 26 Lu Y, Wambach M, Katze MG, *et al.* Binding of the influenza virus NS1 protein to double-stranded RNA inhibits the activation of the protein kinase that phosphorylates the eIF-2 translation initiation factor. *Virology* 1995; 214: 222–228.
- 27 Nakagawa K, Narayanan K, Wada M, *et al.* Inhibition of Stress Granule Formation by Middle East Respiratory Syndrome Coronavirus 4a Accessory Protein Facilitates Viral Translation, Leading to Efficient Virus Replication. *J Virol* 2018; 92: e00902-18.
- 28 Habjan M, Pichlmair A, Elliott RM, *et al.* NSs protein of rift valley fever virus induces the specific degradation of the double-stranded RNA-dependent protein kinase. *J Virol* 2009; 83: 4365–4375.
- 29 Schümann M, Gantke T, Mühlberger E. Ebola virus VP35 antagonizes PKR activity through its C-terminal interferon inhibitory domain. *J Virol* 2009; 83: 8993–8997.
- 30 Brand SR, Kobayashi R, Mathews MB. The Tat protein of human immunodeficiency virus type 1 is a substrate and inhibitor of the interferon-induced, virally activated protein kinase, PKR. *J Biol Chem* 1997; 272: 8388–8395.
- 31 Endo-Munoz L, Warby T, Harrich D, *et al.* Phosphorylation of HIV Tat by PKR increases interaction with TAR RNA and enhances transcription. *Viral J* 2005; 2: 17.
- 32 Gale M, Kwieciszewski B, Dossett M, *et al.* Antiapoptotic and oncogenic potentials of hepatitis C virus are linked to interferon resistance by viral repression of the PKR protein kinase. *J Virol* 1999; 73: 6506–6516.
- 33 Taylor DR, Shi ST, Romano PR, *et al.* Inhibition of the interferon-inducible protein kinase PKR by HCV E2 Protein. *Science* 1999; 285: 107–110.
- 34 Yue Z, Shatkin AJ. Double-Stranded RNA-dependent protein kinase (PKR) is regulated by reovirus structural proteins. *Virology* 1997; 234: 364–371.
- 35 Child SJ, Hakki M, De Niro KL, *et al.* Evasion of cellular antiviral responses by human cytomegalovirus TRS1 and IRS1. *J Virol* 2004; 78: 197–205.
- 36 He B, Gross M, Roizman B. The  $\gamma$ <sub>134.5</sub> protein of herpes simplex virus 1 complexes with protein phosphatase 1 $\alpha$  to dephosphorylate the  $\alpha$  subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. *Proc Natl Acad Sci USA* 1997; 94: 843–848.
- 37 Khoo D, Perez C, Mohr I. Characterization of RNA determinants recognized by the arginine- and proline-rich region of Us11, a herpes simplex virus type 1-encoded double-stranded RNA binding protein that prevents PKR activation. *J Virol* 2002; 76: 11971–11981.
- 38 Burýsek L, Pitha PM. Latently expressed human herpesvirus 8-encoded interferon regulatory factor 2 inhibits double-stranded RNA-activated protein kinase. *J Virol* 2001; 75: 2345–2352.
- 39 Esteban M, García MA, Domingo-Gil E, *et al.* The latency protein LANA2 from Kaposi's sarcoma-associated herpesvirus inhibits apoptosis induced by dsRNA-activated protein kinase but not RNase L activation. *J Gen Virol* 2003; 84: 1463–1470.
- 40 Sharp TV, Schwemmler M, Jeffrey I, *et al.* Comparative analysis of the regulation of the interferon-inducible protein kinase PKR by Epstein–Barr virus RNAs EBER-1 and EBER-2 and adenovirus VAI RNA. *Nucleic Acids Res* 1993; 21: 4483–4490.
- 41 Poppers J, Mulvey M, Perez C, *et al.* Identification of a lytic-cycle Epstein–Barr virus gene product that can regulate PKR activation. *J Virol* 2003; 77: 228–236.
- 42 Romano PR, Zhang F, Tan SL, *et al.* Inhibition of double-stranded RNA-dependent protein kinase PKR by vaccinia virus E3: role of complex formation and the E3 N-terminal domain. *Mol Cell Biol* 1998; 18: 7304–7316.
- 43 Sharp TV, Moonan F, Romashko A, *et al.* The vaccinia virus E3L gene product interacts with both the regulatory and the substrate binding regions of PKR: implications for PKR autoregulation. *Virology* 1998; 250: 302–315.
- 44 Sharp TV, Witzel JE, Jagus R. Homologous regions of the  $\alpha$  subunit of eukaryotic translational initiation factor 2 (eIF2 $\alpha$ ) and the vaccinia virus K3L gene product interact with the same domain within the dsRNA-activated protein kinase (PKR). *Eur J Biochem* 1997; 250: 85–91.
- 45 Schierhorn KL, Jolmes F, Bespalowa J, *et al.* Influenza A virus virulence depends on two amino acids in the N-terminal domain of Its NS1 protein to facilitate inhibition of the RNA-dependent protein kinase PKR. *J Virol* 2017; 91: e00198-1.
- 46 Kindler E, Thiel V, Weber F. Interaction of SARS and MERS coronaviruses with the antiviral interferon response. In: Ziebuhr J, ed. *Advances in Virus Research*. 1st Edn. Elsevier, 2016. Vol. 96: 109–221.

- 47 Iuliano AD, Roguski KM, Chang HH, *et al.* Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *Lancet* 2018; 391: 1285–1300.
- 48 Tan S-L, Gale MJ, Katze MG. Double-stranded RNA-independent dimerization of interferon-induced protein kinase PKR and inhibition of dimerization by the cellular P58IPK inhibitor. *Mol Cell Biol* 1998; 18: 2431–2443.
- 49 Gale M, Tan SL, Wambach M, *et al.* Interaction of the interferon-induced PKR protein kinase with inhibitory proteins P58IPK and vaccinia virus K3L is mediated by unique domains: implications for kinase regulation. *Mol Cell Biol* 1996; 16: 4172–4181.
- 50 Rutkowski DT, Kang SW, Goodman AG, *et al.* The role of p58IPK in protecting the stressed endoplasmic reticulum. *Mol Biol Cell* 2007; 18: 3681–3691.
- 51 Khaperskyy DA, Hatchette TF, McCormick C. Influenza A virus inhibits cytoplasmic stress granule formation. *FASEB J* 2012; 26: 1629–1639.
- 52 Li F, Chen Y, Zhang Z, *et al.* Robust expression of vault RNAs induced by influenza A virus plays a critical role in suppression of PKR-mediated innate immunity. *Nucleic Acids Res* 2015; 43: 10321–10337.
- 53 Nandy C, Mrázek J, Stoiber H, *et al.* Epstein–Barr virus-induced expression of a novel human vault RNA. *J Mol Biol* 2009; 388: 776–784.
- 54 Lee K, Kunkeaw N, Jeon SH, *et al.* Precursor miR-886, a novel noncoding RNA repressed in cancer, associates with PKR and modulates its activity. *RNA* 2011; 17: 1076–1089.
- 55 Krischuns T, Günl F, Henschel L, *et al.* Phosphorylation of TRIM28 enhances the expression of IFN- $\beta$  and proinflammatory cytokines during HPAIV infection of human lung epithelial cells. *Front Immunol* 2018; 9: 2229.
- 56 Mok BWY, Liu H, Chen P, *et al.* The role of nuclear NS1 protein in highly pathogenic H5N1 influenza viruses. *Microbes Infect* 2017; 19: 587–596.
- 57 Fung TS, Liu DX. Coronavirus infection, ER stress, apoptosis and innate immunity. *Front Microbiol* 2014; 5: 296.
- 58 Siu KL, Chan CP, Kok KH, *et al.* Comparative analysis of the activation of unfolded protein response by spike proteins of severe acute respiratory syndrome coronavirus and human coronavirus HKU1. *Cell Biosci* 2014; 4: 3.
- 59 Masters VR. The molecular biology of coronaviruses. *Adv Virus Res* 2006; 66: 193–292.
- 60 Liao Y, Fung TS, Huang M, *et al.* Upregulation of CHOP/GADD153 during coronavirus infectious bronchitis virus infection modulates apoptosis by restricting activation of the extracellular signal-regulated kinase pathway. *J Virol* 2013; 87: 8124–8134.
- 61 Chan C-P, Siu K-L, Chin K-T, *et al.* Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein. *J Virol* 2006; 80: 9279–9287.
- 62 Minakshi R, Padhan K, Rani M, *et al.* The SARS coronavirus 3a protein causes endoplasmic reticulum stress and induces ligand-independent downregulation of the type 1 interferon receptor. *PLoS One* 2009; 4: e8342.
- 63 de Wilde AH, Wannee KF, Scholte FE, *et al.* A kinome-wide small interfering RNA screen identifies proviral and antiviral host factors in severe acute respiratory syndrome coronavirus replication, including double-stranded RNA-activated protein kinase and early secretory pathway proteins. *J Virol* 2015; 89: 8318–8333.
- 64 Krähling V, Stein DA, Spiegel M, *et al.* Severe acute respiratory syndrome coronavirus triggers apoptosis via protein kinase R but is resistant to its antiviral activity. *J Virol* 2009; 83: 2298–2309.
- 65 Kindler E, Gil-Cruz C, Spanier J, *et al.* Early endonuclease-mediated evasion of RNA sensing ensures efficient coronavirus replication. *PLoS Pathog* 2017; 13: e1006195.
- 66 Volk A, Hackbart M, Deng X, *et al.* Coronavirus endoribonuclease and deubiquitinating interferon antagonists differentially modulate the host response during replication in macrophages. *J Virol* 2020; 94: e00178–20.
- 67 Crespillo-Casado A, Chambers JE, Fischer PM, *et al.* PPP1R15A-mediated dephosphorylation of eIF2 $\alpha$  is unaffected by Sephin1 or Guanabenz. *Elife* 2017; 6: e26109.
- 68 Axten JM, Romeril SP, Shu A, *et al.* Discovery of GSK2656157: an optimized PERK inhibitor selected for preclinical development. *ACS Med Chem Lett* 2013; 4: 964–968.
- 69 Rojas-Rivera D, Delvaeye T, Roelandt R, *et al.* When PERK inhibitors turn out to be new potent RIPK1 inhibitors: critical issues on the specificity and use of GSK2606414 and GSK2656157. *Cell Death Differ* 2017; 24: 1100–1110.
- 70 Axten JM, Medina JR, Feng Y, *et al.* Discovery of 7-methyl-5-(1-([3-(trifluoromethyl)phenyl] acetyl)-2,3-dihydro-1H-indol-5-yl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (GSK2606414), a potent and selective first-in-class inhibitor of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). *J Med Chem* 2012; 55: 7193–7207.
- 71 Özcan U, Yilmaz E, Özcan L, *et al.* Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* 2006; 313: 1137–1140.
- 72 Rosen MD, Woods CR, Goldberg SD, *et al.* Discovery of the first known small-molecule inhibitors of heme-regulated eukaryotic initiation factor 2 $\alpha$  (HRI) kinase. *Bioorg Med Chem Lett* 2009; 19: 6548–6551.
- 73 Jammi NV, Whitby LR, Beal PA. Small molecule inhibitors of the RNA-dependent protein kinase. *Biochem Biophys Res Commun* 2003; 308: 50–57.
- 74 Hu Y, Conway TW. 2-Aminopurine inhibits the double-stranded RNA-dependent protein kinase both *in vitro* and *in vivo*. *J Interferon Res* 1993; 13: 323–328.
- 75 Fujimoto J, Kurasawa O, Takagi T, *et al.* Identification of novel, potent, and orally available GCN2 inhibitors with type I half binding mode. *ACS Med Chem Lett* 2019; 10: 1498–1503.
- 76 Nakamura A, Nambu T, Ebara S, *et al.* Inhibition of GCN2 sensitizes ASNS-low cancer cells to asparaginase by disrupting the amino acid response. *Proc Natl Acad Sci USA* 2018; 115: E7776–E7785.
- 77 Sidrauski C, Acosta-Alvear D, Khoutorsky A, *et al.* Pharmacological brake-release of mRNA translation enhances cognitive memory. *Elife* 2013; 2: e00498.
- 78 Sekine Y, Zyryanova A, Crespillo-Casado A, *et al.* Stress responses. Mutations in a translation initiation factor identify the target of a memory-enhancing compound. *Science* 2015; 348: 1027–1030.
- 79 Sidrauski C, Tsai JC, Kampmann M, *et al.* Pharmacological dimerization and activation of the exchange factor eIF2 $\beta$  antagonizes the integrated stress response. *Elife* 2015; 4: e07314.
- 80 Halliday M, Radford H, Zents KAM, *et al.* Repurposed drugs targeting eIF2 $\alpha$ -P-mediated translational repression prevent neurodegeneration in mice. *Brain* 2017; 140: 1768–1783.
- 81 Stockwell SR, Platt G, Barrie SE, *et al.* Mechanism-based screen for G1/S checkpoint activators identifies a selective activator of EIF2AK3/PERK signalling. *PLoS One* 2012; 7: e28568.

- 82 Bueno M, Lai YC, Romero Y, *et al.* PINK1 deficiency impairs mitochondrial homeostasis and promotes lung fibrosis. *J Clin Invest* 2015; 125: 521–538.
- 83 Obeng EA, Carlson LM, Gutman DM, *et al.* Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. *Blood* 2006; 107: 4907–4916.
- 84 Landeras-Bueno S, Fernández Y, Falcón A, *et al.* Chemical genomics identifies the PERK-mediated unfolded protein stress response as a cellular target for influenza virus inhibition. *mBio* 2016; 7: e000085-16.
- 85 Burwick N, Zhang MY, de la Puente P, *et al.* The eIF2-alpha kinase HRI is a novel therapeutic target in multiple myeloma. *Leuk Res* 2017; 55: 23–32.
- 86 Yefidoff-Freedman R, Fan J, Yan L, *et al.* Development of 1-((1,4-trans)-4-aryloxy-cyclohexyl)-3-aryleurea activators of heme-regulated inhibitor as selective activators of the eukaryotic initiation factor 2 alpha (eIF2 $\alpha$ ) phosphorylation arm of the integrated endoplasmic reticulum stress response. *J Med Chem* 2017; 60: 5392–5406.
- 87 Thomis DC, Samuel CE. Mechanism of interferon action: autoregulation of RNA-dependent P1/eIF-2 $\alpha$  protein kinase (PKR) expression in transfected mammalian cells. *Proc Natl Acad Sci USA* 1992; 89: 10837–10841.
- 88 Oganeyan G, Saha SK, Guo B, *et al.* Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. *Nature* 2006; 439: 208–211.
- 89 Hu W, Hofstetter W, Wei X, *et al.* Double-stranded RNA-dependent protein kinase-dependent apoptosis induction by a novel small compound. *J Pharmacol Exp Ther* 2009; 328: 866–872.
- 90 Zhang P, McGrath BC, Reinert J, *et al.* The GCN2 eIF2 $\alpha$  kinase is required for adaptation to amino acid deprivation in mice. *Mol Cell Biol* 2002; 22: 6681–6688.
- 91 Barnes NA, Stephenson SJ, Tooze RM, *et al.* Amino acid deprivation links BLIMP-1 to the immunomodulatory enzyme indoleamine 2,3-dioxygenase. *J Immunol* 2009; 183: 5768–5777.
- 92 Sundrud MS, Korolov SB, Feuerer M, *et al.* Halofuginone inhibits TH17 cell differentiation by activating the amino acid starvation response. *Science* 2009; 324: 1334–1338.
- 93 Reinert RB, Oberle LM, Wek SA, *et al.* Role of glutamine depletion in directing tissue-specific nutrient stress responses to L-asparaginase. *J Biol Chem* 2006; 281: 31222–31233.
- 94 Boyce M, Bryant KF, Jousse C, *et al.* A selective inhibitor of eIF2 $\alpha$  dephosphorylation protects cells from ER stress. *Science* 2005; 307: 935–939.
- 95 Tsaytler P, Harding HP, Ron D, *et al.* Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science* 2011; 332: 91–94.
- 96 Perego J, Mendes A, Bourbon C, *et al.* Guanabenz inhibits TLR9 signaling through a pathway that is independent of eIF2 $\alpha$  dephosphorylation by the GADD34/PP1c complex. *Sci Signal* 2018; 11: eaam8104.
- 97 Crespillo-Casado A, Claes Z, Choy MS, *et al.* A Sephin1-insensitive tripartite holophosphatase dephosphorylates translation initiation factor 2 $\alpha$ . *J Biol Chem* 2018; 293: 7766–7776.
- 98 Das I, Krzyzosiak A, Schneider K, *et al.* Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. *Science* 2015; 348: 239–242.
- 99 Chambers JE, Dalton LE, Clarke HJ, *et al.* Actin dynamics tune the integrated stress response by regulating eukaryotic initiation factor 2 $\alpha$  dephosphorylation. *Elife* 2015; 4: e04872.
- 100 Lindquist ME, Lifland AW, Utley TJ, *et al.* Respiratory syncytial virus induces host RNA stress granules to facilitate viral replication. *J Virol* 2010; 84: 12274–12284.
- 101 Cheng J, Gao S, Zhu C, *et al.* Typical stress granule proteins interact with the 3' untranslated region of enterovirus D68 to inhibit viral replication. *J Virol* 2020; 94: e02041-19.
- 102 Xiao Y, Ma Z, Wang R, *et al.* Downregulation of protein kinase PKR activation by porcine reproductive and respiratory syndrome virus at its early stage infection. *Vet Microbiol* 2016; 187: 1–7.
- 103 Gao P, Chai Y, Song J, *et al.* Reprogramming the unfolded protein response for replication by porcine reproductive and respiratory syndrome virus. *PLoS Pathog* 2019; 15: e1008169.
- 104 Zhou Y, Fang L, Wang D, *et al.* Porcine reproductive and respiratory syndrome virus infection induces stress granule formation depending on protein kinase R-like endoplasmic reticulum kinase (PERK) in MARC-145 cells. *Front Cell Infect Microbiol* 2017; 7: 111.
- 105 Chen W-Y, Schnitzlein WM, Calzada-Nova G, *et al.* Genotype 2 strains of porcine reproductive and respiratory syndrome virus dysregulate alveolar macrophage cytokine production via the unfolded protein response. *J Virol* 2017; 92: e01251-17.
- 106 van 't Wout EFA, van Schadewijk A, van Boxel R, *et al.* Virulence factors of *Pseudomonas aeruginosa* induce both the unfolded protein and integrated stress responses in airway epithelial cells. *PLoS Pathog* 2015; 11: e1004946.
- 107 Vasil ML, Ochsner UA. The response of *Pseudomonas aeruginosa* to iron: genetics, biochemistry and virulence. *Mol Microbiol* 1999; 34: 399–413.
- 108 Yang ZS, Ma LQ, Zhu K, *et al.* *Pseudomonas* toxin pyocyanin triggers autophagy: implications for pathoadaptive mutations. *Autophagy* 2016; 12: 1015–1028.
- 109 Perra L, Balloy V, Foussignière T, *et al.* CHAC1 is differentially expressed in normal and cystic fibrosis bronchial epithelial cells and regulates the inflammatory response induced by *Pseudomonas aeruginosa*. *Front Immunol* 2018; 9: 2823.
- 110 Fusade-Boyer M, Arora J, Diehl K, *et al.* Evaluation of the antiviral activity of Sephin1 treatment and its consequences on eIF2 $\alpha$  phosphorylation in response to viral infections. *Front Immunol* 2019; 10: 134.
- 111 Hayward SL, Scharer CD, Cartwright EK, *et al.* Environmental cues regulate epigenetic reprogramming of airway-resident memory CD8<sup>+</sup> T cells. *Nat Immunol* 2020; 21: 309–320.
- 112 Cláudio N, Dalet A, Gatti E, *et al.* Mapping the crossroads of immune activation and cellular stress response pathways. *EMBO J* 2013; 32: 1214–1224.
- 113 Deng J, Lu PD, Zhang Y, *et al.* Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. *Mol Cell Biol* 2004; 24: 10161–10168.
- 114 Mijošek V, Lasitschka F, Warth A, *et al.* Endoplasmic reticulum stress is a danger signal promoting innate inflammatory responses in bronchial epithelial cells. *J Innate Immun* 2016; 8: 464–478.
- 115 Hattori T, Ohoka N, Hayashi H, *et al.* C/EBP homologous protein (CHOP) up-regulates IL-6 transcription by trapping negative regulating NF-IL6 isoform. *FEBS Lett* 2003; 541: 33–39.

Q5

Q6

- 116 Caristi S, Piraino G, Cucinotta M, *et al.* Prostaglandin E2 induces interleukin-8 gene transcription by activating C/EBP homologous protein in human T lymphocytes. *J Biol Chem* 2005; 280: 14433–14442.
- 117 Liu H, Huang L, Bradley J, *et al.* GCN2-dependent metabolic stress is essential for endotoxemic cytokine induction and pathology. *Mol Cell Biol* 2014; 34: 428–438.
- 118 Li Y, Xiao J, Tan Y, *et al.* Inhibition of PKR ameliorates lipopolysaccharide-induced acute lung injury by suppressing NF- $\kappa$ B pathway in mice. *Immunopharmacol Immunotoxicol* 2017; 39: 165–172.
- 119 Zeng Y, Qin Q, Li K, *et al.* PKR suppress NLRP3-pyoptosis pathway in lipopolysaccharide-induced acute lung injury model of mice. *Biochem Biophys Res Commun* 2019; 519: 8–14.
- 120 Cabanski M, Steinmüller M, Marsh LM, *et al.* PKR regulates TLR2/TLR4-dependent signaling in murine alveolar macrophages. *Am J Respir Cell Mol Biol* 2008; 38: 26–31.
- 121 Broz P, Dixit VM. Inflammasomes: mechanism of assembly, regulation and signalling. *Nat Rev Immunol* 2016; 16: 407–420.
- 122 Denk S, Neher MD, Messerer DAC, *et al.* Complement C5a functions as a master switch for the pH balance in neutrophils exerting fundamental immunometabolic effects. *J Immunol* 2017; 198: 4846–4854.
- 123 Lu B, Nakamura T, Inouye K, *et al.* Novel role of PKR in inflammasome activation and HMGB1 release. *Nature* 2012; 488: 670–674.
- 124 Yu S, Wang D, Huang L, *et al.* The complement receptor C5aR2 promotes protein kinase R expression and contributes to NLRP3 inflammasome activation and HMGB1 release from macrophages. *J Biol Chem* 2019; 294: 8384–8394.
- 125 Li R, Shang Y, Yu Y, *et al.* High-mobility group box 1 protein participates in acute lung injury by activating protein kinase R and inducing M1 polarization. *Life Sci* 2020; 246: 117415.
- 126 Dolinay T, Himes BE, Shumyatcher M, *et al.* Integrated stress response mediates epithelial injury in mechanical ventilation. *Am J Respir Cell Mol Biol* 2017; 57: 193–203.
- 127 Dolinay T, Idahosa C, Gupta K, *et al.* Protein kinase R-like endoplasmic reticulum kinase is a mediator of stretch in ventilator-induced lung injury. *Respir Res* 2018; 19: 157.
- 128 Kim SR, Kim DI, Kang MR, *et al.* Endoplasmic reticulum stress influences bronchial asthma pathogenesis by modulating nuclear factor  $\kappa$ B activation. *J Allergy Clin Immunol* 2013; 132: 1397–1408.
- 129 Yuan X, de Sousa JR, Araujo MTF, *et al.* Allergy immunotherapy restores airway epithelial barrier dysfunction through suppressing IL-25-induced endoplasmic reticulum stress in asthma. *Sci Rep* 2018; 8: 7950.
- 130 Ravindran R, Loebbermann J, Nakaya HI, *et al.* The amino acid sensor GCN2 controls gut inflammation by inhibiting inflammasome activation. *Nature* 2016; 531: 523–527.
- 131 Woo CW, Cui D, Arellano J, *et al.* Adaptive suppression of the ATF4–CHOP branch of the unfolded protein response by toll-like receptor signalling. *Nat Cell Biol* 2009; 11: 1473–1480.
- 132 Woo CW, Kutzler L, Kimball SR, *et al.* Toll-like receptor activation suppresses ER stress factor CHOP and translation inhibition through activation of eIF2 $\beta$ . *Nat Cell Biol* 2012; 14: 192–200.
- 133 Hengstermann A, Müller T. Endoplasmic reticulum stress induced by aqueous extracts of cigarette smoke in 3T3 cells activates the unfolded-protein-response-dependent PERK pathway of cell survival. *Free Radic Biol Med* 2008; 44: 1097–1107.
- 134 Jorgensen E, Stinson A, Shan L, *et al.* Cigarette smoke induces endoplasmic reticulum stress and the unfolded protein response in normal and malignant human lung cells. *BMC Cancer* 2008; 8: 229.
- 135 Tagawa Y, Hiramatsu N, Kasai A, *et al.* Induction of apoptosis by cigarette smoke via ROS-dependent endoplasmic reticulum stress and CCAAT/enhancer-binding protein-homologous protein (CHOP). *Free Radic Biol Med* 2008; 45: 50–59.
- 136 Tagawa Y, Hiramatsu N, Kato H, *et al.* Induction of CCAAT/enhancer-binding protein-homologous protein by cigarette smoke through the superoxide anion-triggered PERK–eIF2 $\alpha$  pathway. *Toxicology* 2011; 287: 105–112.
- 137 Zarcone MC, Duistermaat E, van Schadewijk A, *et al.* Cellular response of mucociliary differentiated primary bronchial epithelial cells to diesel exhaust. *Am J Physiol Lung Cell Mol Physiol* 2016; 311: L111–L123.
- 138 Zarcone MC, van Schadewijk A, Duistermaat E, *et al.* Diesel exhaust alters the response of cultured primary bronchial epithelial cells from patients with chronic obstructive pulmonary disease (COPD) to non-typeable *Haemophilus influenzae*. *Respir Res* 2017; 18: 27.
- 139 Ni L, Chuang C-C, Zuo L. Fine particulate matter in acute exacerbation of COPD. *Front Physiol* 2015; 6: 294.
- 140 Laing S, Wang G, Briazova T, *et al.* Airborne particulate matter selectively activates endoplasmic reticulum stress response in the lung and liver tissues. *Am J Physiol Cell Physiol* 2010; 299: 736–749.
- 141 Zarcone MC, Duistermaat E, Alblas MJ, *et al.* Effect of diesel exhaust generated by a city bus engine on stress responses and innate immunity in primary bronchial epithelial cell cultures. *Toxicol in Vitro* 2018; 48: 221–231.
- 142 Min T, Bodas M, Mazur S, *et al.* Critical role of proteostasis-imbalance in pathogenesis of COPD and severe emphysema. *J Mol Med* 2011; 89: 577–593.
- 143 Gan G, Hu R, Dai A, *et al.* The role of endoplasmic reticulum stress in emphysema results from cigarette smoke exposure. *Cell Physiol Biochem* 2011; 28: 725–732.
- 144 Yun JH, Morrow J, Owen CA, *et al.* Transcriptomic analysis of lung tissue from cigarette smoke-induced emphysema murine models and human chronic obstructive pulmonary disease show shared and distinct pathways. *Am J Respir Cell Mol Biol* 2017; 57: 47–58.
- 145 Tan SX, Jiang DX, Hu RC, *et al.* Endoplasmic reticulum stress induces HRD1 to protect alveolar type II epithelial cells from apoptosis induced by cigarette smoke extract. *Cell Physiol Biochem* 2017; 43: 1337–1345.
- 146 Tanel A, Pallepati P, Bettaieb A, *et al.* Acrolein activates cell survival and apoptotic death responses involving the endoplasmic reticulum in A549 lung cells. *Biochim Biophys Acta* 2014; 1843: 827–835.
- 147 Taylor M, Vella L, Carr T. Assessment of an *in vitro* model of lung epithelial cell stress responses. *Free Radic Biol Med* 2014; 75: Suppl. 1, S51.
- 148 van Rijt SH, Keller IE, John G, *et al.* Acute cigarette smoke exposure impairs proteasome function in the lung. *Am J Physiol Cell Mol Physiol* 2012; 303: L814–L823.
- 149 Kenche H, Ye ZW, Vedagiri K, *et al.* Adverse outcomes associated with cigarette smoke radicals related to damage to protein-disulfide isomerase. *J Biol Chem* 2016; 291: 4763–4778.
- 150 Chen Z, Wang D, Liu X, *et al.* Oxidative DNA damage is involved in cigarette smoke-induced lung injury in rats. *Environ Health Prev Med* 2015; 20: 318–324.

Q7

Q8



- 151 Kenche H, Baty CJ, Vedagiri K, *et al.* Cigarette smoking affects oxidative protein folding in endoplasmic reticulum by modifying protein disulfide isomerase. *FASEB J* 2013; 27: 965–977.
- 152 Sun Y, Ito S, Nishio N, *et al.* Enhancement of the acrolein-induced production of reactive oxygen species and lung injury by GADD34. *Oxid Med Cell Longev* 2015; 2015: 170309.
- 153 Yuan T, Luo B, Wei T, *et al.* Salubrinal protects against cigarette smoke extract-induced HBEpC apoptosis likely via regulating the activity of PERK-eIF2 $\alpha$  signaling pathway. *Arch Med Res* 2012; 43: 522–529.
- 154 Ye P, Mimura J, Okada T, *et al.* Nrf2- and ATF4-dependent upregulation of xCT modulates the sensitivity of T24 bladder carcinoma cells to proteasome inhibition. *Mol Cell Biol* 2014; 34: 3421–3434.
- 155 He CH, Gong P, Hu B, *et al.* Identification of activating transcription factor 4 (ATF4) as an Nrf2-interacting protein. Implication for heme oxygenase-1 gene regulation. *J Biol Chem* 2001; 276: 20858–20865.
- 156 DeNicola GM, Chen PH, Mullarky E, *et al.* NRF2 regulates serine biosynthesis in non-small cell lung cancer. *Nat Genet* 2015; 47: 1475–1481.
- 157 Afonyushkin T, Oskolkova OV, Philippova M, *et al.* Oxidized phospholipids regulate expression of ATF4 and VEGF in endothelial cells via NRF2-dependent mechanism: novel point of convergence between electrophilic and unfolded protein stress pathways. *Arterioscler Thromb Vasc Biol* 2010; 30: 1007–1013.
- 158 Cullinan SB, Zhang D, Hannink M, *et al.* Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol Cell Biol* 2003; 23: 7198–7209.
- 159 Cullinan SB, Diehl JA. PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress. *J Biol Chem* 2004; 279: 20108–20117.
- 160 Lu PD, Jousse C, Marciniak SJ, *et al.* Cytoprotection by pre-emptive conditional phosphorylation of translation initiation factor 2. *EMBO J* 2004; 23: 169–179.
- 161 Steiling K, van den Berge M, Hijazi K, *et al.* A dynamic bronchial airway gene expression signature of chronic obstructive pulmonary disease and lung function impairment. *Am J Respir Crit Care Med* 2013; 187: 933–942.
- 162 Thenappan T, Ormiston ML, Ryan JJ, *et al.* Pulmonary arterial hypertension: pathogenesis and clinical management. *BMJ* 2018; 360: j5492.
- 163 Eyries M, Montani D, Girerd B, *et al.* EIF2AK4 mutations cause pulmonary veno-occlusive disease, a recessive form of pulmonary hypertension. *Nat Genet* 2014; 46: 65–69.
- 164 Best DH, Sumner KL, Austin ED, *et al.* EIF2AK4 mutations in pulmonary capillary hemangiomatosis. *Chest* 2014; 145: 231–236.
- 165 Abou Hassan OK, Haidar W, Arabi M, *et al.* Novel EIF2AK4 mutations in histologically proven pulmonary capillary hemangiomatosis and hereditary pulmonary arterial hypertension. *BMC Med Genet* 2019; 20: 176.
- 166 Montani D, Price LC, Dorfmueller P, *et al.* Pulmonary veno-occlusive disease. *Eur Respir J* 2009; 33: 189–200.
- 167 Eichstaedt CA, Song J, Benjamin N, *et al.* EIF2AK4 mutation as “second hit” in hereditary pulmonary arterial hypertension. *Respir Res* 2016; 17: 141.
- 168 Gómez J, Reguero JR, Alvarez C, *et al.* A semiconductor chip-based next generation sequencing procedure for the main pulmonary hypertension genes. *Lung* 2015; 193: 571–574.
- 169 Tenorio J, Navas P, Barrios E, *et al.* A founder EIF2AK4 mutation causes an aggressive form of pulmonary arterial hypertension in Iberian Gypsies. *Clin Genet* 2015; 88: 579–583.
- 170 Hadinnapola C, Bleda M, Haimel M, *et al.* Phenotypic characterization of EIF2AK4 mutation carriers in a large cohort of patients diagnosed clinically with pulmonary arterial hypertension. *Circulation* 2017; 136: 2022–2033.
- 171 Best DH, Sumner KL, Smith BP, *et al.* EIF2AK4 mutations in patients diagnosed with pulmonary arterial hypertension. *Chest* 2017; 151: 821–828.
- 172 Montani D, Girerd B, Jais X, *et al.* Clinical phenotypes and outcomes of heritable and sporadic pulmonary veno-occlusive disease: a population-based study. *Lancet Respir Med* 2017; 5: 125–134.
- 173 Manaud G, Nossent EJ, Lambert M, *et al.* Comparison of human and experimental pulmonary veno-occlusive disease. *Am J Respir Cell Mol Biol* 2020; 63: 118–131.
- 174 Nossent EJ, Antigny F, Montani D, *et al.* Pulmonary vascular remodeling patterns and expression of general control nonderepressible 2 (GCN2) in pulmonary veno-occlusive disease. *J Heart Lung Transplant* 2018; 37: 647–655.
- 175 Perros F, Günther S, Ranchoux B, *et al.* Mitomycin-induced pulmonary veno-occlusive disease: evidence from human disease and animal models. *Circulation* 2015; 132: 834–847.
- 176 Malzer E, Dominicus CS, Chambers JE, *et al.* The integrated stress response regulates BMP signalling through effects on translation. *BMC Biol* 2018; 16: 34.
- 177 Taraseviciene-Stewart L, Kasahara Y, Alger L, *et al.* Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death-dependent pulmonary endothelial cell proliferation and severe pulmonary hypertension. *FASEB J* 2001; 15: 427–438.
- 178 Sakao S, Taraseviciene-Stewart L, Lee JD, *et al.* Initial apoptosis is followed by increased proliferation of apoptosis-resistant endothelial cells. *FASEB J* 2005; 19: 1178–1180.
- 179 McMurtry MS, Archer S, Altieri DC, *et al.* Gene therapy targeting survivin selectively induces pulmonary vascular apoptosis and reverses pulmonary arterial hypertension. *J Clin Invest* 2005; 115: 1479–1491.
- 180 Long L, Ormiston ML, Yang X, *et al.* Selective enhancement of endothelial BMPR-II with BMP9 reverses pulmonary arterial hypertension. *Nat Med* 2015; 21: 777–785.
- 181 Soon E, Holmes AM, Treacy CM, *et al.* Elevated levels of inflammatory cytokines predict survival in idiopathic and familial pulmonary arterial hypertension. *Circulation* 2010; 122: 920–927.
- 182 Song Y, Jones JE, Beppu H, *et al.* Increased susceptibility to pulmonary hypertension in heterozygous BMPR2-mutant mice. *Circulation* 2005; 112: 553–562.
- 183 Sawada H, Saito T, Nickel NP, *et al.* Reduced BMPR2 expression induces GM-CSF translation and macrophage recruitment in humans and mice to exacerbate pulmonary hypertension. *J Exp Med* 2014; 211: 263–280.
- 184 Moore BB, Hogaboam CM. Murine models of pulmonary fibrosis. *Am J Physiol Cell Mol Physiol* 2008; 294: L152–L160.
- 185 Bueno M, Brands J, Voltz L, *et al.* ATF3 represses PINK1 gene transcription in lung epithelial cells to control mitochondrial homeostasis. *Aging Cell* 2018; 17: e12720.
- 186 Jiang HY, Wek SA, McGrath BC, *et al.* Activating transcription factor 3 is integral to the eukaryotic initiation factor 2 kinase stress response. *Mol Cell Biol* 2004; 24: 1365–1377.

- 187 Burman A, Kropski JA, Calvi CL, *et al.* Localized hypoxia links ER stress to lung fibrosis through induction of C/EBP homologous protein. *JCI Insight* 2018; 3: e99543.
- 188 Zhang L, Xu D, Li Q, *et al.* N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) attenuates silicotic fibrosis by suppressing apoptosis of alveolar type II epithelial cells *via* mediation of endoplasmic reticulum stress. *Toxicol Appl Pharmacol* 2018; 350: 1–10.
- 189 Xiaojun W, Yan L, Hong X, *et al.* Acetylated  $\alpha$ -tubulin regulated by N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) exerts the anti-fibrotic effect in rat lung fibrosis induced by silica. *Sci Rep* 2016; 6: 32257.
- 190 Hinnebusch AG. Translational regulation of GCN4 and the general amino acid control of yeast. *Annu Rev Microbiol* 2005; 59: 407–450.
- 191 Bi M, Naczki C, Koritzinsky M, *et al.* ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. *EMBO J* 2005; 24: 3470–3481.
- 192 Song B, Scheuner D, Ron D, *et al.* Chop deletion reduces oxidative stress, improves  $\beta$  cell function, and promotes cell survival in multiple mouse models of diabetes. *J Clin Invest* 2008; 118: 3378–3389.
- 193 Bobrovnikova-Marjon E, Grigoriadou C, Pytel D, *et al.* PERK promotes cancer cell proliferation and tumor growth by limiting oxidative DNA damage. *Oncogene* 2010; 29: 3881–3895.
- 194 Kutomi G, Tamura Y, Tanaka T, *et al.* Human endoplasmic reticulum oxidoreductin 1- $\alpha$  is a novel predictor for poor prognosis of breast cancer. *Cancer Sci* 2013; 104: 1091–1096.
- 195 Ye J, Kumanova M, Hart LS, *et al.* The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation. *EMBO J* 2010; 29: 2082–2096.
- 196 Igarashi T, Izumi H, Uchiumi T, *et al.* Clock and ATF4 transcription system regulates drug resistance in human cancer cell lines. *Oncogene* 2007; 26: 4749–4760.
- 197 Tanabe M, Izumi H, Ise T, *et al.* Activating transcription factor 4 increases the cisplatin resistance of human cancer cell lines. *Cancer Res* 2003; 63: 8592–8595.
- 198 Rouschop KM, Dubois LJ, Keulers TG, *et al.* PERK/eIF2 $\alpha$  signaling protects therapy resistant hypoxic cells through induction of glutathione synthesis and protection against ROS. *Proc Natl Acad Sci USA* 2013; 110: 4622–4627.
- 199 Rouschop KMA, van den Beucken T, Dubois L, *et al.* The unfolded protein response protects human tumor cells during hypoxia through regulation of the autophagy genes MAP1LC3B and ATG5. *J Clin Invest* 2010; 120: 127–141.
- 200 Yang H, Kowalski D, Kudlac K, *et al.* HSP90/AXL/eIF4E-regulated unfolded protein response as an acquired vulnerability in drug-resistant KRAS-mutant lung cancer. *Oncogenesis* 2019; 8: 45.
- 201 Albert AE, Adua SJ, Cai WL, *et al.* Adaptive protein translation by the integrated stress response maintains the proliferative and migratory capacity of lung adenocarcinoma cells. *Mol Cancer Res* 2019; 17: 2343–2355.
- 202 Kaira K, Oriuchi N, Imai H, *et al.* Prognostic significance of L-type amino acid transporter 1 expression in resectable stage I–III nonsmall cell lung cancer. *Br J Cancer* 2008; 98: 742–748.
- 203 Cormerais Y, Giuliano S, LeFloch R, *et al.* Genetic disruption of the multifunctional CD98/LAT1 complex demonstrates the key role of essential amino acid transport in the control of mTORC1 and tumor growth. *Cancer Res* 2016; 76: 4481–4492.
- 204 Dey S, Sayers CM, Verginadis II, *et al.* ATF4-dependent induction of heme oxygenase 1 prevents anoikis and promotes metastasis. *J Clin Invest* 2015; 125: 2592–2608.
- 205 Forsythe JA, Jiang BH, Iyer NV, *et al.* Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* 1996; 16: 4604–4613.
- 206 Blais JD, Addison CL, Edge R, *et al.* Perk-dependent translational regulation promotes tumor cell adaptation and angiogenesis in response to hypoxic stress. *Mol Cell Biol* 2006; 26: 9517–9532.
- 207 Ghosh R, Lipson KL, Sargent KE, *et al.* Transcriptional regulation of VEGF-A by the unfolded protein response pathway. *PLoS One* 2010; 5: e9575.
- 208 Wang Y, Alam GN, Ning Y, *et al.* The unfolded protein response induces the angiogenic switch in human tumor cells through the PERK/ATF4 pathway. *Cancer Res* 2012; 72: 5396–5406.
- 209 Atkins C, Liu Q, Minthorn E, *et al.* Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res* 2013; 73: 1993–2002.
- 210 Aerts JG, Hegmans JP. Tumor-specific cytotoxic T cells are crucial for efficacy of immunomodulatory antibodies in patients with lung cancer. *Cancer Res* 2013; 73: 2381–2388.
- 211 Pataer A, Raso MG, Correa AM, *et al.* Prognostic significance of RNA-dependent protein kinase on non-small cell lung cancer patients. *Clin Cancer Res* 2010; 16: 5522–5528.
- 212 Pataer A, Vorburger SA, Barber GN, *et al.* Adenoviral transfer of the melanoma differentiation-associated gene 7 (mda7) induces apoptosis of lung cancer cells *via* up-regulation of the double-stranded RNA-dependent protein kinase (PKR). *Cancer Res* 2002; 62: 2239–2243.
- 213 Gannon HS, Zou T, Kiessling MK, *et al.* Identification of ADAR1 adenosine deaminase dependency in a subset of cancer cells. *Nat Commun* 2018; 9: 5450.
- 214 Dalton LE, Clarke HJ, Knight J, *et al.* The endoplasmic reticulum stress marker CHOP predicts survival in malignant mesothelioma. *Br J Cancer* 2013; 108: 1340–1347.
- 215 Xu D, Liang SQ, Yang H, *et al.* Increased sensitivity to apoptosis upon endoplasmic reticulum stress-induced activation of the unfolded protein response in chemotherapy-resistant malignant pleural mesothelioma. *Br J Cancer* 2018; 119: 65–75.
- 216 Han J, Back SH, Hur J, *et al.* ER-stress-induced transcriptional regulation increases protein synthesis leading to cell death. *Nat Cell Biol* 2013; 15: 481–490.
- 217 Lin W, Lin Y, Li J, *et al.* A deregulated integrated stress response promotes interferon- $\gamma$ -induced medulloblastoma. *J Neurosci Res* 2011; 89: 1586–1595.
- 218 Chitnis NS, Pytel D, Bobrovnikova-Marjon E, *et al.* miR-211 is a prosurvival microRNA that regulates CHOP expression in a PERK-dependent manner. *Mol Cell* 2012; 48: 353–364.

Q9

Q10

# Author Queries

*Journal:* ERR

*Manuscript:* ERR-0184-2020

- Q1 Is the suggested definition of siRNA ('short interfering RNA') correct?
- Q2 Please check that the abbreviated form of nonstructural protein is presented correctly throughout.
- Q3 Please provide missing publisher location for Ref. [4].
- Q4 Please provide missing publisher location for Ref. [46].
- Q5 Ref [109] listed authors Marshall C and Darby J; however, these are not named as authors in the published article. The names have been replaced with authors Balloy and Foussigniere - please check that this is correct. <https://pubmed.ncbi.nlm.nih.gov/30555487/>
- Q6 The author names provided for reference [110] do not appear to match those in the published document. Please check and confirm the correct names of authors 2 and 3
- Q7 The author names provided for reference [127] do not appear to match those in the published document. Please check and confirm the correct names of authors 2 and 3
- Q8 The author names provided for reference [129] do not appear to match those in the published document. Please check and confirm the correct names of authors 2 and 3.
- Q9 The title 'Suppression of ribosomal reinitiation at upstream open reading frames in amino acid-starved cells forms the basis for GCN4 translational control.' was included within reference [190]. Should this reference be added to the citation list?
- Q10 The author names provided for reference [200] do not appear to match those in the published document. Please check and confirm the correct names of authors 2 and 3
- Q11 References [45-47] have been re-ordered to reflect the position of table 1.
- Q12 References [68-218] have been re-ordered to reflect the position of table 2.