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Endoplasmic Reticulum Stress, a Target for Drug Design and Drug Resistance in Parasitosis

Mei Peng[1,2,3], *Fang Chen*[4], *Zhongdao Wu*[1,2,3] *and Jia Shen*[1,2,3]*

[1] Department of Parasitology of Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China, [2] Key Laboratory of Tropical Disease Control (SYSU), Ministry of Education, Guangzhou, China, [3] Provincial Engineering Technology Research Center for Biological Vector Control, Guangzhou, China, [4] School of Medicine, South China University of Technology, Guangzhou, China

Endoplasmic reticulum stress (ER stress) can be induced when cellular protein homeostasis is damaged, and cells can activate the unfolded protein response (UPR) to restore protein homeostasis or induce cell death to facilitate the survival of the whole system. Globally, parasites are a constant threat to human health and are therefore considered a serious public health problem. Parasitic infection can cause ER stress in host cells, and parasites also possess part or all of the UPR under ER stress conditions. In this review, we aim to clarify the role of ER stress pathways and related molecules in parasites for their survival and development, the pathogenesis of parasitosis in hosts, and the artemisinin resistance of *Plasmodium*, which provides some potential drug design targets to inhibit survival of parasites, relieves pathological damage of parasitosis, and solves the problem of artemisinin resistance.

Keywords: endoplasmic reticulum stress, drug targets, parasite, parasitosis, drug resistance

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***Correspondence:**
Jia Shen
shenj29@mail.sysu.edu.cn

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ER STRESS AND UPR

The endoplasmic reticulum (ER), a vital organelle in eukaryotic cells, is the site of synthesis and processing of membrane and secretory proteins, synthesis of lipids, and storage of Ca^{2+} (Dolai and Adak, 2014). Therefore, it is important to maintain ER homeostasis. Yet, many factors influence the protein homeostasis of ER, such as plasma cell differentiation (Gass et al., 2002), tunicamycin (Pahl and Baeuerle, 1995), and parasite infection (Galluzzi et al., 2017) which result in accumulated misfolded or unfolded proteins that exceed the folding capacity of ER and trigger endoplasmic reticulum stress (ER stress). Endoplasmic reticulum-associated degradation (ERAD) and unfolded protein response (UPR) are the two major quality control processes of ER stress (Bukau et al., 2006). UPR reduces the synthesis of proteins and eliminates misfolded proteins within the ER by increasing the expression of the ER chaperone proteins.

In mammalian cells, the UPR is mediated by three signaling pathways and activated by three ER-transmembrane proteins: inositol-requiring kinase/endoribonuclease 1 (IRE1), protein kinase RNA-like ER kinase (PERK), and activating transcription factor 6 (ATF6) (**Figure 1**; Hwang and Qi, 2018). Glucose-regulated proteins 78 (GRP78) (Bertolotti et al., 2000; Shen et al., 2002), also called immunoglobulin heavy chain binding protein (Bip) (Bertolotti et al., 2000; Shen et al., 2002), binds to these transmembrane proteins in unstressed cells, while it dissociates from them and binds to unfolded or misfolded proteins in stressed cells (Bertolotti et al., 2000; Shen et al., 2002; Grootjans et al., 2016). After dissociation from Bip, IRE1α will be activated by forming IRE1α

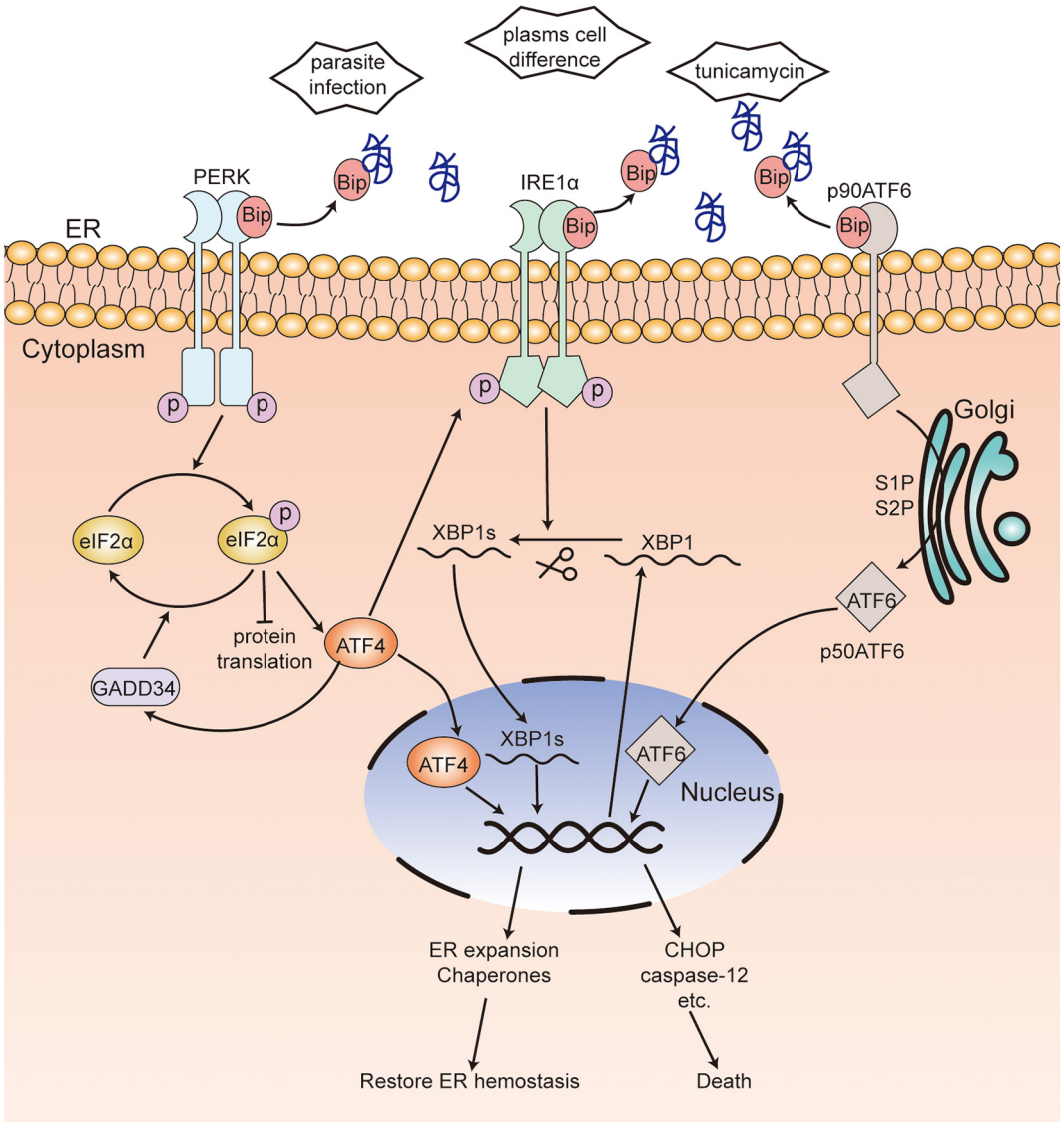

FIGURE 1 | Unfolded protein response in mammalian cells. In unstressed cells, Bip binds to three transmembrane proteins PERK, IRE1α and ATF6. When ER stress occurs, Bip dissociates from these transmembrane proteins and binds to unfolded or misfolded proteins. Activated PERK, induced by oligomerization following dissociation from Bip, phosphorylates eIF2α, which reduces protein translation while upregulating the expression of ATF4. eIF2α phosphorylation can be dephosphorylated by GADD34. After dissociation from Bip, IRE1α is activated by forming IRE1α homodimers and incises XBP1 mRNA into XBP1s. Bip releases from ATF6, which leads to the translocation to Golgi and activation of ATF6. ATF4, XBP1s, and activated ATF6 enter the nucleus and activate the transcription of ER chaperones or various death effectors, which results in the restoration of ER hemostasis or cell death. [ab], unfolded or misfolded proteins; PERK, protein kinase RNA-like ER kinase; IRE1, inositol-requiring kinase/endoribonuclease 1; eIF2α, α-subunit of eukaryotic translational initiation factor 2; ATF6, activating transcription factor 6; ATF4, activating transcription factor 4; XBP1, X box-binding protein 1; CHOP, C/EBP-homologous protein; Bip, immunoglobulin heavy chain binding protein; GADD34, DNA damage-inducible protein-34.

homodimers and incise the transcription factor X box-binding protein 1 (XBP1) mRNA into spliced XBP1 (XBP1s) (Bertolotti et al., 2000; Calfon et al., 2002). The function of XBP1s is to maintain the ER function and response to UPR and regulate the expansion of the secretory apparatus (Acosta-Alvear et al., 2007). Activated PERK, induced by oligomerization and autophosphorylation following dissociation from Bip (Bertolotti et al., 2000), phosphorylates the α-subunit of eukaryotic translational initiation factor 2 (eIF2α) and attenuates protein translation, which will reduce the load of newly synthesized proteins within the ER while upregulating the expression of activating transcription factor 4 (ATF4). In addition, the phosphorylation of eIF2α can be dephosphorylated by growth arrest and DNA damage-inducible protein-34 (GADD34). Further, ATF4 is required for the transactivation of GADD34, which will promote the recovery of translation

(Novoa et al., 2001; Ma and Hendershot, 2003). Under ER stress, ATF6 translocates from the ER to the Golgi apparatus (Shen et al., 2002). A 90-kDa protein ATF6 (p90ATF6) is converted to a 50-kDa protein ATF6 (p50ATF6, an active and mature form of ATF6) through the cleavage of Golgi-resident proteases—site 1 protease (S1P) and site 2 protease (S2P). P50ATF6 further activates the transcription of ER chaperone genes after entering the nucleus (Haze et al., 1999; Ron and Walter, 2007). Interactions among the three UPR pathways have been found, wherein ATF6 induces the transcription of XBP1 (Yoshida et al., 2001) and PERK-ATF4 upregulates the expression of IRE1α (Tsuru et al., 2016). These interactions will promote UPR to be stronger and more persistent in order to deal with various types of ER stress. Nevertheless, prolonged and severe ER stress can activate various cell death effectors such as BAK, BAX, caspase-12, C/EBP-homologous protein (CHOP), and GADD34 and induce cell death (Ron and Walter, 2007). The mechanism of UPR is evolutionary conservatism across eukaryotes.

PARASITIC INFECTION AND UPR

Parasitosis, caused by parasitic infections, has been harmful to human health and economic development since very long and is still a major global public health problem. As is known, the effective control of parasitic diseases is mainly dependent on the application of parasitic drugs and disruption of the pathogen's life cycle, such as praziquantel and artemisinin. Unfortunately, drug resistance of parasitic drugs has been reported in recent years (Fallon and Doenhoff, 1994; Dondorp et al., 2009). Therefore, new anti-parasitic drugs including those to alleviate the pathology of the host caused by parasite infection and those to kill parasites should be identified urgently, along with determination of the mechanisms of drug resistance.

When parasites infect the host and obtain nutrients, they will perturb ER homeostasis and induce ER stress and UPR of the host. On the one hand, the induced ER stress of the host is beneficial to the survival and infection of the parasites. For instance, it has been reported that *Plasmodium berghei* infection induced ER stress of hepatocytes and activated UPR through the XBP1 and cAMP responsive element-binding protein (CREBH, a hepatocyte specific UPR mediator) pathways, which contributed to the infection of *Plasmodium* by providing phosphatidylcholine and regulating iron level (Inacio et al., 2015). In addition, *Leishmania* infection induced ER stress of the host to facilitate infection through the PERK-eIF2α-ATF4 and IRE1-XBP1 pathways (Dias-Teixeira et al., 2016, 2017; Galluzzi et al., 2016; Abhishek et al., 2018). And *Toxoplasma* triggered the UPR in host cells, which affected calcium release from ER, can enhance host cell migration and dissemination of the parasite to host organs (Augusto et al., 2020). However, Poncet et al. (2021) have showed that the IRE1a/XBP1s branch of the UPR was a key regulator of host defense upon *Toxoplasma gondii* infection, that mice deficient for IRE1a and XBP1 in DCs displayed a severe susceptibility to *T. gondii* infection, which indicates that the UPR induced by parasites also plays an important role in host immune defense. Anyhow, on the other hand, excessive ER stress and UPR will cause severe pathological damage to the host. Yu et al. (2014) found that the levels of GRP78, CHOP, cleaved caspase-12, and phosphorylated-JNK in the intestine of *Trichinella spiralis*-infected mice were significantly upregulated, which indicated that the ER stress-induced apoptotic pathway participated in intestinal lesions caused by *T. spiralis* infection. Thus, inhibition of excessive UPR in the host may be a therapeutic target to alleviate the pathological symptoms.

Additionally, the parasites can sense ER stress and either induce UPR to facilitate their survival when attacked by the host immune system or adapt to the host environment (such as changes in pH and temperature, oxidative stress, nutrient deficiency) (Zuzarte-Luís and Mota, 2018). Therefore, the UPR signaling pathway may be a potential target for inhibiting the survival and development of parasites.

THE UPR IN THE HOST MAY BE A THERAPEUTIC TARGET FOR RELIEVING PATHOLOGICAL DAMAGE OF PARASITOSIS

Different parasitic infections result in different pathological damage to different host tissues and organs. Nowadays, increasing reports show that ER stress and UPR play an important role in the development of pathology of parasitosis (Anand and Babu, 2013; Ayyappan et al., 2019).

Plasmodium

Plasmodium spp., which are the causative agents of malaria, are obligate intracellular protozoan parasites. Anand and Babu (2013) reported that experimental cerebral malaria (ECM), caused by *P. berghei* ANKA (PbA) infection, was related to ER stress. They found that PbA infection-induced ER stress could cause the apoptosis of neuronal cells in mice by activating the three branches of UPR—PERK-eIF2α-ATF4/GADD34, IRE1-XBP1s, and ATF6—along with upregulating the levels of CHOP, cleaved caspase-3 and caspase-12 and downregulating the expression of Bip, calreticulin, and calnexin.

Trypanosome

Trypanosome cruzi is the causative pathogen of Chagas disease in humans. Reportedly, the trypomastigotes of *T. cruzi* infection could induce ER stress in the heart of mice, with an increase in the levels of Bip, PERK, eIF2α, ATF4, and CHOP, thereby causing damage to the host. Interestingly, 2-aminopurine (2-APB, an ER stress inhibitor) treatment could alleviate the pathological damage to the heart by decreasing the phosphorylation of eIF2α and its downstream signaling. Therefore, this indicates that inhibition of ER stress may be a therapeutic target for cardiomyopathy in Chagas patients (Ayyappan et al., 2019).

Toxoplasma

Toxoplasma is an obligate intracellular parasite and opportunistic pathogenic parasite (Sullivan et al., 2004). *Toxoplasma* encephalitis is the most serious outcome of toxoplasmosis,

which may be fatal to immunocompromised individuals. Some studies have found that *Toxoplasma* encephalitis was related to ER stress. It has been reported that the tachyzoites of *T. gondii* RH strain and TgCtwh3 (a representative Chinese 1 *Toxoplasma* strain) induced apoptosis of neural stem cells and neural stem cell line C17.2 by activating CHOP, caspase-12, and JNK (Wang et al., 2014; Zhou et al., 2015). Pretreatment with tauroursodeoxycholic acid (TUDCA, an ER stress inhibitor) and Z-ATAD-FMK (a caspase-12 inhibitor) led to the inhibition of apoptosis (Wang et al., 2014; Zhou et al., 2015), which suggested that neural stem cell apoptosis induced by both TgCtwh3 and RH strain infection was dependent on the ER stress pathway, and ER stress inhibitors could be used to alleviate *Toxoplasma* encephalitis. In addition, Wan et al. (2015) showed that virulence factor rhoptry protein 18 (ROP18) secreted by *T. gondii* was involved in nerve cell apoptosis *via* the ER stress pathway, characterized by an increase in the expression of cleaved caspase-12, CHOP, and cleaved caspase-3. Ran et al. further indicated that ROP18 induced apoptosis of neural cells by phosphorylating reticulon 1-C [RTN1-C, a protein localized in the ER that is preferentially expressed in the neural cells of the central nervous system (CNS) at Ser7/134 and Thr4/8/118], which led to the acetylation of GRP78 and induced ER stress (An et al., 2018). These results suggest that inhibition of ROP18 of *T. gondii* can be used as a drug target for the treatment of *Toxoplasma* encephalitis to inhibit the ER stress-induced apoptosis of host cells.

Schistosoma japonicum

Schistosoma japonicum is the causative agent of schistosomiasis. The pathogenic mechanism of schistosomiasis is primarily attributed to egg-induced hepatic granuloma and fibrosis and cirrhosis (Yu et al., 2016; Duan et al., 2019). Duan et al. (2019) showed that the level of CHOP, a vital factor in the ER stress-mediated apoptosis pathway, was significantly increased in mice at 6 and 10 weeks following infection with *S. japonicum*. The study indicated that ER stress may be involved in *S. japonicum* infection-induced hepatic fibrosis. Moreover, Yu et al. (2016) showed that treatment with taurine, an inhibitor of ER stress, significantly suppressed the egg-induced hepatic granuloma and alleviated hepatic fibrosis in mice at 8 weeks post-infection, along with marked reduction of the expression of GRP78. Therefore, ER stress inhibitors may be a therapeutic drug for hepatic fibrosis.

The summary of ER stress in hosts caused by parasitic infection is shown in **Figure 2**. Therefore, the UPR signaling pathway may be a therapeutic target to alleviate pathological symptoms.

THE UPR IN PARASITES SUGGESTS POTENTIAL DRUG TARGETS FOR INHIBITING THE SURVIVAL AND DEVELOPMENT OF PARASITES

Parasites can sense ER stress and induce UPR of themselves to facilitate their survival and development. Different parasites may have different components of ER stress pathway.

Plasmodium

Plasmodium has a complicated life cycle, including the merozoite, ring, trophozoite, schizont, and gametophyte stages in humans and the ookinete and sporozoite stages in mosquitoes. Chaubey et al. (2014) showed that *Plasmodium falciparum* lacked the orthologs of XBP1, IRE1, ATF6, and ATF4, and only retained the PERK-eIF2α pathway to regulate translation under ER stress. Three eIF2α kinases have been identified, namely IK1, IK2, and PK4 (eIF2α kinase of *Plasmodium* (Möhrle et al., 1997), a PERK homolog of mammals) (Ward et al., 2004). It has been reported that increased phosphorylation of eIF2α leads to reduced levels of protein translation, which is associated with the formation of *P. falciparum* gametophytes and the conversion of the *P. berghei* gametophytes into ookinetes when treated with dithiothreitol (DTT) (Chaubey et al., 2014; Duran-Bedolla et al., 2017). In addition, Zhang et al. (2012) have shown that PK4 was involved in the invasion of new red blood cells of merozoite-containing schizonts and the gametocyte infecting *Anopheles* mosquitoes. The inhibition of PK4 of *P. berghei* by generating a PK4 conditional mutant (PbPK4cKO) would alleviate the symptoms of malaria and inhibit disease transmission. Another study indicated that treatment of GSK2606414 (a small molecule inhibitor of PERK (Axten et al., 2012), which specifically inhibits PK4 instead of IK1 and IK2 *in vitro*) could block the transformation of *P. falciparum* from trophozoites to schizonts (Zhang et al., 2017). The transformation between different forms increased the ability of translational regulation of *Plasmodium*. In addition, Chen et al. (2018) reported that apoptozole, a novel chemical scaffold, was lethal to the chloroquine-sensitive and chloroquine -resistant *P. falciparum* parasite strains by inhibiting GRP78 function *in vitro*. Compared to human GRP78, *P. falciparum* GRP78 showed a lower affinity to the endogenous ligands, ADP and ATP, which indicated that the competitive inhibitors of GRP78 can be investigated for *P. falciparum* control (Chen et al., 2018).

According to the above mentioned studies, it appears that the PK4-eIF2α pathway plays an important role in both morphological transformation and host transmission in *Plasmodium*. Thus, PK4 inhibition would inhibit the development of *Plasmodium*, which implies that PK4 inhibitors may be a potential target in malaria treatment. However, Bridgford et al. (2018) found that dihydroartemisinin (DHA) increased the toxicity to *Plasmodium* by prolonging PK4 activation and eIF2α phosphorylation. Therefore, appropriate ER stress is beneficial to the development of *Plasmodium*, while excessive ER stress would be lethal to the parasites.

Leishmania

Leishmania is the pathogen causing Leishmaniasis and has two forms—promastigote and amastigote. Gosline et al. (2011) proved that *Leishmania* lacked a transcriptional regulation response to UPR, and only retained the translational regulation in ER stress. They also showed an increased level of phosphorylation of eIF2α in *L. donovani* after treatment of DTT (Gosline et al., 2011). Moreover, Chow et al. (2011) found that the PERK homolog of *Leishmania* largely colocalized with Bip in

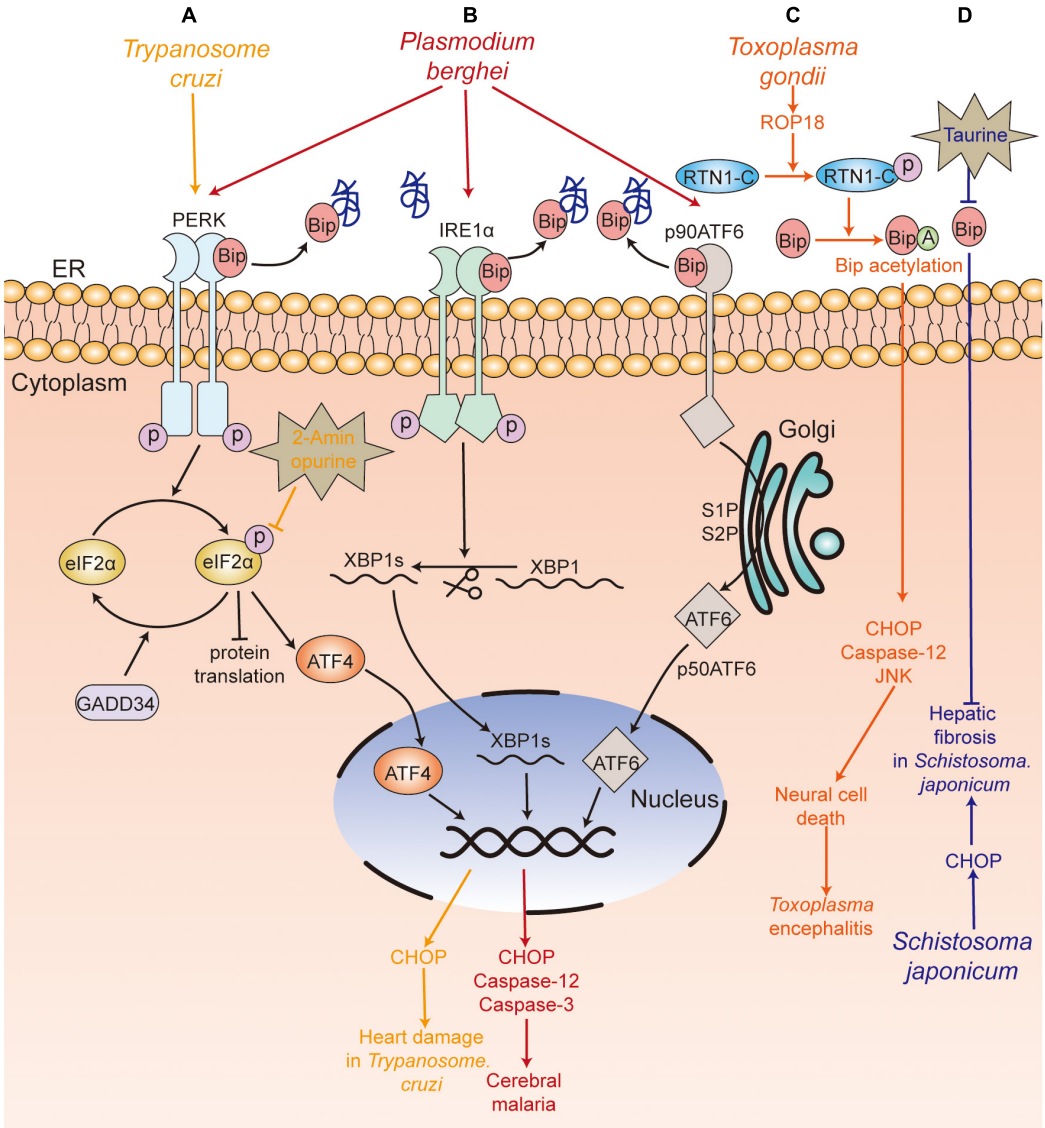

FIGURE 2 | UPR participates in the pathological damage caused by parasite infection. **(A)** *T. Cruzi* (yellow) infection caused heart damage, with upregulation of PERK-eIF2α-ATF4-CHOP pathway, and 2-aminopurine treatment alleviated the heart's pathological damage. **(B)** *P. berghei* (red) infection induced neuronal cell death and caused experimental cerebral malaria by activating the three branches of the UPR (PERK-eIF2α-ATF4/GADD34, IRE1-XBP1s, ATF6). **(C)** Rhoptry protein 18 (ROP18) of *T. gondii* (orange) phosphorylated reticulon 1-C (RTN1-C), which led to the acetylation of GRP78 and further upregulated the expression of cleaved caspase-12, CHOP, cleaved caspase-3, and induced the apoptosis of neural cells. **(D)** *S. japonicum* (blue) infection led to increased levels of CHOP, which was involved in hepatic fibrosis, and the treatment with taurine suppressed the egg-induced hepatic granuloma and fibrosis. PERK, protein kinase RNA-like ER kinase; IRE1, inositol-requiring kinase/endoribonuclease 1; eIF2α, α-subunit of eukaryotic translational initiation factor 2; ATF6, activating transcription factor 6; ATF4, activating transcription factor 4; XBP1, X box-binding protein 1; CHOP, C/EBP-homologous protein; Bip, immunoglobulin heavy chain binding protein;GADD34, DNA damage-inducible protein-34.

ER, which can phosphorylate eIF2α at threonine 166. They further confirmed that PERK-dependent eIF2α phosphorylation was vital for *Leishmania* to switch from the promastigote to amastigote form *in vitro* (Chow et al., 2011). Unlike host macrophages having intact UPR pathway, the mere presence of the PERK pathway in *L. donovani* promoted the parasite's susceptibility to DTT-induced ER stress (Gosline et al., 2011), which suggests that inhibition of the PERK pathway and induction of ER stress in *Leishmania* are both potential targets to kill the parasite. Dolai et al. (2011) proved that tunicamycin treatment induced apoptosis of *Leishmania major*, with an increase in the level of Bip.

Trypanosome

Trypanosome brucei is a protozoan parasite that cycles between the tsetse fly (procyclic form) and mammalian host (blood stream form), which causes African sleeping sickness in humans and nagana in livestock (Zhang et al., 2019). Goldshmidt et al. (2010)

reported that the expression of Bip of *T. brucei* was increased in both procyclic and blood stream forms in DTT-induced ER stress, and irrecoverable ER stress could induce spliced leader RNA silencing pathway (SLS pathway, a unique process in *T. brucei*), which may accelerate programmed cell death (PCD). Besides, Messias Sandes et al. (2019) showed that both DTT and tunicamycin could induce PCD in *T. cruzi*.

There were three putative eIF2α kinases (TbeIF2K1-K3) in *T. brucei*, though its genome lacked the homologs of IRE1/XBP1. It was reported that TbeIF2K2, a transmembrane glycoprotein expressed both in the procyclic and bloodstream forms of *Trypanosome* (Moraes et al., 2007), shared no similar sequence with known eIF2 kinases of mammals and was localized to the flagellar pocket, where endocytosis and exocytosis occur, and all proteins were transported from the flagellar pocket to the cell membrane (Gull, 2003). Therefore, the localization of TbeIF2K2 indicated that it could sense proteins and regulate protein synthesis near the flagellar pocket of the *Trypanosome* (Moraes et al., 2007), which suggests that TbeIF2K2 may be a good drug target to destroy *T. brucei*. In addition, Hope et al. (2014) showed that SEC63 (a factor participating in protein translocation machinery in ER) silence-induced ER stress could activate PK3 (TbeIF2K3) and trigger the release of PK3 from the ER to nucleus in the procyclic form of *T. brucei*. The deletion of PK3 reduced the death of *T. brucei* in SEC63 silence-induced ER stress, which suggests that PK3 is required for ER stress-induced PCD. Thus, the results indicate that TbeIF2K2 and TbeIF2K3 could be potential drug targets to eliminate *T. brucei*.

The PERK-eIF2α pathway is also involved in the form transformation of *T. cruzi* at different developmental stages. Tonelli et al. (2011) reported that the differentiation of non-infective epimastigotes into infective metacyclic trypomastigotes in *T. cruzi* requires the phosphorylation of Tc-eIF2α .

In conclusion, the results show that eIF2α phosphorylation plays an important role in the survival and development of *Trypanosome*, while excessive ER stress induced by DTT or tunicamycin can lead to the death of *Trypanosome*.

Toxoplasma

Toxoplasma shows two forms in the human host: tachyzoite (a rapidly growing form) and bradyzoite (a quiescent cyst form) (Black and Boothroyd, 2000; Sullivan et al., 2004). It has been reported that *T. gondii* lacked the homologs of IRE1 and ATF6 (Joyce et al., 2013), while it possessed four TgIF2α kinases, namely TgIF2K-A, TgIF2K-B, TgIF2K-C, and TgIF2K-D (Narasimhan et al., 2008; Konrad et al., 2014). Narasimhan et al. (2008) showed that only TgIF2K-A was a transmembrane protein localized in the ER and bonded to Bip under unstressed conditions. When ER stress occurred, the binding of Bip to TgIF2K-A was reduced, similar to the binding of BiP to PERK in mammals, which suggests that part of the UPR was conserved in *T. gondii* (Narasimhan et al., 2008).

ER stress is also involved in the differentiation of *Toxoplasma*. Narasimhan et al. (2008) reported that the phosphorylation of TgIF2α induced by tunicamycin treatment resulted in the differentiation of *T. gondii* from tachyzoite to bradyzoite cysts. Treatment with salubrinal, an inhibitor of eIF2α dephosphorylation, could also induce the differentiation of bradyzoite cysts, which indicated that TgIF2α phosphorylation was involved in the differentiation of bradyzoite cysts (Narasimhan et al., 2008). Cyst formation is a good way to escape from the host's immune attack. Therefore, the formation of bradyzoite cysts induced by TgIF2α phosphorylation promotes the survival of *T. gondii* under stressful conditions. Similar results were confirmed by Joyce et al. (2013). Besides, Joyce et al. (2010) reported that the TgIF2α mutant strain of *Toxoplasma* (i.e., TgIF2α-S71A, which cannot be phosphorylated) showed a lower virulence to the host cell, a lower survival rate and a slower transmitting speed, compared with the control strain of *Toxoplasma*. Moreover, Augusto et al. (2018) showed that specific inhibition of TgIF2K-A with GSK2606414 could inhibit the lytic cycle of tachyzoites, including attachment/invasion, replication, egress, and differentiation, which prolonged the survival time of mice with acute toxoplasmosis at a lethal dose of 100 RH strain tachyzoites. Interestingly, GSK2606414 did not show apparent detrimental effects on the host cell though with a high concentration *in vitro*. Therefore, the results suggest that TgIF2K-A and TgIF2α can be used as drug targets to inhibit *Toxoplasma* survival.

However, DTT treatment and stearoyl-coenzyme A (CoA) desaturase (SCD) accumulation at the ER could trigger ER stress with increasing phosphorylation of TgIF2α and mediated the apoptosis or autophagy of *T. gondii* (Nguyen et al., 2017; Hao et al., 2019). Therefore, although the TgIF2K-A/TgIF2α pathway plays a protective role in *T. gondii* under stress conditions, severe disruption of ER homeostasis can lead to the death of *T. gondii*.

Entamoeba histolytica

Entamoeba histolytica infection, caused by ingestion of cysts in contaminated water and food, usually induces amoebic dysentery and liver abscesses in humans (Pineda and Perdomo, 2017). Santi-Rocca et al. (2012) found that no genes encoded the orthologs of PERK and ATF6 in *E. histolytica* amoeba, while the expression of gene encoding eIF2α was upregulated upon treatment with nitric oxide (NO). Hendrick et al. (2016) showed that eIF2α could be phosphorylated at serine-59 in *E. histolytica*, with a decrease in translation levels during long-term serum starvation, long-term heat shock, and oxidative stress instead of short-term serum starvation, short-term heat shock, and glucose deprivation, and the viability of EheIF2α-S59D (a phosphomimetic variant of eIF2α) was significantly increased during long-term serum starvation. This study suggests that EheIF2α phosphorylation promotes the survival of *E. histolytica* under stress conditions. DTT treatment can also induce distinct fragmentation of ER and phosphorylation of EheIF2α, while treatment with SNP and DPTA-NON-Oate (NO donors) did not induce phosphorylation of EheIF2α (Walters et al., 2019). Besides, Kumari et al. (2018) identified the ortholog of IRE1 in *E. histolytica* (EhIre1) and reported that treatment with

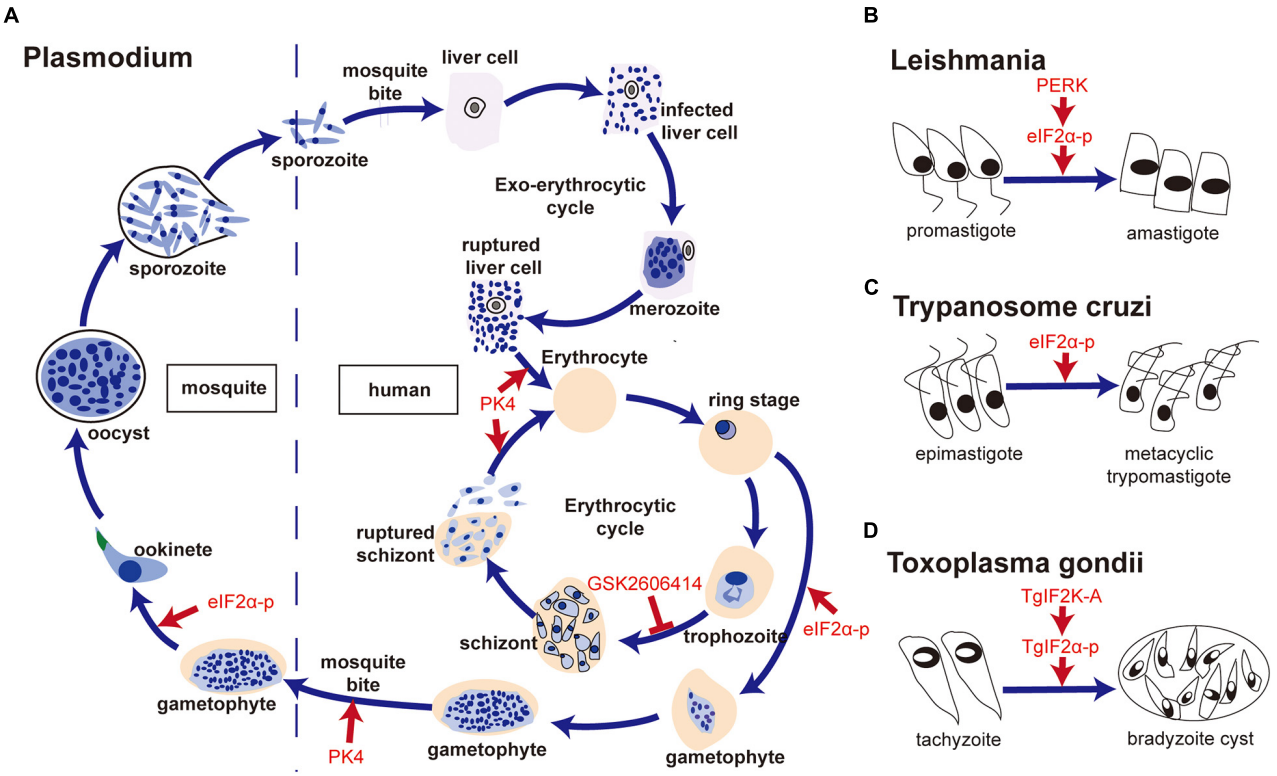

FIGURE 3 | The PERK-eIF2α pathway is involved in forms transformation of parasites. **(A)** PK4-eIF2α was associated with the formation of gametophytes, conversion of *Plasmodium* gametophytes into ookinetes, invasion of new red blood cells by merozoite-containing schizonts and the infection of *Anopheles* mosquitoes by gametocytes; **(B)** PERK- eIF2α phosphorylation was vital for the switch from promastigote to amastigote in *Leishmania*; **(C)** Tc-eIF2α phosphorylation was required for the differentiation of non-infective epimastigotes into infective metacyclic trypomastigotes of *T. cruzi*; **(D)** TgIF2K-A/TgIF2α phosphorylation was related to bradyzoite cyst differentiation in *T. gondii*.

tunicamycin resulted in the upregulation of EhIre1. In addition, the level of eIF2α phosphorylation was increased during encystation of *Entamoeba invadens*, but whether eIF2α is necessary for encystation still needs further investigation (Hendrick et al., 2016).

Echinococcus granulosus

Echinococcus granulosus is the causative cestode of hydatidosis or cystic echinococcosis (CE) and is a worldwide zoonotic infection that affects many organs in human and mammals (Loos et al., 2018). Nicolao et al. (2017) have identified the ortholog of IRE2, XBP1, and ATF6 in the genome of *E. granulosus*, but the ortholog of PERK/ATF4 was not found. Treatment with bortezomib (a proteasome inhibitor) led to lower viability of *E. granulosus* in the larval stage *in vitro* than that in the control group, with an increase of EgGRP78 and EgIRE2/EgXBP1 mRNA levels in protoscoleces; however, no changes were found in the metacestodes (Nicolao et al., 2017). Another study also showed that arsenic trioxide (As_2O_3) could disturb the intracellular Ca^{2+} homeostasis and activated ER stress-related apoptosis of protoscoleces *in vitro*, with an increase in the expression of GRP78, caspase-3, and caspase-12 (Li et al., 2018). These studies show that the induction of ER stress can lead to the apoptosis of protoscoleces *in vitro*.

In sum, the components of the UPR response such as the PERK-eIF2α pathway of some parasites (**Figures 3**, **4**), including *Plasmodium*, *Leishmania*, *Trypanosome*, *Toxoplasma*, and *E. histolytica,* play an important role in their survival and development. However, excessive ER stress could induce the death of parasites such as *Plasmodium*, *Leishmania*, *Trypanosome*, *Toxoplasma,* and *E. granulosus* (**Figure 4**). Considering the toxicity of commonly used ER stress inducers such as DTT and tunicamycin, it is difficult to use them to kill parasites *in vivo*. For those parasites that are more sensitive to ER stress inducers than their hosts, it is necessary to explore the appropriate concentration of these inducers. TUDCA, a bile salt and chemical chaperone used to treat biliary cirrhosis clinically (Lazaridis et al., 2001), partially inhibits ER stress by lowering the levels of PERK, Bip (Malo et al., 2010; Liu et al., 2015; Li et al., 2019). Thus, TUDCA may be an alternative therapy for parasitosis.

Endoplasmic reticulum-associated degradation is another way for maintaining ER homeostasis, which can degrade misfolded protein (Hwang and Qi, 2018). And ERAD also exists in parasites, such as trypanosomes (Tiengwe et al., 2016). In addition, some apicomplexan parasites, including *P. falciparum*, *T. gondii* and *cryptosporidium,* harbor an apicoplast, which is important for parasite survival (Agrawal et al., 2013). Reportedly, ERAD

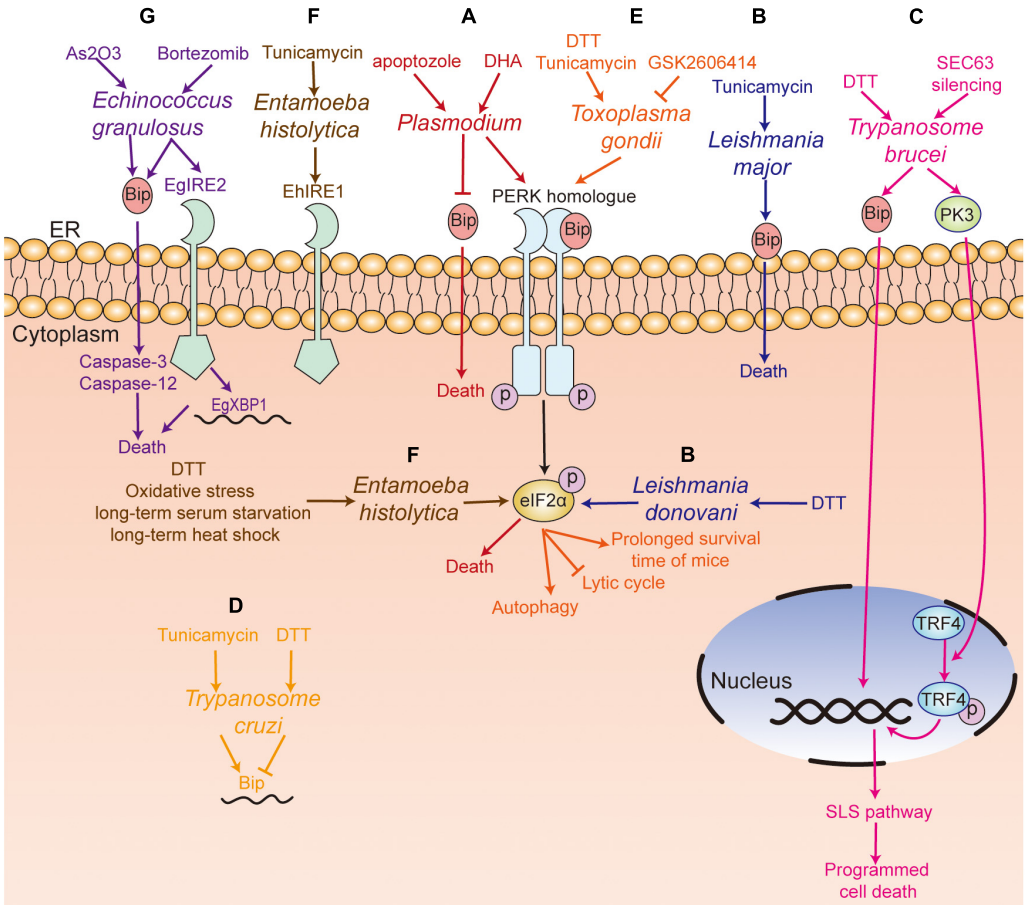

FIGURE 4 | UPR pathway in parasites. **(A)** *Plasmodium* spp. (red) with the PERK homolog; apoptozole was lethal to the chloroquine-sensitive and chloroquine-resistant *P. falciparum* parasite strains by inhibiting GRP78 function *in vitro*, and DHA treatment could induce the death of *P. falciparum* through the PERK-eIF2α pathway; **(B)** Tunicamycin treatment induced the death of *L. major* (blue) with an increased level of Bip, and DTT treatment upregulated the phosphorylation of eIF2α in *L. donovani*; **(C)** DTT treatment induced programmed cell death. SEC63 silencing activated PK3, which further induced programmed cell death by phosphorylating TRF4 and inducing the SLS pathway in *T. brucei* (pink); **(D)** Tunicamycin treatment increased the level of Bip mRNA, while DTT treatment decreased the level of Bip in *T. Cruzi* (yellow); **(E)** GSK2606414 inhibited the lytic cycle of tachyzoites, including attachment/invasion, replication, egress, and prolonged the survival time of infected mice. DTT treatment mediated the apoptosis or autophagy of *T. gondii* (orange) by increasing the phosphorylation of TgIF2α; **(F)** DTT treatment, long-term serum starvation, long-term heat shock and oxidative stress induced the phosphorylation of eIF2α, and the ortholog of IRE1 in *E. histolytica* (EhIre1) (gray) was identified; **(G)** Bortezomib treatment induced the death of *E. granulosus* (purple) with an increase of EgGRP78 and EgIRE2/EgXBP1 mRNA. Arsenic trioxide treatment induced the death of *E. granulosus* (purple) with upregulation of the expression of GRP78, caspase-3, and caspase-12 in protoscoleces. PERK, protein kinase RNA-like ER kinase; IRE1, inositol-requiring kinase/endoribonuclease 1; XBP1, X box-binding protein 1; Bip, immunoglobulin heavy chain binding protein; eIF2α, α-subunit of eukaryotic translational initiation factor 2; TRF4, TBP-related factor 4; SLS pathway, spliced leader RNA silencing pathway; DTT, dithiothreitol; DHA, dihydroartemisinin.

components were associated with importing the apicoplast protein, and lose of ERAD components would lead to the death of parasites (Agrawal et al., 2009; Spork et al., 2009). Thus, apicoplast is a potential anti-parasitic drug target. Ubiquitin-dependent ERAD is essential for the survival of *Plasmodium* (Chung et al., 2012). Harbut et al. found that the inhibitors of signal peptide peptidase (SPP, a protein of ERAD) was lethal to *P. falciparum* (Harbut et al., 2012). It was reported that NITD731, a SPP inhibitor, was effective against *T. cruzi* and *T. gondii*, and it showed no toxicity to human cell lines (Harbut et al., 2012). Above studies further showed that parasites were much more sensitive to the disruption of protein homeostasis. Thus, inhibition of the two key quality-control mechanisms, UPR and ERAD, may be a potential way for parasites control.

UPR IN PARASITES IS INVOLVED IN ARTEMISININ RESISTANCE AND RECRUDESCENCE OF *Plasmodium*

As is well known, UPR can restore ER homeostasis. Therefore, when parasites are exposed to external risk factors such as drugs, they are capable of restoring their own homeostasis by inducing ER stress and activating UPR; hence, it is not surprising that ER stress and UPR are involved in the mechanism of drug resistance.

TABLE 1 | Potential drug targets of UPR for treatment of parasitosis.

Inhibitors	Target molecules	Function/mechanism of inhibitor	Effects	References
2-aminopurine	PERK-eIF2α- CHOP pathway	Inhibiting eIF2α phosphorylation and its downstream signaling	Alleviating *T. cruzi* infection induced-heart damage	Ayyappan et al., 2019
TUDCA/Taurine	CHOP-cleaved caspase-12 pathway; GRP78-CHOP pathway	Inhibiting ER stress induced cell apoptosis	Alleviating *T. gondii* infection induced-*Toxoplasma* encephalitis; Alleviating *S. japonicum* infection induced-hepatic granuloma and fibrosis	Wang et al., 2014; Yu et al., 2016
ROP18 inhibitors	ROP18 of *T. gondii*	Inhibiting *T. gondii* infection induced-nerve cell apoptosis by ER stress pathway	Alleviating *Toxoplasma* encephalitis	Wan et al., 2015; Tang et al., 2017; An et al., 2018
GSK2606414	PERK homolog PK4 of *Plasmodium;* PERK homolog TgIF2K-A of *Toxoplasma*	Inhibiting the activation of PK4 and phosphorylation of eIF2α; inhibiting the lytic cycle of tachyzoites	Alleviating the symptoms of malaria, preventing the recurrence of *Plasmodium* and inhibit the transmission of this disease; Inhibiting the invasion, replication and differentiation of *T. gondii*	Zhang et al., 2017; Augusto et al., 2018
apoptozole	GRP78 of *P. falciparum*	Inhibiting GRP78 function	Leading to the death of chloroquine-sensitive and -resistant *P. falciparum* strains	Chen et al., 2018
ER stress inducer	PERK pathway of *Leishmania*; eIF2α of *Plasmodium*; Bip of *T. brucei;* PERK pathway of *T. gondii;* TgIF2α of *T. gondii;* Bip of *Leishmania major*	Inducing eIF2α phosphorylation; Inducing eIF2α phosphorylation of *Plasmodium;* Increasing the expression of Bip of *T. brucei;* Inducing eIF2α phosphorylation of *T. gondii;* Inducing the phosphorylation of TgIF2α; Increasing the expression of Bip of *Leishmania major*	Kill parasites (The parasite is more susceptible to ER stress than host due to the mere presence of the PERK pathway); Participating in the formation of *P. falciparum* gametophytes and the conversion of the *P. berghei;* Inducing programmed cell death of *T. brucei;* Inducing apoptosis or autophagy of *T. gondii;* Inhibiting the differentiation of *T. gondii* from tachyzoite to bradyzoite cysts; Inducing the apoptosis of *Leishmania major*	Narasimhan et al., 2008; Goldshmidt et al., 2010; Dolai et al., 2011; Gosline et al., 2011; Chaubey et al., 2014; Duran-Bedolla et al., 2017; Nguyen et al., 2017; Hao et al., 2019
TbeIF2K2 inhibitors	PERK homolog TbeIF2K2 of *T. brucei*	May suppress the function of sensing protein and regulating protein synthesis near flagellar pocket of *Trypanosome*	Inhibiting the survival of parasites	Gull, 2003; Moraes et al., 2007
PK3 activator	PERK homolog PK3 of *T. brucei*	Increasing ER stress-induced PCD	Lead to the death of *T. brucei*	Hope et al., 2014
Tc-eIF2α phosphorylation inhibitor	Tc-eIF2α of *T. cruzi*	Inhibiting the phosphorylation of Tc-eIF2α	Inhibiting the differentiation of non-infective epimastigotes into infective metacyclic trypomastigotes	Tonelli et al., 2011
TgIF2α phosphorylation inhibitor Salubrinal	TgIF2α of *T. gondii*	Inhibiting the phosphorylation of TgIF2α	Inhibiting the survival of *Toxoplasma* and decreasing virulence to host cell	Narasimhan et al., 2008; Joyce et al., 2010
Bortezomib	GRP78- IRE2/XBP1 pathway of protoscoleces of *E. granulosus*	Inducing ER stress and apoptosis	Reducing the viability of *E. granulosus*	Nicolao et al., 2017
PI3P tubules/vesicles inhibitor	PI3P tubules/vesicles of *Plasmodium*	Inhibiting the formation and diffusion of PI3P tubules/vesicles	Inhibiting UPR mediated artemisinin resistance	Mok et al., 2015; Bhattacharjee et al., 2018

PERK, protein kinase RNA-like ER kinase; IRE1, inositol-requiring kinase/endoribonuclease 1; ATF6, activating transcription factor 6; ATF4, activating transcription factor 4; XBP1, X box-binding protein 1; CHOP, C/EBP-homologous protein; GRP78, Glucose regulated proteins 78; TUDCA, tauroursodeoxycholic acid; ROP18, rhoptry protein 18; eIF2α, α-subunit of eukaryotic translational initiation factor 2; PCD, programmed cell death; PI3P, phosphatidylinositol-3-phosphate.

Artemisinin-based combination therapies (ACTs) are efficient frontline drugs to treat malaria. However, since artemisinin resistance was first discovered *in vivo* in western Cambodia (Dondorp et al., 2009), it gradually become a great challenge in malaria treatment. In recent years, many researches have focused on the mechanism of artemisinin resistance, and some of them have suggested that UPR is an important mechanism for artemisinin resistance. By analyzing 1,043 *P. falciparum* samples isolated from the peripheral blood of patients with acute malaria, Mok et al. (2015) found that artemisinin-resistant parasites exhibited decelerated development in the early ring stage and the expression of two molecular chaperone complexes of UPR were upregulated, such as *Plasmodium* reactive oxidative stress complex (PROSC, BiP belonging to the family) (Haldar et al., 2018) and TCP-1 ring complex (TRiC) (Mok et al., 2015). Thus, they speculated that the decelerated development of artemisinin-resistant *P. falciparum* may be associated with the upregulation of their UPR, which as a proteostatic mechanism that can repair the artemisinin induced impaired protein and reduce artemisinin-induced toxic proteopathy (Mok et al., 2015). Souvik et al. further clarified that the amplified phosphatidylinositol-3-phosphate (PI3P) tubules/vesicles in the parasite's ER in infected red cells extensively spread the proteostatic capacity of UPR, which may neutralize artemisinin's toxic proteopathy and participate in artemisinin resistance (Bhattacharjee et al., 2018). Therefore, ER stress inhibitors or PI3P tubules/vesicles inhibitors may be used in patients with artemisinin resistance.

Zhang et al. (2017) studied the relationship between PK4-eIF2α pathway and recrudescence of *Plasmodium* and found that treatment of ARTs could activate the phosphorylation of PK4-eIF2α and promote latency in the ring stage. Treatment with salubrinal significantly increased the recrudescence rate, while the PK4 inhibitor GSK2606414 abolished recrudescence after ARTs treatment in *P. berghei*-infected mice. Furthermore, they also showed that eIF2α phosphorylation was only observed in the young ring stage of $Dd2^{C580Y}$ but not in Dd2, an ART-sensitive and chloroquine-resistant *Plasmodium* line. This study indicated that the recrudescence of *Plasmodium* was related to the activation of PK4 and phosphorylation of eIF2α following ART treatment. The results show that artemisinin can be combined with PK4 inhibitor to prevent the recurrence of *Plasmodium*.

CONCLUSION AND PERSPECTIVES

Parasitic infection-induced pathological damage in hosts largely depends on ER stress. Therefore, inhibition of ER stress in hosts can be an effective treatment approach for parasitic diseases. In addition, considering that ER stress of parasites participates in their survival, development, and infection, the components or molecules of ER stress of parasites may be used as drug targets to kill or inhibit the development of parasites. The ER stress components or molecules that may be potential targets for the treatment of parasitic diseases are summarized in **Table 1**. Chemical chaperones TUDCA, trehalose, and 4-phenylbutyrate (4-PBA) have been used to reduce ER stress (Hetz et al., 2013), and they may be available for clinical application. Besides, clinical trials have shown that TUDCA therapy improved the sensitivity of insulin in the liver and muscle of insulin-resistant obese patients by affecting ER stress (Kars et al., 2010). Low dose of naltrexone treatment improved the function of epithelial barrier in IBD patients by reducing ER stress (Lie et al., 2018). These studies further demonstrate the feasibility of ER stress inhibitors as a treatment for parasitic diseases. In addition, the selectively target molecules of ER stress are more likely to be used, such as the inhibitors of ER stress molecules that play a key role in the survival and development of parasites (e.g., GSK2606414, PERK inhibitor) (Zhang et al., 2017), or the molecules of ER stress of parasites that are different from the host genes (e.g., GRP78 of *P. falciparum*) (Chen et al., 2018), or the parasites that are more sensitive to ER stress inducers than the host (e.g., DTT-induced ER stress in *Leishmania*) (Gosline et al., 2011). Moreover, because ER stress is involved in drug resistance, the inhibitors of ER stress molecules can be used in combination with anti-parasite drugs, such as ER stress inhibitors or PI3P tubules/vesicles inhibitors that may be used in patients with artemisinin resistance (Mok et al., 2015; Bhattacharjee et al., 2018).

AUTHOR CONTRIBUTIONS

JS advocated writing this review, reviewed, edited, and approved its final version. MP collected literature and wrote the manuscript. FC collected and reviewed literature. ZW provided some suggestions for this review. All authors contributed to the article and approved the submitted version.

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