Advances in Medical Sciences 64 (2019) 315–323

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Review article

Role of endoplasmic reticulum stress and protein misfolding in disorders of the liver and pancreas

Jan Lukas[a], Jola Pospech[b], Christina Oppermann[d], Christina Hund[a], Katharina Iwanov[a], Supansa Pantoom[a], Janine Petters[a], Moritz Frech[a], Susanne Seemann[a], Franziska-Gisela Thiel[c], Jana-Marielle Modenbach[c], Robert Bolsmann[c], Laura de Freitas Chama[c], Franziska Kraatz[c], Firas El-Hage[b], Manuel Gronbach[d], Annelie Klein[e], Regina Müller[f], Sabine Salloch[f], Frank-Ulrich Weiss[c], Peter Simon[c], Preshit Wagh[c], Almuth Klemenz[c], Elke Krüger[g], Julia Mayerle[h,c], Mihaela Delcea[e], Udo Kragl[d], Matthias Beller[b], Arndt Rolfs[a], Markus M. Lerch[c], Matthias Sendler[c,*]

[a] Albrecht-Kossel-Institute for Neuroregeneration, Rostock University Medical Centre, Rostock, Mecklenburg-Vorpommern, Germany
[b] Leibniz-Institute for Catalysis at the University of Rostock, Rostock, Germany
[c] Department of Medicine A, University Medicine Greifswald, Greifswald, Germany
[d] Department of Chemistry, University of Rostock, Rostock, Germany
[e] ZIK HIKE Innovation Center, Humoral Immune Responses in Cardiovascular Disorders, University of Greifswald, Germany
[f] Institute of Ethics and History of Medicine, University Medicine Greifswald, Greifswald, Germany
[g] Department of Medical Biochemistry and Molecular Biology, University Medicine Greifswald, Greifswald, Germany
[h] Department of Medicine II, Ludwigs-Maximilians University Munich, Munich, Germany

ARTICLE INFO

Keywords:
Endoplasmic reticulum associated protein degradation-ERAD
ER stress
Wilson's disease
Pancreatitis
Unfolded protein response

ABSTRACT

The endoplasmic reticulum (ER) is the site of synthesis and folding of membrane and secretory proteins. The fraction of protein passing through the ER represents a large proportion of the total protein in the cell. Protein folding, glycosylation, sorting and transport are essential tasks of the ER and a compromised ER folding network has been recognized to be a key component in the disease pathogenicity of common neurodegenerative, metabolic and malignant diseases. On the other hand, the ER protein folding machinery also holds significant potential for therapeutic interventions. Many causes can lead to ER stress. A disturbed calcium homeostasis, the generation of reactive oxygen species (ROS) and a persistent overload of misfolded proteins within the ER can drive the course of adisease. In this review the role of ER-stress in diseases of the liver and pancreas will be examined using pancreatitis and Wilson´s disease as examples. Potential therapeutic targets in ER-stress pathways will also be discussed.

1. Introduction

Protein misfolding is a field of translational research with increasing importance in basic pathophysiological concepts of gastroenterological diseases [1]. Excessive protein misfolding or the accumulation of defective or superfluous proteins is responsible for the induction of endoplasmic reticulum stress (ER-stress) and activation of the unfolded protein response (UPR) [2]. Massive ER-stress is also an apoptosis inducing signal which directly links UPR to cell death via activation of caspase 12 [3]. A balancing regulation in response to ER-stress is therefore essential for cell survival and may act as a protective mechanism in many disease phenotypes. Three major pathways are involved in the UPR response which regulates the expression of UPR related genes and the induction of cellular protein degradation pathways like autophagy: the inositol requiring enzyme 1α (IRE1α), the protein kinase RNA-like endoplasmic reticulum kinase (PERK) and the activating transcription factor 6 (ATF6). All three proteins share some functions but also induce distinct downstream pathways (Fig.1).

1.1. IRE1α

The release of the ER chaperone GRP-78, also known as binding

* Corresponding author at: Department of Medicine A, University Medicine Greifswald, Forschungscluster III, Fleischmannstr. 41, 17475 Greifswald, Germany.
E-mail address: matthias.sendler@uni-greifswald.de (M. Sendler).

https://doi.org/10.1016/j.advms.2019.03.004
Received 28 September 2018; Accepted 21 March 2019
Available online 09 April 2019

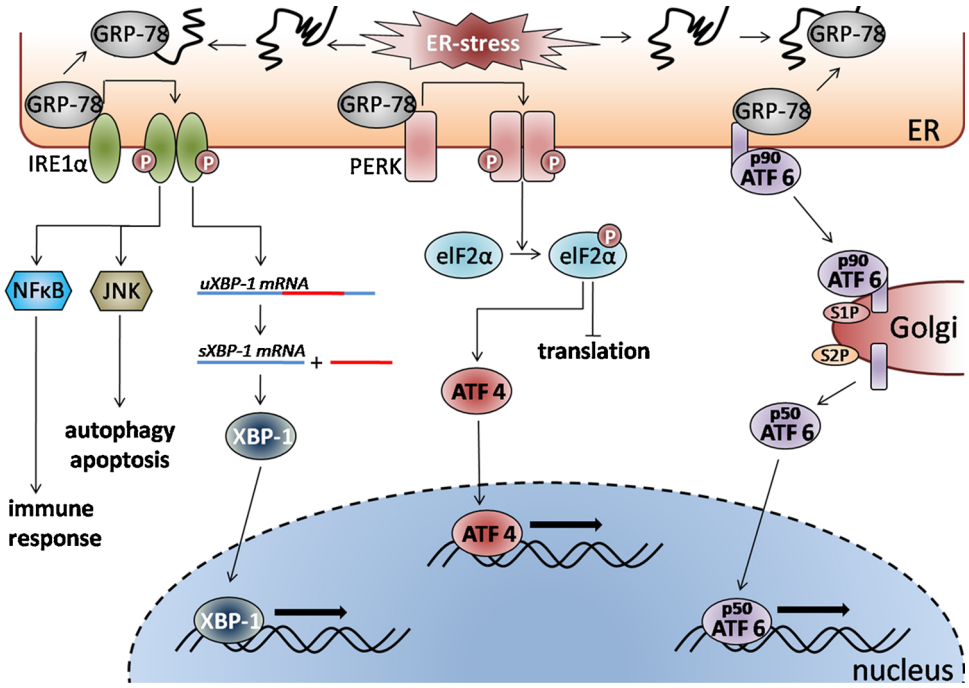

Fig. 1. The three major pathways which recognize ER-stress and induce the unfolded protein response via activation of the transcription factors XBP-1, ATF4 and ATF6.

immunoglobulin protein (BiP) [4], from IRE1α is a consequence of an increase in unfolded or misfolded proteins in the ER-lumen and induces the kinase activity of IRE1α before leading to autophosphorylation and oligomerization of the protein [5]. Activated IRE1α has endoribonuclease activity, which is responsible for the alternative splicing of X-box binding protein 1 (XBP-1) mRNA [6]. XBP-1, a transcription factor of the leucine zipper class of proteins, binds to HLA-DRα and regulates the expression of MHC class II complexes [7]. Alternative splicing of XBP-1 RNA results in a frame shift within the translational region and in synthesis of a 40 kDa XBP-1 s instead of a 33 kDa, XBP1u isoform. This XBP-1 switch regulates the transcription of ER chaperones like GRP-78 or proteins of the ER-associated protein degradation pathway (ERAD) [8]. Beside the autophosphorylation of IRE1α, the cytosolic kinase activity also interacts with the JNK pathway and regulates the induction of autophagy in direct response to UPR activation [9]. This interaction is mediated via TRAF2, which can also activate the NFκB pathway [10]. The nuclear factor NFκB pathway is a prototypical pro-inflammatory signaling pathway which regulates the expression of a plurality of inflammatory genes like cytokines and chemokines. In this manner the interaction between IRE1α and NFκB links ER-stress directly to a pro-inflammatory immune response.

1.2. PERK

PERK is a protein kinase which is located at the ER-membrane. The accumulation of misfolded or unfolded proteins leads to the dissociation of ER-chaperons, such as GRP-78/BiP from PERK and induces the autophosphorylation of the protein [11]. One target of the cytoplasmic kinase domain of PERK is the translation elongation initiation factor-2alpha (eIF2α) which is essential for the initiation of protein translation. The phosphorylation inactivates elF2α which attenuates protein synthesis and prevents additional ER-stress by decreasing the amount of unfolded proteins [12]. Still, not all proteins are affected by the down regulation of translation. Transcription factor 4 (ATF4) is continuously expressed and activates the transcription of proteins of the BCL-2 family and the transcription factor C/EBP homologous protein (CHOP). The growth-arrest- and DNA-damage-induced transcript 34 GADD34, also not influenced by PERK, is able to bind to the serine/threonine protein phosphatase 1 (PP1) and can restore elF2α function by dephosphorylation [13]. The expression of GADD34, as well as the transcription of stress response related genes that prevent ER-stress, or the genes for the resistance to reactive oxygen species, are under the control of the transcription factor ATF4 [14]. The transcription factor CHOP is involved in regulation of apoptosis by transcriptional upregulation of pro-apoptotic genes [15]. The PERK pathway thus provides a direct link to a general, anti-stress response mechanisms as well as to cell death pathways such as apoptosis.

1.3. ATF6

Activation of transcription factor 6 (ATF6) is directly coupled to the ER-membrane by a transmembrane domain. During ER-stress, dissociation of GRP-78 from ATF6 (p90) induces its transport to the Golgi where proteolytic cleavage of the transmembrane part by proteases S1P and S2P release ATF4 (p50) to the cytosol [16]. Mature cytosolic ATF6 translocates to the nucleus and acts as transcription factor for UPR or ERAD pathways similar to CHOP or XBP-1 [17].

Recent data suggest a critical role of protein folding and ER-stress in various diseases of the gastrointestinal tract such as Crohn's disease [18], fatty liver disease [19] or pancreatitis [20]. Misfolding mutations not only lead to a loss of protein function, but their accumulation within the ER is a critical cellular stress signal which can significantly contribute to disease progression. Beside genetic factors also epigenetic factors such as alcohol or cigarette smoking are associated with increased ER-stress and activation of the UPR [21,22].

2. Review

2.1. ER-stress: cause or consequence of pancreatitis

Acute pancreatitis represents a self-digestion of the pancreas by its own digestive proteases. This process is accompanied by local and systemic immune reactions which increase disease severity [23]. The chronic form of pancreatitis (CP) develops from recurrent acute episodes leading to a stepwise replacement of exocrine and endocrine tissue by fibrosis (the so called necrosis fibrosis sequence) which

ultimately results in exocrine pancreatic insufficiency and diabetes [24,25]. Trypsinogen, the inactive precursor form of a pancreatic serine protease, is thought to play a crucial role for the induction of pancreatitis. Premature activation of trypsinogen to trypsin within pancreatic acinar cells by the lysosomal hydrolase cathepsin B, or by enhanced trypsin autoactivation have been suggested to be initial events that cause the onset of acute disease [26]. Genetic data seem to confirm this theory. Mutations within the *PRSS1* gene, which encodes the human cationic trypsinogen, were found to associate with an increased risk of chronic pancreatitis [27,28]. Besides dominant mutations within the *PRSS1* gene, genetic variants in genes involved in trypsinogen processing have been found to increase the risk of developing CP. Mutations within the serine protease inhibitor kazal type 1 (SPINK-1), a potent trypsin inhibitor [29], or chymotrypsin C which is a proteolytic processor of trypsinogen [30], further support the role of trypsinogen in pancreatitis. Recent experimental studies suggest that trypsinogen activation may trigger the onset of disease, but does not influence the immune response or the progression to CP, or tissue replacement by fibrosis [31,32]. It appears that other cellular stress factors, unrelated to proteolytic activities, play important roles in disease onset or progression.

2.1.1. Cellular events resulting in ER-stress in pancreatic acinar cells

Increased ER-stress is thought to be a triggering mechanism, independent of trypsin activity, which can induce acinar cell damage [33]. Pancreatic acinar cells produce large amounts of digestive enzymes and their protein folding and degradation machinery is upregulated under physiological conditions to prevent accumulation of misfolded proteins. ER-stress related transcription factors, such as XBP-1, are also constitutively upregulated and active [34]. Control of protein homeostasis is essential for physiological secretion of digestive enzymes and acinar cell function. ER-related chaperons such as GRP-78 are highly expressed in acinar cells and help to prevent ER-stress [35]. Also heat shock protein HSP-70 is expressed in acinar cells [36] inhibiting intracellular protease activation and acinar cell damage during pancreatitis [37]. Knock down of HSP-70 results in increased disease severity during caerulein induced pancreatitis in mice [37]. This indicates a critical role of the protein folding machinery in disease progression, intracellular protease activation and cell death. Not only chaperons assist in acinar cell protection, but also protein degradation by autophagy is needed to preserve functional protein synthesis and secretion [38]. Autophagy plays an important role in protecting acinar cell function during an episode of acute pancreatitis. Autophagy is a general cellular degradation and recycling mechanism, which is involved in protein homeostasis. During pancreatitis even damaged organelles, such as mitochondria, are removed and their components recycled by autophagosomal degradation [39]. Engulfment of cellular waste and unnecessary proteins by autophagosomes is followed by fusion with lysosomes to autophagolysosomes. The final degradation of proteins is mediated mainly by lysosomal cathepsins. Chaperon mediated autophagy is a special form of protein degradation mechanism which recognizes miss-folded proteins and transports them via lysosomal associated membrane protein 2 (LAMP2) into lysosomal vesicles [40,41]. ER-stress has been shown to induce autophagy in order to decrease accumulated proteins [42]. Animals with pancreas specific deficiency of ATG5, ATG7 or LAMP2 show defective autophagosomal and lysosomal flux which has a severe impact on the integrity of the pancreas [43–45]. During acute pancreatitis the autophagosomal flux has been found to be impaired [45–47]. This results in a decreased ability to degrade damaged or surplus proteins exiting the ER [48]. ER-stress is therefore a consequence of the blockage of pancreatic secretion, and this is thought to contribute to cellular injury. The blockage of secretion, a key event of pancreatitis in acinar cells, and the defective autophagosomal flux together result in a massive protein accumulation which, in turn, affects the integrity of the ER. On the cellular level ER-stress is a result of the earliest pathophysiological events during the disease course, which could be demonstrated in several experimental disease models [33,48–50]. Recent studies reported receptor-mediated autophagosomal degradation of damaged endoplasmic reticulum [51,52], which may play an important role in restoring normal protein turnover. A crosstalk between ER-stress and ER-phagy, the autophagosomal degradation of ER, has recently been described and links ER-stress to autophagy and cell death mechanisms [53].

The blockage of pancreatic secretion, either mechanically by an impacted gallstone [24,54,55] or via a pathological stimulus [26], is a key event during pancreatitis. It leads to a protein traffic jam and a subsequent induction of cellular degradation pathways like autophagy, or proteasomal degradation. Accumulation of proteins within the endoplasmic reticulum triggers transport mechanisms from the ER-lumen into the cytosol. There, proteins are marked for proteasomal degradation by a small 76 aminoacid containing protein – ubiquitin [56]. This process of protein ubiquitination involves three main steps which are catalyzed by E1, the ubiquitin activating enzyme, E2 the ubiquitin converting enzyme and finally E3, the ubiquitin ligase which transfers ubiquitin from E2 to the target protein. Hundreds of different E3 ligases guarantee substrate specific protein degradation [56], and defective proteasomal degradation can be associated with severe clinical phenotypes. Inactivating mutations within the ubiquitin ligase E3 gene *UBR1,* for instance, cause Johanson-Blizzard disease which is characterized by defects in multiple organ systems, including craniofacial abnormalities and major defects of the exocrine pancreas [57], leading to malabsorption and pancreatic insufficiency. Following the N-end rule pathway many digestive enzymes are substrates of UBR1 ligase. Loss of function mutations of the UBR1 gene results in an intrauterine-onset of a destructive form of pancreatitis which emphasizes the importance of a functional degradation apparatus within the secretory compartment of acinar cells [57,58].

ER-stress is also known to induce the release of Ca^{2+} from the endoplasmic reticulum, an intracellular Ca^{2+} storage compartment, into the cytosol. Calcium is a critical intracellular signal transmitter and a sustained increase of cytosolic Ca^{2+} can result in secretion blockage of acinar cells and intracellular protease activation [59]. On the other hand, cytosolic Ca^{2+} can accumulate in mitochondria and induce mitochondrial transition pore opening. As a consequence, a decreased mitochondrial membrane potential and diminished adenosine triphosphate (ATP) generation serve as a trigger for apoptosis [60] or cell necrosis [61]. Notably, mitochondrial damage is associated with decreased autophagy and increased ER-stress [48]. It appears that the ER/mitochondria axis works in both directions and is strongly interconnected in acinar cells. ER-stress can be a consequence of sustained intracellular Ca^{2+} signaling. On the other hand, ER-stress, following an accumulation of misfolded proteins, is a causative trigger factor of acinar cell damage. Compromised UPR or ERAD pathways therefore contribute to disease severity and may play a role in initial acinar cell damage (Fig.2).

2.1.2. ER-stress by misfolding mutations: risk for chronic pancreatitis

The prevalent concept of hereditary pancreatitis is related to mutations within the cationic trypsinogen gene (*PRSS1),* which have been demonstrated to inc rease autoactivation or enhance enzyme activity in vitro [27]. The c.365 G > A (p.R122 H) variant within *PRSS1* enhances trypsinogen autoactivation, whereas a mutation within the same codon, c.364C > T (p.R122C), also associated with pancreatitis, has been regarded as a loss of function mutation [62]. R122C trypsinogen has a misfolded protein structure due to disulfide mispairing in the ER, which is associated with increased ER-stress in the acinar cell. Other *PRSS1* misfolding mutations have been discovered in the last years including c.346C > T (p.R116C) [63] or c.311C > T (p.L104 P) [64,65]. Mutations located within the activation peptide of trypsinogen such as c.65A > G (p.D22 G) or c.68A > G (p.K23R) permit autoactivation of trypsinogen but also diminish enzyme secretion and can therefore elevate ER-stress levels with a subsequent activation of CHOP [63,66]. The

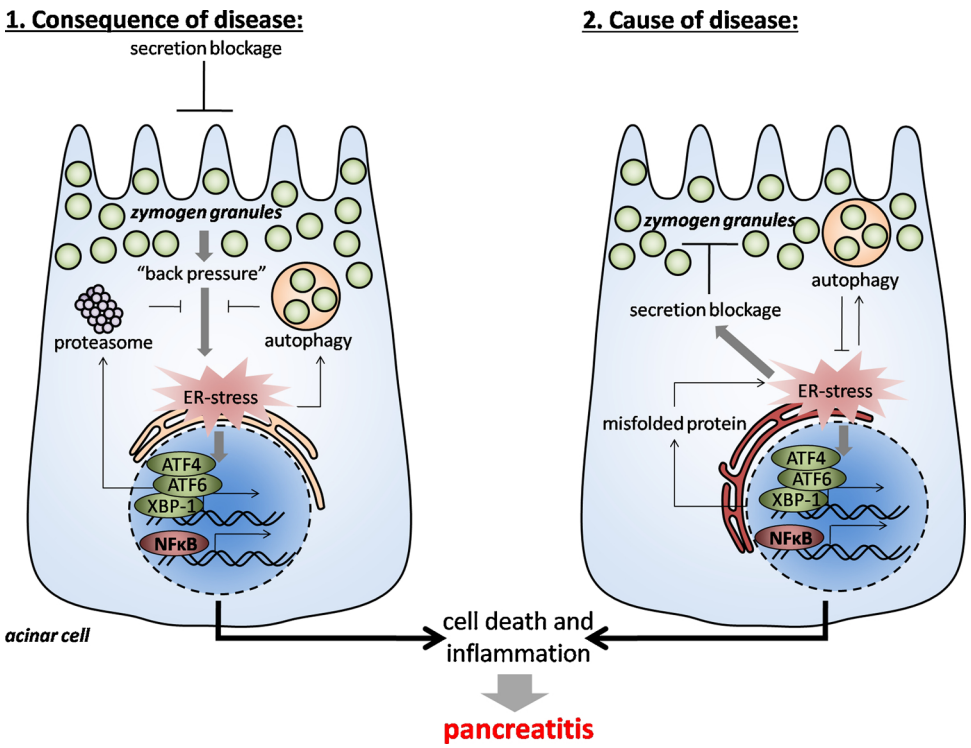

Fig. 2. During pancreatitis ER-stress is induced. On the one hand, it is a consequence of secretion blockage and the accumulation of unnecessary zymogen granules. ER-stress can also be disease causing. Misfolding mutations in highly expressed genes of the secretory pathway like PRSS1, CEL or CPA1 can cause ER-stress which can raise the risk of chronic pancreatitis.

mechanisms behind this observation are poorly understood but it is well known that active trypsin can rapidly degrade poly-ubiquitin residues of proteins marked for proteasomal degradation [67] which leads to an additional accumulation of proteins in acinar cells. This could be one aspect of the observed rise in ER-stress after the onset of experimental pancreatitis in the animal models of the disease [20,68].

Since the initial discovery of the R122H mutation in the *PRSS1* gene, genetic variants in other genes have been identified to be associated with an increased risk of developing chronic pancreatitis. Also for those, ER-stress has been suggested to be a disease triggering mechanism. Hybrid variants (CEL-HYB) of carboxyl ester lipase (CEL) which originate from homologue recombination of the CEL gene and a CEL pseudogene are not efficiently secreted resulting in an increased cellular ER-stress level [69].In the same manner several chymotrypsin C variants (CTRC) have been shown to trigger ER-stress [70]. Loss of function variants of another highly expressed digestive enzyme of the pancreas, carboxypeptidase A1 (CPA1), are strongly associated with an early onset of chronic pancreatitis [71]. The majority of these CPA1 variants has a misfolded phenotype, which is accompanied by decreased enzyme activity and diminished enzyme secretion [72]. In general, misfolded mutations of digestive enzymes are associated with decreased levels of secretion and an increased ER-stress response. The very high expression levels of digestive enzymes in pancreatic acinar cells could explain why mutations in these genes are connected to increased ER-stress in a way that may be causative or permissive for the onset of pancreatitis. ER-stress appears to be a pathomechanism which is independent of protease activity but disease relevant [72].

Beside genetic factors, epigenetic and environmental factors have also been shown to increase ER-stress. It is well known that alcohol consumption can induce ER-stress in hepatocytes [73] and CHOP has been suggested to represent a critical mediator linking ER-stress to hepatocyte cell death in alcoholic liver disease [74]. Alcohol abuse and cigarette smoking are also well established risk factors for the development of chronic pancreatitis [75,76] and recent animal studies imply a critical role of smoking and alcohol on ER-stress induction in pancreatic acinar cells [49]. A combination of ethanol treatment and cigarette smoking strongly activated the PERK pathway in acinar cells and resulted in an upregulation of CHOP [49]. On the other hand, XBP-1 splicing was decreased, which reduces the ER-stress response [49]. Ethanol feeding alone of XBP-1 -/+ mice resulted in increased pancreatic damage because of a diminished ability to induce UPR [50].

In summary, it seems that multiple risk factors can trigger ER-stress and include not only genetic factors such as sequence variants within *PRSS1*, *CPA-1* or *CEL* that give rise to misfolded proteins, but also environmental risk factors such as smoking and alcohol abuse that contribute to increased ER-stress levels. Pancreatitis is a multifactorial disease in which a variety of disease causing factors can combine and act in concert. Recent studies suggest that ER-stress is one of these disease causing factors. A better understanding of the complex ER-stress-related pancreatitis pathophysiology is needed, because UPR and ERAD could be therapeutic targets for the prevention of the disease onset or the reduction of its severity.ER-stress reducing agents could provide a therapeutic option for patients carrying an ER-stress- inducing mutation such as the CEL-HYB or various CTRC variants [30,69]. Patients affected in this manner could benefit from a pharmacological prevention of ER-stress. ER-stress reducing agents could slow the disease progression or the severity of acute episodes, which would ultimately delay pancreatic fibrosis and defer the development of exocrine and endocrine insufficiency. If ER-stress was to be shown to be a general disease-causing mechanism, a prophylactic therapy would become conceivable and could change the natural history of the disease and improve the quality of life of affected patients. This is different in acute pancreatitis; here ER-stress is a consequence of the disease which makes a therapeutic intervention difficult to establish. ER stress-reducing agents would therefore mostly serve as prophylactic agent for patients suffering from chronic or recurrent pancreatitis.

2.2. ER-stress in Wilson´s disease

Wilson´s disease is an inborn error of the copper metabolism. The first description of the clinical symptoms dates back to a case report from 1861, the first series of cases was described in spring 1912 by Samuel Alexander Kinnier Wilson in London [77,78]. In 1948, a perturbation of the copper metabolism was first described as the cause of

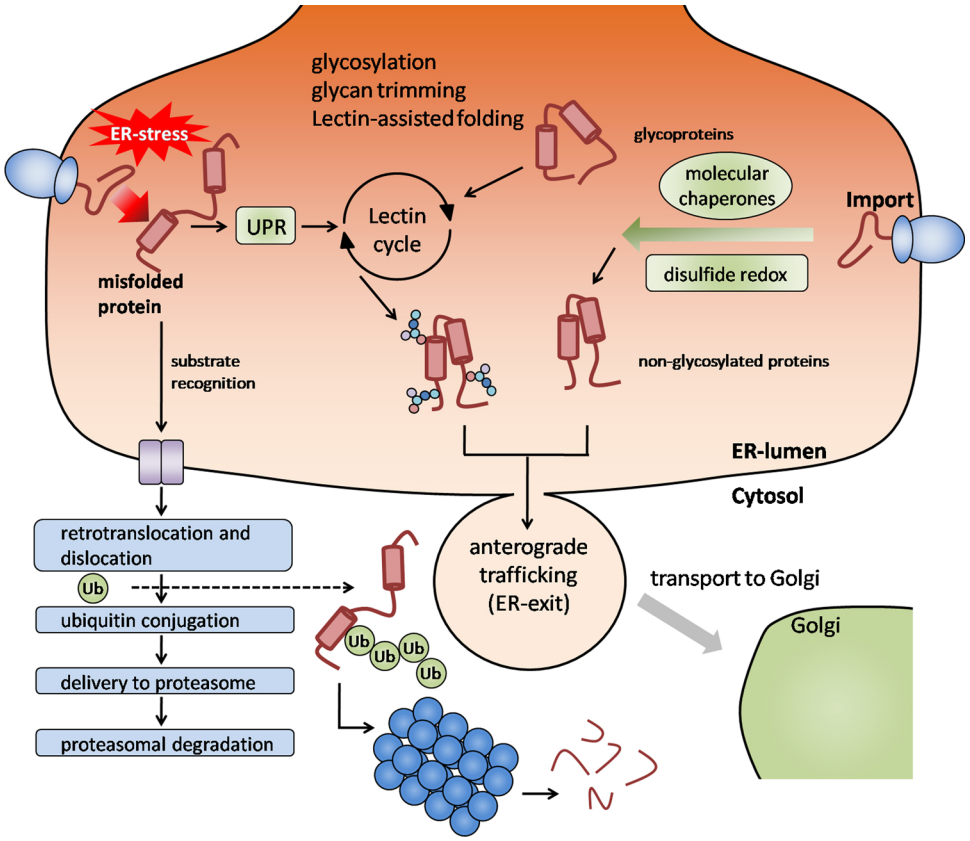

Fig. 3. Protein processing and quality control in the endoplasmic reticulum (ER). Following co-translational import into the ER lumen, nascent polypeptide chains are subjected to sequential quality control, ensuring anterograde trafficking of correctly folded proteins to the Golgi. Precise folding of both glycosylated and non-glycosylated proteins is mediated via reduction of disulphide bonds and assisted by distinct chaperones, including lectins. Misfolded proteins can be redirected to the quality control machinery by the unfolded protein response (UPR) to enable re-folding and prevent ER-stress. Upon increasing accumulation, misfolded proteins are exported from the ER to the cytosol, ubiquitinated and submitted to proteasomal degradation.

the disorders [79]. The disease is caused by a gene defect located on the long arm of chromosome 13 (13q14.3) identified independently by several research groups in 1993 [80–86] coding for copper-carrier ATP7B, also known as Wilson protein. The disease pathophysiology is characterized by the inability of the cells to discharge unused cytosolic copper. Excess copper and copper-derived oxidants contribute to disease progression [87] by influencing a large number of copper-dependent processes in the cell, i.e. oxidative metabolism, free radical detoxification and iron uptake [88]. Copper is normally incorporated into a variety of vital enzymes explaining the vast variety of affected processes. Furthermore, high copper levels were demonstrated to affect the correct function of the proteasome, which leads to the accumulation of ubiquitinated proteins in the cell [89].

ER associated protein processing, quality control involving ERAD of unfolded, misfolded and aggregated proteins (Fig. 3) are well understood for glycosylated proteins, which constitute most of cellular secretory and integral membrane proteins. The membrane-integral ATP7B protein, however, does not undergo glycosylation [90], and the processes facilitating correct folding of non-glycosylated proteins are not fully known. Distinct components of the glycoprotein quality control have been suggested to contribute in monitoring non-glycosylated proteins as well [91,92], potentially within a separate pathway additionally serving as a support machinery under ER stress conditions [93]. The degradation via the ubiquitin proteasome system is a common path without taking into account the glycosylation status of misfolded or aggregated proteins in the ER.

A number of reports provide evidence that protein folding, processing and endocytic trafficking are compromised as part of the Wilson´s disease pathology [94,95]. This finding appears to be largely dependent on the type of mutation. To date, almost 900 ATP7B mutations have been reported (HGMD professional, queried: May 2017) including 471 point mutations that lead to a single amino acid substitution (missense) in the gene product. These mutations may alter protein folding and thus affect proteostasis within the ER. In monogenic diseases, a gene mutation that leads to the expression of a single mutant protein variant can induce the UPR, as is the case of the known gain-of-function mutation P23H in Rhodopsin in *retinitis pigmentosa* [96].There are also a number of examples coming from hepatic disease [97,98]. Nevertheless, the effect on the clinical course of the diseases caused by UPR and ER-stress cannot yet be quantified, even though a relationship between genetically induced protein misfolding and damage to ER homeostasis is evident in a variety of genetic diseases including Wilson´s Disease [99]. It is clear, however, that a permanent ER stress level accelerates the formation of reactive oxygen species, resulting in a self-sustaining fatal cycle that may lead to cellular destruction [100]. The finding that copper-induced disturbance of ER homeostasis was reduced upon administration of chemical chaperones like 4-phenylbutyrate, shown to be effective on the Wilson protein [101], further supports the correlation between oxidative stress, ER stress and hepatotoxicity in Wilson´s disease.

The functions of copper and iron intersect at various points in metabolism and mutually influence the functionality of each other´s pathways [102]. Consequently, related effects were reported on UPR in abnormal copper and iron metabolism [101,103]. The interplay of excess iron, UPR activation and calcium (Ca^{2+}) signaling leads to JNK or CHOP mediated apoptosis [104], indicating again the close connection of the cellular processes involving redox biology in pathophysiology. A systems biology approach showed a number of new targets for pharmacological intervention within the ER homeostasis network related to the p38/JNK-dependent ER quality control [105]. Activation of the p38/JNK ameliorated the course of the disease in Long-Evans Cinnamon (LEC) rats [106]. Importantly, this rodent model of Wilson´s disease carries a genetic defect leading to a complete loss of ATP7B function [107]. Moreover, the increase in levels of the ER-resident molecular chaperone calreticulin (CRT), caused by abnormal iron levels, could suggest iron-induced protein misfolding, possibly secondary to a perturbed Ca^{2+} balance in the ER. The role of CRT in the imbalanced iron metabolism is well examined and CRT has even been

categorized as a cellular marker for clinical iron overload [103].

2.2.1. Polymorphic ATP7B gene and its consequences

As stated above, *ATP7B* has multiple forms and most mutant alleles are private, which means that the mutation is restricted to one or few families. The multi-domain ATP7B protein is composed of an N-terminal copper-binding domain, the transmembrane domain, the ATP-binding domain, and the phosphatase domain [108]. The *ATP7B* gene is widely expressed, but its predominant expression and function is located in the liver [109]. Different splice forms have been localized to distinct cellular compartments [110]. Mutations can thus have various effects. Nucleotide deletions and insertions leading to frameshifts usually have deleterious consequences. Missense mutations within functional residues such as the DKTG motif lead to inability of the protein to execute the copper-induced trafficking between Trans-Golgi Network (TGN) and cell-peripheral loci [111]. Destruction of the copper binding core sequence MxCxxC or the conserved P-Type ATPase-specific TGE, DKTG, TGDN, and MGDGVND motives result in functional impairment of the protein. Multi-disciplinary approaches including systems biological studies, cell-based assays and clinical analysis of patients revealed the molecular defect of individual atypical mutations [112]. However, the specific consequences of hundreds of *ATP7B* mutations have not yet been identified. They may alter folding, stability, transport or subcellular localization of the Wilson protein. In an earlier study by Huster et al. [95], most analyzed Wilson protein variants with the exception of D765 N and the common European variant H1069Q were distributed similarly to the wild type form, while truncated Wilson protein mutants showed an altered, diffuse distribution pattern clearly distinguishable from the wild type protein. H1069Q was found to be retained in the ER and degraded via proteasomal activity [94,113]. Two COOH-terminal mutations, L1373 P and L1373R, displayed a delayed exit from the ER and premature degradation [114]. We have observed an extensive degradation phenotype over a wide range of different mutations utilizing a HEK293H cell over-expression system (Fig. 4).

Importantly an improvement in protein expression and correction of trafficking of *ATP7B* mutations V1262 F, R778 L and H1069Q was achieved, when cells were cultivated at 30 °C instead of 37 °C [115]. This indicates that protein folding in the ER may be a significant milestone in damage assessment of specific Wilson protein mutants. In the same study, curcumin was identified as a chemical chaperone that can functionally increase cellular Wilson protein levels.

In silico predictions whether an *ATP7B* variant is disease-relevant are difficult due to the complex multi-domain organization of the Wilson protein [116]. A large number of novel mutations are still being discovered. Typically, these mutations have to be designated as variants of unknown significance (VUS) as long as no pathological molecular defect or definite clinical phenotype has been established. It has even been disputed, whether variants described as disease-causing mutations really have pathological consequences [114]. Besides the disruption of functional amino acid residues such as ATP- or copper binding sites or mutations that can affect the copper-induced shuttling between TGN and the cell periphery, mutations can abolish folding and the stability of the corresponding protein. The nature of a genetic mutation is not merely suggestive of its contribution to the phenotypic variability [117], it will serve as an important indicator for therapeutic decision making. Even disease-irrelevant polymorphisms are suspected of influencing the phenotypic manifestation of Wilson´s disease, if they are found in alleles with existing Wilson´s disease mutation. Therefore, understanding the exact consequences of ATP7B mutations, especially with reference to the protein folding behavior within the ER and subsequent organelle trafficking, will facilitate the development of advanced mutation-specific therapies.

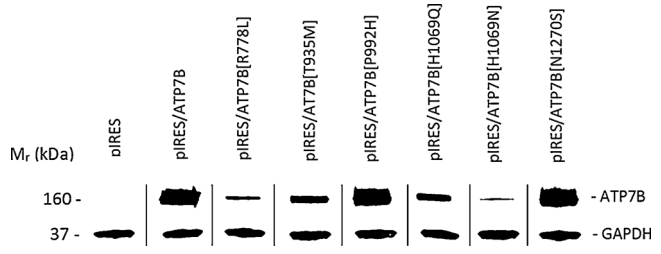

Fig. 4. Different level of ATP7B protein in transfected HEK293H cells. The HEK293 cells were transfected with WT or mutant ATP7B cDNA, lysed, and subjected to Western blot analysis. Most mutations show a clear degradation phenotype. P992H and N1270S appear unaffected. Western blot was carried out using anti-ATP7B (Elabscience, EAP1530; dilution 1:330) and anti-GAPDH (abcam, ab8245; dilution 1:10.000). Both antibodies were applied in TBS-Tween supplemented with 3% skim dry milk.

2.2.2. Potential genetic disease modifiers in Wilson´s disease

Copper Metabolism Murr1 Domain 1 (COMMD1) is a copper chaperone involved in the ATP7B-dependent copper transport. A canine model with a partial COMMD1/MURR1 gene deletion of exon 2 has been reported as showing a Wilson's disease-like phenotype [118,119]. The COMMD family of proteins has been linked to the Ubiquitin pathway of protein degradation. COMMD1 acts via NFκB-mediated regulation of gene transcription [120], whose relation to ER stress is known, even though the mechanism is not well understood [121,122]. Distinct mutants of the Wilson protein showed a stronger binding to COMMD1 [115], possibly indicating a changed lifespan of the protein variants.

A functional rescue of mutant H1069Q was observed when α-crystallin B was over-expressed. The endogenous molecular chaperone protects cells against ER stress-induced apoptosis [123] and is upregulated under excess copper conditions. Counter-regulatory reactions preventing ER-stress are also mediated by the induction of metallothioneins, which have been found to be upregulated especially in the early stages of hepatic Wilson's [124,125].

The presence of modifier genes in Wilson´s disease is indisputable [124,126,127]. However, ER stress is not yet in the focus of clinical assessment for Wilson's disease. There has not yet been much clinical interest in the mainly transcriptionally regulated UPR. Therefore, the assessment of epigenetic factors in a mouse model of Wilson´s disease has been an important contribution to identify the underlying mechanisms of molecular Wilson´s disease [128]. The study involved the investigation of the toxic milk mouse harboring a spontaneous G to A base substitution within exon 8 of the ATP7B gene, leading to a glycine to aspartate substitution in amino acid residue 712 (G712D) inside a putative transmembrane domain. It could be demonstrated that, besides numerous pathways involving oxidative stress functions, both, ubiquitin-mediated proteolysis and the lysosomal pathway are the major dysregulated pathways in Wilson´s disease. Copper clusters form in cellular membranes, such as those of lysosomes [124], and the activation of lysosomal exocytosis stimulates copper clearance and can ameliorate the disease course. This suggests the endosomal/lysosomal system to be an important pathway in copper metabolism and a potential therapeutic target [113]. However, the broad heterogeneity of Wilson´s disease in combination with the considerable diversity of genetic mutations in the ATP7B gene suggests that a single or few mouse models are inadequate to reflect the full spectrum of the disease. For example, it was assumed that specific mutations may influence individual processes of copper recognition, copper-mediated trafficking and copper transport. This may trigger different effects on oxidative phosphorylation, ubiquitination and protein degradation and, hence, contribute to the variable clinical manifestations of clinical Wilson´s disease [88].

Current therapeutic regimens for Wilson´s disease involve penicillamine and the adjunctive N-acetyl cysteine. A recent study found ER-stress and apoptosis related markers elevated as a result of the treatment [129]. Moreover, careful monitoring of risks in patients with neurological symptoms is imperative and the wrong choice of

medication can limit the safety and efficacy [126]. The above reasons justify the continuing need to explore alternative therapeutic approaches to Wilson´s disease. Pharmacological chaperones, for example, appear to reduce the burden of ER stress likely due to a relief of protein overload in the ER lumen [130,131] and, thus, represent an attractive class of active compounds for the treatment of protein folding diseases.

3. Conclusion

It can be postulated that cells affected by permanent ER-stress are exposed to an increased risk for apoptotic and necroptotic processes. Furthermore, ER-stress may promote inflammatory reactions in the surrounding tissues, hence accelerating disease progression or creating secondary damage.

The resulting heterogeneous clinical manifestation impedes diagnosis and may worsen the prognosis for affected patients. A prophylactic therapy of ER-stress could prevent progression to chronic disease in pancreatitis, whereas the use of molecular chaperons, targeting specific misfolding mutations, could reverse the disease phenotype of Wilson's disease.

Conflict of interests

The authors declare no conflict of interests.

Financial disclosure

This work was supported by the European Union (PePPP center of excellence MV: grants ESF/14-BM-A55-0045/16, ESF/14-BM-A55-0046/16, ESF/14-BM-A55-0047/16, ESF/14-BM-A55-0048/16, ESF/14-BM-A55-0049/16, ESF/14-BM-A55-0050/16).

Author contribution

Study Design: Markus M. Lerch, Arndt Rolfs, Matthias Beller, Elke Krüger, Udo Kragel, Mihaela Delcea, Julia Mayerle

Statistical Analysis: n/a

Data Interpretation: n/a

Manuscript Preparation: Jan Lukas, Jola Pospech, Christina Oppermann, Christina Hund, Katharina Iwanov, Supansa Pantoom, Janine Petters, Moritz Frech, Susanne Seemann, Franziska-Gisela Thiel, Jana-Marielle Modenbach, Robert Bolsmann, Laura de Freitas Chama, Franziska Kraatz, Firas El-Hage, Manuel Gronbach, Annelie Klein, Regina Müller, Sabine Salloch, Frank-Ulrich Weiss, Peter Simon, Preshit Wagh, Almuth Klemenz

Literature Search: Jan Lukas, Jola Pospech, Christina Oppermann, Matthias Sendler

Funds Collection: Markus M. Lerch, Arndt Rolfs, Matthias Beller, Elke Krüger, Udo Kragel, Mihaela Delcea, Julia Mayerle, Frank-Ulrich Weiss

References

[1] Lindholm D, Korhonen L, Eriksson O, Kõks S. Recent insights into the role of unfolded protein response in ER stress in health and disease. Front Cell Dev Biol 2017;5:48.
[2] McDuffee AT, Senisterra G, Huntley S, Lepock JR, Sekhar KR, Meredith MJ, et al. Proteins containing non-native disulfide bonds generated by oxidative stress can act as signals for the induction of the heat shock response. J Cell Physiol 1997;171(May (2)):143–51.
[3] Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, et al. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature 2000;403(January (6765)):98–103.
[4] Liu CY, Xu Z, Kaufman RJ. Structure and intermolecular interactions of the luminal dimerization domain of human IRE1alpha. J Biol Chem 2003;278(May (20)):17680–7.
[5] Kaufman RJ. Stress signaling from the lumen of the endoplasmic reticulum: coordination of gene transcriptional and translational controls. Genes Dev 1999;13(May (10)):1211–33.
[6] Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, et al. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature 2002;415(January (6867)):92–6.
[7] Liou HC, Boothby MR, Finn PW, Davidon R, Nabavi N, Zeleznik-Le NJ, et al. A new member of the leucine zipper class of proteins that binds to the HLA DR alpha promoter. Science 1990;247(March(4950)):1581–4.
[8] Lee A-H, Iwakoshi NN, Glimcher LH. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol 2003;23(November (21)):7448–59.
[9] Castillo K, Rojas-Rivera D, Lisbona F, Caballero B, Nassif M, Court FA, et al. BAX inhibitor-1 regulates autophagy by controlling the IRE1α branch of the unfolded protein response. EMBO J 2011;30(September(21)):4465–78.
[10] Tam AB, Mercado EL, Hoffmann A, Niwa M. ER stress activates NF-κB by integrating functions of basal IKK activity, IRE1 and PERK. PLoS One 2012;7(10):e45078.
[11] Harding HP, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 1999;397(January (6716)):271–4.
[12] Iwawaki T, Hosoda A, Okuda T, Kamigori Y, Nomura-Furuwatari C, Kimata Y, et al. Translational control by the ER transmembrane kinase/ribonuclease IRE1 under ER stress. Nat Cell Biol 2001;3(February (2)):158–64.
[13] Brush MH, Weiser DC, Shenolikar S. Growth arrest and DNA damage-inducible protein GADD34 targets protein phosphatase 1 alpha to the endoplasmic reticulum and promotes dephosphorylation of the alpha subunit of eukaryotic translation initiation factor 2. Mol Cell Biol 2003;23(February (4)):1292–303.
[14] Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell 2003;11(March (3)):619–33.
[15] Zinszner H, Kuroda M, Wang X, Batchvarova N, Lightfoot RT, Remotti H, et al. CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 1998;12(April (7)):982–95.
[16] Haze K, Yoshida H, Yanagi H, Yura T, Mori K. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. Mol Biol Cell 1999;10(November (11)):3787–99.
[17] Yoshida H, Okada T, Haze K, Yanagi H, Yura T, Negishi M, et al. ATF6 activated by proteolysis binds in the presence of NF-Y (CBF) directly to the cis-acting element responsible for the mammalian unfolded protein response. Mol Cell Biol 2000;20(September (18)):6755–67.
[18] Kaser A, Lee A-H, Franke A, Glickman JN, Zeissig S, Tilg H, et al. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell 2008;134(September (5)):743–56.
[19] González-Rodríguez A, Mayoral R, Agra N, Valdecantos MP, Pardo V, Miquilena-Colina ME, et al. Impaired autophagic flux is associated with increased endoplasmic reticulum stress during the development of NAFLD. Cell Death Dis 2014;17(April 5):e1179.
[20] Kubisch CH, Logsdon CD. Endoplasmic reticulum stress and the pancreatic acinar cell. Expert Rev Gastroenterol Hepatol 2008;2(April (2)):249–60.
[21] Jorgensen E, Stinson A, Shan L, Yang J, Gietl D, Albino AP. Cigarette smoke induces endoplasmic reticulum stress and the unfolded protein response in normal and malignant human lung cells. BMC Cancer 2008;11(August 8):229.
[22] Galligan JJ, Smathers RL, Shearn CT, Fritz KS, Backos DS, Jiang H, et al. Oxidative stress and the ER stress response in a murine model for early-stage alcoholic liver disease. J Toxicol 2012;2012:207594.
[23] Sendler M, Dummer A, Weiss FU, Krüger B, Wartmann T, Scharffetter-Kochanek K, et al. Tumour necrosis factor α secretion induces protease activation and acinar cell necrosis in acute experimental pancreatitis in mice. Gut 2013;62(March (3)):430–9.
[24] Sendler M, Beyer G, Mahajan UM, Kauschke V, Maertin S, Schurmann C, et al. Complement component 5 mediates development of fibrosis, via activation of stellate cells, in 2 mouse models of chronic pancreatitis. Gastroenterology 2015;149(September (3)):765–76. e10.
[25] Kleeff J, Whitcomb DC, Shimosegawa T, Esposito I, Lerch MM, Gress T, et al. Chronic pancreatitis. Nat Rev Dis Primer 2017;7(September 3):17060.
[26] Halangk W, Lerch MM, Brandt-Nedelev B, Roth W, Ruthenbuerger M, Reinheckel T, et al. Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. J Clin Invest 2000;106(September (6)):773–81.
[27] Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 1996;14(Octuber (2)):141–5.
[28] Witt H, Luck W, Becker M. A signal peptide cleavage site mutation in the cationic trypsinogen gene is strongly associated with chronic pancreatitis. Gastroenterology 1999;117(July (1)):7–10.
[29] Witt H, Luck W, Hennies HC, Classen M, Kage A, Lass U, et al. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. Nat Genet 2000;25(June (2)):213–6.
[30] Rosendahl J, Witt H, Szmola R, Bhatia E, Ozsvári B, Landt O, et al. Chymotrypsin C (CTRC) variants that diminish activity or secretion are associated with chronic pancreatitis. Nat Genet 2008;40(January (1)):78–82.
[31] Dawra R, Sah RP, Dudeja V, Rishi L, Talukdar R, Garg P, et al. Intra-acinar trypsinogen activation mediates early stages of pancreatic injury but not inflammation in mice with acute pancreatitis. Gastroenterology 2011;141(December (6)):2210–7. e2.
[32] Sah RP, Dudeja V, Dawra RK, Saluja AK. Cerulein-induced chronic pancreatitis does not require intra-acinar activation of trypsinogen in mice. Gastroenterology 2013;144(May (5)):1076–85. e2.

[33] Sah RP, Garg SK, Dixit AK, Dudeja V, Dawra RK, Saluja AK. Endoplasmic reticulum stress is chronically activated in chronic pancreatitis. J Biol Chem 2014;289(October (40)):27551–61.
[34] Iwawaki T, Akai R, Kohno K, Miura M. A transgenic mouse model for monitoring endoplasmic reticulum stress. Nat Med 2004;10(January (1)):98–102.
[35] Bruneau N, Lombardo D, Levy E, Bendayan M. Roles of molecular chaperones in pancreatic secretion and their involvement in intestinal absorption. Microsc Res Tech 2000;49(May (4)):329–45.
[36] Bhagat L, Singh VP, Hietaranta AJ, Agrawal S, Steer ML, Saluja AK. Heat shock protein 70 prevents secretagogue-induced cell injury in the pancreas by preventing intracellular trypsinogen activation. J Clin Invest 2000;106(July (1)):81–9.
[37] Bhagat L, Singh VP, Song AM, van Acker GJD, Agrawal S, Steer ML, et al. Thermal stress-induced HSP70 mediates protection against intrapancreatic trypsinogen activation and acute pancreatitis in rats. Gastroenterology 2002;122(January (1)):156–65.
[38] Antonucci L, Fagman JB, Kim JY, Todoric J, Gukovsky I, Mackey M, et al. Basal autophagy maintains pancreatic acinar cell homeostasis and protein synthesis and prevents ER stress. Proc Natl Acad Sci U S A 2015;112(November (45)):E6166–74.
[39] Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008;451(February (7182)):1069–75.
[40] Cuervo AM. Autophagy: in sickness and in health. Trends Cell Biol 2004;14(February (2)):70–7.
[41] Kiffin R, Kaushik S, Zeng M, Bandyopadhyay U, Zhang C, Massey AC, et al. Altered dynamics of the lysosomal receptor for chaperone-mediated autophagy with age. J Cell Sci 2007;120(March (Pt 5)):782–91.
[42] Yorimitsu T, Nair U, Yang Z, Klionsky DJ. Endoplasmic reticulum stress triggers autophagy. J Biol Chem 2006;281(October (40)):30299–304.
[43] Zhou X, Xie L, Xia L, Bergmann F, Büchler MW, Kroemer G, et al. RIP3 attenuates the pancreatic damage induced by deletion of ATG7. Cell Death Dis 2017;8(July (7)):e2918.
[44] Diakopoulos KN, Lesina M, Wörmann S, Song L, Aichler M, Schild L, et al. Impaired autophagy induces chronic atrophic pancreatitis in mice via sex- and nutrition-dependent processes. Gastroenterology 2015;148(March (3)):626–38. e17.
[45] Mareninova OA, Sendler M, Malla SR, Yakubov I, French SW, Tokhtaeva E, et al. Lysosome associated membrane proteins maintain pancreatic acinar cell homeostasis: LAMP-2 deficient mice develop pancreatitis. Cell Mol Gastroenterol Hepatol 2015;1(November (6)):678–94.
[46] Fortunato F, Bürgers H, Bergmann F, Rieger P, Büchler MW, Kroemer G, et al. Impaired autolysosome formation correlates with Lamp-2 depletion: role of apoptosis, autophagy, and necrosis in pancreatitis. Gastroenterology 2009;137(July (1)):350–60. 360-5.
[47] Mareninova OA, Hermann K, French SW, O'Konski MS, Pandol SJ, Webster P, et al. Impaired autophagic flux mediates acinar cell vacuole formation and trypsinogen activation in rodent models of acute pancreatitis. J Clin Invest 2009;119(November (11)):3340–55.
[48] Biczo G, Vegh ET, Shalbueva N, Mareninova OA, Elperin J, Lotshaw E, et al. Mitochondrial dysfunction, through impaired autophagy, leads to endoplasmic reticulum stress, deregulated lipid metabolism, and pancreatitis in animal models. Gastroenterology 2017;23(Octuber).
[49] Lugea A, Gerloff A, Su H-Y, Xu Z, Go A, Hu C, et al. The combination of alcohol and cigarette smoke induces endoplasmic reticulum stress and cell death in pancreatic acinar cells. Gastroenterology 2017;153(December (6)):1674–86.
[50] Lugea A, Tischler D, Nguyen J, Gong J, Gukovsky I, French SW, et al. Adaptive unfolded protein response attenuates alcohol-induced pancreatic damage. Gastroenterology 2011;140(March (3)):987–97.
[51] Mochida K, Oikawa Y, Kimura Y, Kirisako H, Hirano H, Ohsumi Y, et al. Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. Nature 2015;522(June (7556)):359–62.
[52] Khaminets A, Heinrich T, Mari M, Grumati P, Huebner AK, Akutsu M, et al. Regulation of endoplasmic reticulum turnover by selective autophagy. Nature 2015;522(June (7556)):354–8.
[53] Song S, Tan J, Miao Y, Zhang Q. Crosstalk of ER stress-mediated autophagy and ER-phagy: involvement of UPR and the core autophagy machinery. J Cell Physiol 2017;4(August).
[54] Hernández CA, Lerch MM. Sphincter stenosis and gallstone migration through the biliary tract. Lancet Lond Engl. 1993;341(May (8857)):1371–3.
[55] Sendler M, Weiss F-U, Golchert J, Homuth G, van den Brandt C, Mahajan UM, et al. Cathepsin B-Mediated Activation of Trypsinogen in Endocytosing Macrophages Increases Severity of Pancreatitis in Mice. Gastroenterology 2018;154(3):704–18. e10.
[56] Pickart CM, Eddins MJ. Ubiquitin: structures, functions, mechanisms. Biochim Biophys Acta 2004;1695(November (1–3)):55–72.
[57] Zenker M, Mayerle J, Lerch MM, Tagariello A, Zerres K, Durie PR, et al. Deficiency of UBR1, a ubiquitin ligase of the N-end rule pathway, causes pancreatic dysfunction, malformations and mental retardation (Johanson-Blizzard syndrome). Nat Genet 2005;37(December (12)):1345–50.
[58] Zenker M, Mayerle J, Reis A, Lerch MM. Genetic basis and pancreatic biology of Johanson-Blizzard syndrome. Endocrinol Metab Clin North Am 2006;35(June (2)):243–53. vii–viii.
[59] Krüger B, Albrecht E, Lerch MM. The role of intracellular calcium signaling in premature protease activation and the onset of pancreatitis. Am J Pathol 2000;157(July (1)):43–50.
[60] Deniaud A, Sharaf el dein O, Maillier E, Poncet D, Kroemer G, Lemaire C, et al. Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. Oncogene 2008;27(January (3)):285–99.
[61] Gukovskaya AS, Gukovsky I. Which way to die: the regulation of acinar cell death in pancreatitis by mitochondria, calcium, and reactive oxygen species. Gastroenterology 2011;140(June (7)):1876–80.
[62] Simon P, Weiss FU, Sahin-Toth M, Parry M, Nayler O, Lenfers B, et al. Hereditary pancreatitis caused by a novel PRSS1 mutation (Arg-122 –& Cys) that alters autoactivation and autodegradation of cationic trypsinogen. J Biol Chem 2002;277(February (7)):5404–10.
[63] Kereszturi E, Szmola R, Kukor Z, Simon P, Weiss FU, Lerch MM, et al. Hereditary pancreatitis caused by mutation-induced misfolding of human cationic trypsinogen: a novel disease mechanism. Hum Mutat 2009;30(April (4)):575–82.
[64] Balázs A, Hegyi P, Sahin-Tóth M. Pathogenic cellular role of the p.L104P human cationic trypsinogen variant in chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol 2016;310(April (7)):G477–86.
[65] Németh BC, Patai ÁV, Sahin-Tóth M, Hegyi P. Misfolding cationic trypsinogen variant p.L104P causes hereditary pancreatitis. Gut 2017;66(September (9)):1727–8.
[66] Kereszturi E, Sahin-Tóth M. Intracellular autoactivation of human cationic trypsinogen mutants causes reduced trypsinogen secretion and acinar cell death. J Biol Chem 2009;284(November (48)):33392–9.
[67] Burke MC, Wang Y, Lee AE, Dixon EK, Castaneda CA, Fushman D, et al. Unexpected trypsin cleavage at ubiquitinated lysines. Anal Chem 2015;87(August (16)):8144–8.
[68] Kubisch CH, Logsdon CD. Secretagogues differentially activate endoplasmic reticulum stress responses in pancreatic acinar cells. Am J Physiol Gastrointest Liver Physiol 2007;292(June (6)):G1804–12.
[69] Fjeld K, Weiss FU, Lasher D, Rosendahl J, Chen J-M, Johansson BB, et al. A recombined allele of the lipase gene CEL and its pseudogene CELP confers susceptibility to chronic pancreatitis. Nat Genet 2015;47(May (5)):518–22.
[70] Szmola R, Sahin-Tóth M. Pancreatitis-associated chymotrypsinogen C (CTRC) mutant elicits endoplasmic reticulum stress in pancreatic acinar cells. Gut 2010;59(March (3)):365–72.
[71] Witt H, Beer S, Rosendahl J, Chen J-M, Chandak GR, Masamune A, et al. Variants in CPA1 are strongly associated with early onset chronic pancreatitis. Nat Genet 2013;45(October (10)):1216–20.
[72] Sahin-Tóth M. Genetic risk in chronic pancreatitis: the misfolding-dependent pathway. Curr Opin Gastroenterol 2017;33(September (5)):390–5.
[73] Ji C, Kaplowitz N. Betaine decreases hyperhomocysteinemia, endoplasmic reticulum stress, and liver injury in alcohol-fed mice. Gastroenterology 2003;124(May (5)):1488–99.
[74] Ji C, Mehrian-Shai R, Chan C, Hsu Y-H, Kaplowitz N. Role of CHOP in hepatic apoptosis in the murine model of intragastric ethanol feeding. Alcohol Clin Exp Res 2005;29(August (8)):1496–503.
[75] Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology 2013;144(June (6)):1252–61.
[76] Yadav D, Slivka A, Sherman S, Hawes RH, Anderson MA, Burton FR, et al. Smoking is underrecognized as a risk factor for chronic pancreatitis. Pancreatol Off J Int Assoc Pancreatol IAP Al. 2010;10(6):713–9.
[77] Frota NAF, Caramelli P, Barbosa ER. Cognitive impairment in Wilson's disease. Dement Neuropsychol 2009;3(March (1)):16–21.
[78] Wilson SAK. Progressive lenticular degeneration (a familial nervous disease associated with cirrhosis of the liver). Lancet Lond Engl. 1912;1:1115–9.
[79] Cumings JN. The copper and iron content of brain and liver in the normal and in hepato-lenticular degeneration. Brain J Neurol. 1948;71(December (Pt. 4)):410–5.
[80] Yuzbasiyan-Gurkan V, Wagnitz S, Blanton SH, Brewer GJ. linkage studies of the esterase D and retinoblastoma genes to canine copper toxicosis: a model for Wilson disease. Genomics 1993;15(January (1)):86–90.
[81] Houwen RH, Roberts EA, Thomas GR, Cox DW. DNA markers for the diagnosis of Wilson disease. J Hepatol 1993;17(March (3)):269–76.
[82] Yamaguchi Y, Heiny ME, Gitlin JD. Isolation and characterization of a human liver cDNA as a candidate gene for wilson disease. Biochem Biophys Res Commun 1993;197(November (1)):271–7.
[83] Chelly J, Monaco AP. Cloning the Wilson disease gene. Nat Genet 1993;5(December (4)):317–8.
[84] Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P–type ATPase similar to the Menkes gene. Nat Genet 1993;5(December (4)):327–37.
[85] Petrukhin K, Fischer SG, Pirastu M, Tanzi RE, Chernov I, Devoto M, et al. Mapping, cloning and genetic characterization of the region containing the Wilson disease gene. Nat Genet 1993;5(December (4)):338–43.
[86] Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, et al. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. Nat Genet 1993;5(December (4)):344–50.
[87] Medici V, Shibata NM, Kharbanda KK, LaSalle JM, Woods R, Liu S, et al. Wilson's disease: changes in methionine metabolism and inflammation affect global DNA methylation in early liver disease. Hepatology 2013;57(February (2)):555–65.
[88] Riordan SM, Williams R. The Wilson's disease gene and phenotypic diversity. J Hepatol 2001;34(January (1)):165–71.
[89] Hait-Darshan R, Babushkin T, Malik Z. Regulation of heme synthesis and proteasomal activity by copper: possible implications for Wilson's disease. J Environ Pathol Toxicol Oncol 2009;28(3):209–21.
[90] Liu Y, Pilankatta R, Hatori Y, Lewis D, Inesi G. Comparative features of copper ATPases ATP7A and ATP7B heterologously expressed in COS-1 cells. Biochemistry Mosc 2010;49(November (46)):10006–12.
[91] Okuda-Shimizu Y, Hendershot LM. Characterization of an ERAD pathway for nonglycosylated BiP substrates, which require Herp. Mol Cell 2007;28(November (4)):544–54.

[92] Shenkman M, Groisman B, Ron E, Avezov E, Hendershot LM, Lederkremer GZ. A shared endoplasmic reticulum-associated degradation pathway involving the EDEM1 protein for glycosylated and nonglycosylated proteins. J Biol Chem 2013;288(January (4)):2167–78.
[93] Ushioda R, Hoseki J, Nagata K. Glycosylation-independent ERAD pathway serves as a backup system under ER stress. Mol Biol Cell 2013;24(October (20)):3155–63.
[94] Harada M, Sakisaka S, Terada K, Kimura R, Kawaguchi T, Koga H, et al. A mutation of the Wilson disease protein, ATP7B, is degraded in the proteasomes and forms protein aggregates. Gastroenterology 2001;120(March (4)):967–74.
[95] Huster D, Hoppert M, Lutsenko S, Zinke J, Lehmann C, Mössner J, et al. Defective cellular localization of mutant ATP7B in Wilson's disease patients and hepatoma cell lines. Gastroenterology 2003;124(February (2)):335–45.
[96] Lipinski DM, Thake M, MacLaren RE. Clinical applications of retinal gene therapy. Prog Retin Eye Res 2013;32(January):22–47.
[97] Lawless MW, Mankan AK, White M, O'Dwyer MJ, Norris S. Expression of hereditary hemochromatosis C282Y HFE protein in HEK293 cells activates specific endoplasmic reticulum stress responses. BMC Cell Biol 2007;24(July 8):30.
[98] Wang L, Dong H, Soroka CJ, Wei N, Boyer JL, Hochstrasser M. Degradation of the bile salt export pump at endoplasmic reticulum in progressive familial intrahepatic cholestasis type II. Hepatol Baltim Md. 2008;48(November (5)):1558–69.
[99] Guerriero CJ, Brodsky JL. The delicate balance between secreted protein folding and endoplasmic reticulum-associated degradation in human physiology. Physiol Rev 2012;92(April (2)):537–76.
[100] Ozgur R, Turkan I, Uzilday B, Sekmen AH. Endoplasmic reticulum stress triggers ROS signalling, changes the redox state, and regulates the antioxidant defence of Arabidopsis thaliana. J Exp Bot 2014;65(March (5)):1377–90.
[101] Oe S, Miyagawa K, Honma Y, Harada M. Copper induces hepatocyte injury due to the endoplasmic reticulum stress in cultured cells and patients with Wilson disease. Exp Cell Res 2016;347(September (1)):192–200.
[102] Collins JF, Prohaska JR, Knutson MD. Metabolic crossroads of iron and copper. Nutr Rev 2010;68(March (3)):133–47.
[103] Oliveira SJ, de Sousa M, Pinto JP. ER stress and Iron homeostasis: a new frontier for the UPR. Biochem Res Int 2011;2011:1–10.
[104] Lovejoy DB, Guillemin GJ. The potential for transition metal-mediated neurodegeneration in amyotrophic lateral sclerosis. Front Aging Neurosci 2014;23(July) [cited 2018 Apr 3];6. Available from:. http://journal.frontiersin.org/article/10.3389/fnagi.2014.00173/abstract.
[105] Concilli M, Iacobacci S, Chesi G, Carissimo A, Polishchuk R. A systems biology approach reveals new endoplasmic reticulum-associated targets for the correction of the ATP7B mutant causing Wilson disease. Metallomics 2016;8(9):920–30.
[106] Kadowaki S, Meguro S, Imaizumi Y, Sakai H, Endoh D, Hayashi M. Role of p38 mapk in development of acute hepatic injury in long-evans cinnamon (LEC) rats, an animal model of human Wilson's disease. J Vet Med Sci 2013;75(12):1551–6.
[107] Huster D, Finegold MJ, Morgan CT, Burkhead JL, Nixon R, Vanderwerf SM, et al. Consequences of copper accumulation in the livers of the Atp7b−/− (Wilson disease gene) knockout mice. Am J Pathol 2006;168(February (2)):423–34.
[108] Achila D, Banci L, Bertini I, Bunce J, Ciofi-Baffoni S, Huffman DL. Structure of human Wilson protein domains 5 and 6 and their interplay with domain 4 and the copper chaperone HAH1 in copper uptake. Proc Natl Acad Sci 2006;103(April (15)):5729–34.
[109] Buiakova OI, Xu J, Lutsenko S, Zeitlin S, Das K, Das S, et al. Null mutation of the murine ATP7B (Wilson disease) gene results in intracellular copper accumulation and late-onset hepatic nodular transformation. Hum Mol Genet 1999;8(September (9)):1665–71.
[110] Yang XL, Miura N, Kawarada Y, Terada K, Petrukhin K, Gilliam T, et al. Two forms of Wilson disease protein produced by alternative splicing are localized in distinct cellular compartments. Biochem J 1997;326(September (Pt 3)):897–902.
[111] de Bie P, Muller P, Wijmenga C, Klomp LWJ. Molecular pathogenesis of Wilson and Menkes disease: correlation of mutations with molecular defects and disease phenotypes. J Med Genet 2007;44(August (11)):673–88.
[112] Braiterman LT, Murthy A, Jayakanthan S, Nyasae L, Tzeng E, Gromadzka G, et al. Distinct phenotype of a Wilson disease mutation reveals a novel trafficking determinant in the copper transporter ATP7B. Proc Natl Acad Sci. 2014;111(April (14)). E1364–73.
[113] Polishchuk EV, Concilli M, Iacobacci S, Chesi G, Pastore N, Piccolo P, et al. Wilson disease protein ATP7B utilizes lysosomal exocytosis to maintain copper homeostasis. Dev Cell 2014;29(June (6)):686–700.
[114] Braiterman L, Nyasae L, Leves F, Hubbard AL. Critical roles for the COOH terminus of the Cu-ATPase ATP7B in protein stability, trans-Golgi network retention, copper sensing, and retrograde trafficking. Am J Physiol Gastrointest Liver Physiol. 2011;301(July (1)). G69–81.
[115] van den Berghe PVE, Stapelbroek JM, Krieger E, de Bie P, van de Graaf SFJ, de Groot REA, et al. Reduced expression of ATP7B affected by Wilson disease-causing mutations is rescued by pharmacological folding chaperones 4-phenylbutyrate and curcumin. Hepatology 2009;50(December (6)):1783–95.
[116] Laurila K, Vihinen M. Prediction of disease-related mutations affecting protein localization. BMC Genomics 2009;10(1):122.
[117] Dmitriev OY, Bhattacharjee A, Nokhrin S, Uhlemann E-ME, Lutsenko S. Difference in stability of the N-domain underlies distinct intracellular properties of the E1064A and H1069Q mutants of copper-transporting ATPase ATP7B. J Biol Chem 2011;286(May (18)):16355–62.
[118] van De Sluis B, Rothuizen J, Pearson PL, van Oost BA, Wijmenga C. Identification of a new copper metabolism gene by positional cloning in a purebred dog population. Hum Mol Genet 2002;11(January (2)):165–73.
[119] de Bie P, van de Sluis B, Burstein E, van de Berghe PVE, Muller P, Berger R, et al. Distinct Wilson's disease mutations in ATP7B are associated with enhanced binding to COMMD1 and reduced stability of ATP7B. Gastroenterology 2007;133(Octuber (4)):1316–26.
[120] Maine GN, Burstein E. COMMD proteins: COMMing to the scene. Cell Mol Life Sci 2007;64(August (15)):1997–2005.
[121] Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. Nature 2008;454(July (7203)):455–62.
[122] Zhu X, Huang L, Gong J, Shi C, Wang Z, Ye B, et al. NF-κB pathway link with ER stress-induced autophagy and apoptosis in cervical tumor cells. Cell Death Discov 2017;11(September 3):17059.
[123] D'Agostino M, Lemma V, Chesi G, Stornaiuolo M, Cannata Serio M, D'Ambrosio C, et al. The cytosolic chaperone -crystallin B rescues folding and compartmentalization of misfolded multispan transmembrane proteins. J Cell Sci 2013;126(September (18)):4160–72.
[124] Lutsenko S. Modifying factors and phenotypic diversity in Wilson's disease: modifying factors in Wilson's disease. Ann N Y Acad Sci 2014;1315(May (1)):56–63.
[125] Yang L, Wang J, Yang J, Schamber R, Hu N, Nair S, et al. Antioxidant metallothionein alleviates endoplasmic reticulum stress-induced myocardial apoptosis and contractile dysfunction. Free Radic Res 2015;49(Octuber (10)):1187–98.
[126] Bandmann O, Weiss KH, Kaler SG. Wilson's disease and other neurological copper disorders. Lancet Neurol 2015;14(January (1)):103–13.
[127] Gupta A, Das S, Ray K. A glimpse into the regulation of the Wilson disease protein, ATP7B, sheds light on the complexity of mammalian apical trafficking pathways. Metallomics 2018;10(3):378–87.
[128] Medici V, Kieffer DA, Shibata NM, Chima H, Kim K, Canovas A, et al. Wilson Disease: epigenetic effects of choline supplementation on phenotype and clinical course in a mouse model. Epigenetics 2016;11(November (11)):804–18.
[129] Guan D, Xu Y, Yang M, Wang H, Wang X, Shen Z. *N* -acetyl cysteine and penicillamine induce apoptosis via the ER stress response-signaling pathway. Mol Carcinog 2009. n/a-n/a.
[130] Parenti G, Moracci M, Fecarotta S, Andria G. Pharmacological chaperone therapy for lysosomal storage diseases. Future Med Chem 2014;6(June (9)):1031–45.
[131] Mc Donald JM, Krainc D. Lysosomal proteins as a therapeutic target in neurodegeneration. Annu Rev Med 2017;68(January (1)):445–58.