Impeded protein folding and function in active inflammatory bowel disease

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Abstract

The intestinal tract is covered by a total of 300 square metres of IECs (intestinal epithelial cells) that covers the entire intestinal mucosa. For protection against luminal xenobiotics, pathogens and commensal microbes, these IECs are equipped with membrane-bound transporters as well as the ability to secrete specific protective proteins. In patients with active IBD (inflammatory bowel disease), the expression of these proteins, e.g. ABC (ATP-binding cassette) transporters such as ABCG2 (ABC transporter G2) and defensins, is decreased, thereby limiting the protection against various luminal threats. Correct ER (endoplasmic reticulum)-dependent protein folding is essential for the localization and function of secreted and membrane-bound proteins. Inflammatory triggers, such as cytokines and nitric oxide, can impede protein folding, which causes the accumulation of unfolded proteins inside the ER. As a result, the unfolded protein response is activated which can lead to a cellular process named ER stress. The protein folding impairment affects the function and localization of several proteins, including those involved in protection against xenobiotics. In the present review, we discuss the possible inflammatory pathways affecting protein folding and eventually leading to IEC malfunction in patients with active IBD.

Introduction

IECs (intestinal epithelial cells) and their products form a physical barrier between the host and the luminal content. They have to deal with the complex situation where they absorb nutrients and water, and also protect the host from commensal microbes, pathogens and toxic compounds. Various specific adaptations, such as tight junctions and microvilli, as well as specialized IECs such as goblet cells and Paneth cells, enable these cells to carry out their complex task [1].

To protect themselves from toxic compounds, such as xenobiotics, IECs are able to sense, degrade and actively transport various toxic compound out of the cell by specific membrane-bound exporters [2]. Mucosal disorders such as IBD (inflammatory bowel disease) are associated with a disturbance of this well-regulated intestinal homeostasis [3]. One of the cellular processes that seem to be affected by inflammation is ER (endoplasmic reticulum)-dependent protein folding. The present review focuses on how inflammation may affect protein folding and function leading to ER stress followed by IEC malfunction.

Key words: endoplasmic reticulum stress (ER stress), inflammatory bowel disease (IBD), intestinal epithelial cell, protein misfolding, unfolded protein response (UPR), xenobiotic.

Abbreviations used: ABCG2, ATP-binding cassette transporter G2; ER, endoplasmic reticulum; ERAD, ER-associated degradation; HIF1, hypoxia-inducible factor 1; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; PDI, protein disulfide-isomerase; ROS, reactive oxygen species; UPR, unfolded protein response.

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ER stress

Secreted and membrane-bound proteins undergo a folding quality check before they are released from the ER. Un- or mis-folded proteins are extracted from the ER and degraded. This process is called ERAD (ER-associated degradation). However, when un- and mis-folded proteins accumulate inside the ER, the UPR (unfolded protein response) is activated. Upon UPR activation, three major intracellular pathways are activated, namely IRE1 (inositol-requiring enzyme 1), PERK [PKR (double-stranded-RNA-dependent protein kinase)-like ER kinase] and ATF6 (activating transcription factor 6). All three proximal UPR effectors start transcription of genes that are involved in reducing the unfolded protein load in the ER, e.g. ERAD. The mechanism of these three pathways have been described previously [4].

A proportion of proteins, translated in the ER, will always be misfolded due to the complexity of the protein folding process and in the case of high protein production demand. As such, cells with a high secretory activity, e.g. intestinal goblet and Paneth cells, have a constitutively activated ER-stress response. Hence, these cells are extremely sensitive to environmental changes, such as inflammation, which could affect protein synthesis or folding [5]. Interestingly, mutations in genes involved in the ER-stress response are associated with an increased risk for developing IBD [6]. Genetic defects in the ER-stress pathway may therefore contribute to a malfunction of these highly active secretory cells that play a crucial role in intestinal homeostasis.
Intestinal inflammation, protein folding and ER stress

Patients with IBD, which includes Crohn’s disease and ulcerative colitis, are at risk for a chronically inflamed intestinal tract. Active intestinal inflammation is associated with an increased intestinal levels of cytokines, chemokines [7,8] and nitric oxide (NO) [9]. These innate inflammatory responses, such as TNFα (tumour necrosis factor α) and ROS (reactive oxygen species), can directly affect protein folding [10]. At the same time, IEC stimulation is provoked by inflammatory cytokines [11], microbial product recognition by IECs [12] and secreted growth factors [13], which all may increase cellular protein synthesis. These triggers increase the protein production which can be catastrophic by IECs [12] and secreted growth factors [13], leading to intestinal inflammation. A large number of high-impact research papers and reviews have been published on the molecular pathogenesis of inflammation provoked by ER stress, genetic predisposition for ER stress and the ability of ER stress to aggravate inflammation [4–6,14–16]. However, the effect of ER stress on protein expression and function in cells is less well known.

Inflammatory triggers that can provoke ER stress

Chaperones and heat-shock proteins mediate protein folding inside the ER. For example, GRP78 (glucose-regulated protein 78) binds and secures unfolded proteins inside the ER until they are properly folded [17]. The PDI (protein disulfide-isomerase) family catalyses the obligatory disulfide bonds within or between proteins [18]. These helper proteins are crucial for the ER to deal with the high protein concentration. A disturbance in the function of these helper proteins can cause unfolded proteins to accumulate and activate ER-stress pathways.

Interestingly, NO inhibits the function of PDI by S-nitrosylation of its catalytic domains, which leads to the accumulation of unfolded proteins inside the ER and subsequently activates ER stress [19,20]. Furthermore, NO is a small and reactive molecule that can affect protein folding through different approaches as well [21]. Activated innate immune cells in active IBD are associated with an increased NO concentration [9]. The inhibitory effect of high concentrations of NO on protein folding is considered to affect protein folding in IECs during IBD. Other inflammatory triggers such as bacterial cytokotoxins can also affect protein folding and thereby induce ER stress [22].

As discussed above, protein synthesis in general is stimulated by chemokines, cytokines and bacterial compounds [11–13]. It is conceivable that this increased protein synthesis leads to the accumulation of unfolded proteins whereupon ER stress signals are activated.

Other well-described factors that can provoke proteins to misfold are glucose and oxygen deprivation. N-glycosylation of proteins inside the ER is an important post-translational protein modification. N-glycosylation increases the stability and solubility of proteins and protects them from proteases. Before ER-dependent proteins pass through the ER quality check, a large group of linked sugar molecules are added to specific amino acids [23]. Active mucosal inflammation increases the need for nutrients and oxygen as activated immune cells are in considerable need of oxygen and nutrients to defend the mucosa from invading microbes [24]. The increased proliferation of IECs [25] also requires a significant amount of energy. As such, inflammation limits the amount of glucose thereby affecting correct protein folding [26].

In addition to this, intestinal inflammation results in oxygen deprivation, e.g. hypoxia and production of ROS such as NO. The epithelium is protected against inflammation-induced hypoxia by activating the transcription factor HIF1 (hypoxia-inducible factor 1) [27]. Nonetheless, in patients with active IBD, HIF1 is abundantly expressed [28], indicating that actively inflamed intestinal lesions are under severe hypoxia. The exact molecular role of hypoxia during inflammation has been described previously [24,29]. Protein folding inside the ER is dependent on an electron-relay system that requires oxygen as electron acceptor. Folding proteins such as PDI are dependent on oxygen as a terminal electron acceptor in the formation of protein disulfide bonds [30]. Interestingly, HIF1 induces the expression of proteins in the relay of protein disulfide bond formation, providing a link between hypoxia and UPR.

In summary, there are several inflammation-associated triggers that provoke impeded protein folding and eventually lead to UPR and ER stress and these can be found in Figure 1.

Effect of inflammation-induced ER stress

As mentioned above, different specialized proteins protect IECs from toxins present in luminal content. The function and expression of the membrane-bound exporters are depending on proper N-glycosylation and disulfide bonds [31,32]. It has been described previously that the expression of xenobiotic and bile acid transporters, such as P-glycoprotein [MDR1 (multidrug-resistance 1)/ABC1 (ATP-binding cassette transporter B1)] [33] and ABCG2 (ATP-binding cassette transporter G2) [BCRP (breast cancer resistance protein)] [34,35], are decreased in mucosal biopsies from patients with active IBD. This process is linked to a decreased mRNA expression. Despite these findings, the reduced mRNA expression is unlikely to be sufficient to explain the complete loss of protein expression as was seen using immunohistochemical staining [34,35]. Inflammation-induced protein misfolding (ER stress) provides a possible additional mechanism. As the creation of disulfide bonds is inhibited by NO [20,21] and these disulfide bonds are essential for the function of xenobiotic transporters, such as ABCG2 [31,32], it can be assumed that these proteins are not properly folded and therefore reduced in expression during inflammation. A schematic overview of ABCG2 folding and misfolding is shown in Figure 2.
ER-dependent protein folding in IECs of patients with active IBD is affected by several inflammatory triggers.

The function and cellular location of ABCG2 is dependent on proper protein folding. Protein modifications such as inter- and intra-molecular disulfide bonds and N-glycosylation are executed before ABCG2 is localized on the membrane. During active inflammation, the increased NO concentrations and low sugar concentrations affect ABCG2 protein folding whereupon improper folded ABCG2 will be degraded.

This reduction in transporter protein expression indicates that IECs with inflammation-induced ER stress are impeded in their protection from toxic bile acids and other luminal toxic compounds. As a result, the risk for genomic DNA damages and thus eventually neoplastic progression is increased.

In addition to the inflammation-associated decrease in xenobiotic transporter expression, other proteins are also decreased during inflammation. Two non-intestinal examples of affected protein folding induced by NO have been found. Insulin, a small excreted protein with at least three disulfide bonds, is reduced in expression by an increased...
NO concentration triggered by inflammation [36]. A similar reduced protein excretion of vascular endothelial growth factor, a protein with at least five disulfide bonds, has been described under the influence of high NO concentrations [37]. Both unrelated excreted proteins are reduced in expression because of impeded protein folding in the presence of a high NO concentration.

Diarrhoea can be a symptom of patients with IBD, and is caused by changes in electrolyte transport during intestinal inflammation. This is underlined by the fact that membrane-bound sodium transporters and their regulating proteins are decreased in expression during active IBD [38]. Moreover, these sodium transporters, e.g. Na\(^+\)/H\(^+\) exchangers and epithelial Na\(^+\) channel, require multiple amino acid modifications such as N-glycosylation. Nutrition deprivation in the intestinal inflammatory lesions apparently reduces the ability to undergo N-glycosylation of ER-dependent proteins, whereupon unfolded proteins accumulate and ER stress is activated [26]. Thus, upon glucose deprivation, these sodium transporters are likely to be misfolded and degraded from the ER. The inflammation-associated decrease in protein expression of Toll-like receptor 5 [39] and monocarboxylate transporter 1 [40] could be explained by the same mechanism.

Highly active secretory cells, e.g. Paneth cells, are sensitive to changes in protein folding mechanisms because of their constitutive ER stress activation [14]. In addition to the production of mucus-soluble defensins and lysozymes, Paneth cells have important functions in maintaining the stem cell niche in intestinal crypts [41]. Consequently, inflammation-induced protein folding issues inhibit the regular function of Paneth cells and probably diminish intestinal stem cell homeostasis. This will reduce the ability of a fast regenerative IEC seal in covering the inflamed intestinal mucosa, leaving the lamina propria exposed.

**ER stress as therapeutic target for intestinal inflammation**

Studies in metabolic diseases discuss different approaches to regulate ER stress [42]. In the management of IBD, however, there are almost no ER stress-regulating strategies proposed. From studies in metabolic diseases, chemical chaperones, such as 4-phenylbutyric acid, DMSO and bile acid conjugates of ursodeoxycholic acid, are found to provide protein stability and improve the ER protein folding capacity [43]. Inhibition of phosphorylation of the UPR downstream target eIF2\(\alpha\) (eukaryotic initiation factor 2a) by a small molecule named salubralin protects cells from ER stress-associated apoptosis [44]. Although these approaches are promising, their molecular effect on human disease remains unclear. In contrast, conventional therapy for IBD aims to get IBD patients in inflammatory remission. As such, it can be argued that, as a result of reducing inflammatory activity, fewer proteins will misfold and the stress inside the ER is diminished. Thus the question remains whether it is necessary to investigate the possibilities of reducing protein misfolding in IECs specifically for patients with IBD not suffering from a genetic defect in their ER-stress response.

**Conclusions**

Current knowledge provides insight into the substantial effects of impeded protein folding in inflammatory intestinal disease. Xenobiotic transporters such as ABCG2, but also excreted proteins such as lysozyme, are reduced in expression in IECs due to impeded protein folding.

Overall, much more research is needed to fully understand the consequence of impeded protein folding during active IBD. Questions such as why are proteins prone to misfold by high concentrations of NO and which other proteins are affected in expression by misfolding are interesting areas of research.

**References**


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