Animal Nutrition 15 (2023) 225–232

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Review Article

New insights into the unfolded protein response (UPR)-anterior gradient 2 (AGR2) pathway in the regulation of intestinal barrier function in weaned piglets

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ARTICLE INFO

Article history:
Received 8 December 2022
Received in revised form
5 July 2023
Accepted 11 August 2023
Available online 9 September 2023

Keywords:
Weaned piglet
Endoplasmic reticulum stress
Unfolded protein response
Anterior gradient 2
Intestinal barrier function

ABSTRACT

Sustained dysfunction of the intestinal barrier caused by early weaning is a major factor that induces postweaning diarrhea in weaned piglets. In both healthy and diseased states, the intestinal barrier is regulated by goblet cells. Alterations in the characteristics of goblet cells are linked to intestinal barrier dysfunction and inflammatory conditions during pathogenic infections. In this review, we summarize the current understanding of the mechanisms of the unfolded protein response (UPR) and anterior gradient 2 (AGR2) in maintaining intestinal barrier function and how modifications to these systems affect mucus barrier characteristics and goblet cell dysregulation. We highlight a novel mechanism underlying the UPR-AGR2 pathway, which affects goblet cell differentiation and maturation and the synthesis and secretion of mucin by regulating epidermal growth factor receptor and mucin 2. This study provides a theoretical basis and new insights into the regulation of intestinal health in weaned piglets.

1. Introduction

In swine production, bacterial infections are commonly caused by external stress (Wang et al., 2023). A critical period for the postnatal development of the gastrointestinal barrier function in piglets (*Sus scrofa*) is between 3 and 4 weeks of age (Moeser et al., 2017). However, piglets on commercial pig farms are typically weaned from sows at approximately 3–4 weeks of age. In piglets, the gastrointestinal barrier is not yet fully developed and growth retardation, increased diarrhea, gastrointestinal dysfunction, and damage to the intestinal mucosal barrier may be associated with weaning stress (Blavi et al., 2021). Furthermore, post-weaning diarrhea (PWD) in piglets is often caused by enterotoxigenic *Escherichia coli* (ETEC), salmonellosis, and anaerobic spirochetes, particularly under conditions that induce gastrointestinal dysfunction. PWD is the main cause of suboptimal piglet production in the first two weeks after weaning (He et al., 2019), causing huge economic losses to the swine industry worldwide. Recently, strengthening the intestinal barrier function in weaned piglets has become an important research topic.

The small intestine acts as an innate barrier against luminal pathogens and physiological stress in piglets (Ding et al., 2022). Under a stress response, the Kelch-like epichlorohydrin-associated protein 1/nuclear factor erythroid 2-related factor 2 signaling pathway, nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK), and protein kinase B (AKT) pathways activated and generated oxidative stress (Hao et al., 2021; Wang et al., 2023); meanwhile, the NF-κB and MAPK signaling pathways c-Jun N-terminal kinases (JNK) both activated initiation of inflammatory cytokines (Wang et al., 2023), resulting in intestinal mucosal barrier damage, further leading to structural and functional impairments of the small intestinal in piglets.

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2023.08.007

Intestinal homeostasis is a prerequisite for maintaining intestinal function in the small intestine (Ding et al., 2022; Tan et al., 2023). Surprisingly, many studies have shown that cell stress response is linked to endoplasmic reticulum (ER) stress (Gao et al., 2022). In response to ER stress, the deregulation of ER homeostasis can trigger the unfolded protein response (UPR) pathways to restore homeostasis by activating genes involved in protein folding (Yu et al., 2023). For example, the anterior gradient 2 (*AGR2*) gene has recently been determined to be an important regulator of ER stress (Viladomiu et al., 2022). Therefore, it is not surprising that ER stress and UPR signaling are essential for shaping intestinal homeostasis and maintaining intestinal barrier function. However, there are relatively few studies on the UPR-AGR2 pathway in the regulation of intestinal barrier function.

In this study, we review the mechanisms by which endoplasmic reticulum stress (ERS) regulates and maintains the intestinal barrier downstream of the UPR signaling pathway. We also discuss the potentially crucial role of AGR2 in goblet cell differentiation, maturation, and mucin 2 (MUC2) secretion. Finally, we present a novel mechanism underlying the UPR-AGR2 pathway in the regulation of mucus barrier and goblet cell properties.

2. ERS has a potential impact on intestinal barrier function via the downstream UPR signaling pathway in early-weaned piglets

Studies have revealed that an appropriate intestinal barrier function is crucial for maintaining the general wellness of weaned piglets (Modina et al., 2019; Moeser et al., 2017; Pluske et al., 2018). At weaning, the dietary pattern shifted from high-fat/low-carbohydrate breast milk to low-fat/high-carbohydrate feed; approximately 50% of weaning piglets began eating within 24 h, and almost 10% of piglets did not start eating until 48 h after weaning. Within 3–5 d of weaning, the nutrient and energy intake of these piglets decreased significantly (Yang et al., 2016). Early weaned piglets have lower nutrient absorption in their small intestines, resulting in intestinal villous atrophy, crypt hyperplasia, and significantly reduced enzymatic activity of brush border enzymes (e.g., lactose, sucrose, and maltose) in the intestinal epithelium (Chen et al., 2019a, 2019b; Wang et al., 2019; Xiong et al., 2019). Additionally, a reduction in nutrient absorption causes a pronounced decrease in goblet cells and mucus secretion in the small intestine, and the expression levels of intestinal tight junction proteins are reduced, leading to increased intestinal permeability. Bacteria and endotoxins may enter the systemic circulation because of this lack of barrier function. The intestinal immune system responds to various challenges (Pluske et al., 2018), including increased disease susceptibility (Gao et al., 2019; Wang and Ji, 2019). Studies on the potential impact of damage to intestinal barrier function caused by an internal nutritional deficiency in weaned piglets deserve sustained attention.

The ER is the primary organelle in mammalian cells that is responsible for secretory and membrane protein synthesis, folding, and modification. A functioning ER is vital in cells with rapid turnover and metabolism, such as enterocytes and immune cells. A well-developed system of the ER exerts various important cellular functions in the small intestinal epithelial cells of piglets (Oakes and Papa, 2015; Reverendo et al., 2019; Wang and Kaufman, 2014). Early weaning results in several variable conditions, including nutrient deficiency and exogenous or endogenous stressors, that interfere with ER function and create a build-up of unfolded or misfolded proteins in the ER lumen, which is referred to as ER stress (Ma et al., 2017). Even under normal feeding conditions, ERS is often triggered in the intestinal cells of early-weaning piglets (Jiang et al., 2017, 2019). Apoptosis signaling pathways and the proinflammatory response can be activated by severe or persistent ER stress (Cao, 2016; Grootjans et al., 2011), which dysregulates the mucosal immune response to the intestinal microflora (Ma et al., 2017). This ultimately leads to intestinal mucosal barrier damage and dysfunction in weaned piglets (He et al., 2019; Hetz, 2012). The capacity of mammalian cells to improve protein folding and modify or eliminate misfolded proteins through ER-associated signaling pathways for proteolysis is known as the UPR (Kroeger et al., 2019; Vincenz-Donnelly and Hipp, 2017). The UPR is a critical factor in the regulation of intestinal homeostasis (Cao et al., 2013; Hetz, 2012).

There are three primary signaling cascades in the UPR pathways, including inositol requiring enzyme 1α (IRE1α)/X-box binding protein 1 (XBP1), protein kinase RNA-like ER kinase (PERK)/eukaryotic translation initiation factor 2 subunit-α (eIF2α)/activating transcription factor 4 (ATF4)/CCAAT enhancer-binding protein homologous protein (CHOP), and activating transcription factor 6 (ATF6), which are initiated by the ER transmembrane protein sensors IREIα, PERK and ATF6α (Hetz et al., 2020). Through autophagy and ER-associated protein degradation (ERAD) pathways, the activation of the UPR eliminates misfolded proteins, thereby reestablishing ER homeostasis (Hetz et al., 2020) (Fig. 1).

Under homeostatic conditions, IRE1α, PERK, and ATF6 bind to immunoglobulin heavy chain-binding protein (Bip, also known as glucose-regulated protein 78), which maintains them in an inactive state (Hotamisligil, 2010; Ma et al., 2017). Bip dissociates from these three transmembrane proteins in response to ER stress and binds to misfolded or unfolded proteins in the ER, activating downstream signaling pathways such as IRE1α, PERK, and ATF6 (Ma et al., 2017) (Fig. 1).

2.1. IRE1α-XBP1 signaling

IRE1α is activated after its release from Bip via homodimerization and trans-autophosphorylation. Activated IRE1α splices the XBP1 mRNA and generates a functionally active isoform of XBP1 (XBP1s). XBP1s is a transcription factor that regulates the expression of genes encoding components involved in lipid biosynthesis such as ERAD members, ER chaperones, ER translocases, and disulfide isomerases. Tumor necrosis factor receptor-associated factor 2 (TRAF2) is activated by binding to IRE1, resulting in the activation of the JNK pathway (Fig. 1), thereby contributing to pro-apoptotic signaling and an inflammatory response to ER stress (Hetz et al., 2020; Hooper et al., 2019). Studies have revealed that genetic ablation of *Ire1α* in intestinal epithelial cells (IECs) leads to spontaneous colitis, loss of goblet cells, and failure of the intestinal epithelial barrier function in mice (Zhang et al., 2015, 2022a). Mice with IRE1α deficiency are more susceptible to the development of colitis caused by chemicals (Zhang et al., 2022a). The lack of XBP1, the master regulator of the UPR, leads to unresolved ER stress-mediated spontaneous enteritis in mice (Foerster et al., 2022; Kaser et al., 2008), characterized by the loss of Paneth cells, reduced goblet cells, and increased susceptibility to experimental colitis (Adolph et al., 2013; Foerster et al., 2022). This pathway predominantly regulates the secretion of MUC2 in goblet cells (Dai et al., 2022; Lopez-Cauce et al., 2022). These results suggest that IRE1α-XBP1 signaling plays an essential role in regulating intestinal barrier function.

2.2. PERK-eIF2α-ATF4-CHOP signaling

Both homodimerization and trans autophosphorylation activate PERK. By phosphorylating eIF2, activated PERK alleviates the ER burden by attenuating global protein synthesis. PERK-eIF2α induces autophagy via the mammalian target of rapamycin complex 1 (mTORC1) to degrade misfolded proteins (Liu et al., 2019). The

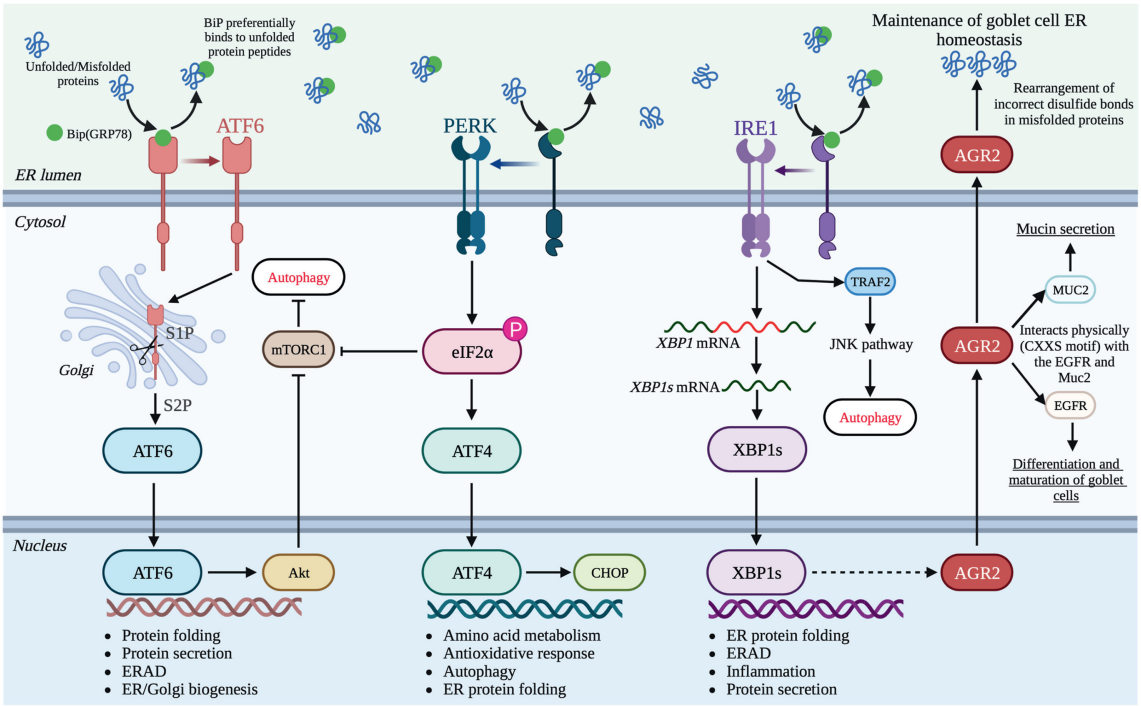

Fig. 1. Major UPR pathways initiated from the ER and the potential role of AGR2 in interacting with EGFR and MUC2. ATF6 = activating transcription factor 6; AKT = protein kinase B; ATF4 = activating transcription factor 4; AGR2 = anterior gradient 2; Bip = immunoglobulin heavy chain-binding protein; CHOP = CCAAT enhancer-binding protein homologous protein; ER = endoplasmic reticulum; eIF2α = eukaryotic translation initiation factor 2 subunit-α; EGFR = epidermal growth factor receptor; ERAD = ER-associated protein degradation; GRP78 = glucose regulated protein 78; IRE1 = inositol requiring enzyme 1; JNK = c-Jun N-terminal kinase; MUC2 = mucin 2; mTORC1 = mammalian target of rapamycin complex 1; PERK = protein kinase RNA-like ER kinase; S1P = site-1 protease; S2P = site-2 protease; TRAF2 = tumor necrosis factor receptor-associated factor 2; UPR = unfolded protein response; XBP1s = X-box binding protein 1s.

transcription factor ATF4 can bypass this inhibition and activate the expression of *Chop*, a master regulator of ER stress-induced apoptosis (Hetz et al., 2020; Hooper et al., 2019) (Fig. 1). Previous studies showed that the protective effects of selenium nanoparticles (SeNPs) on intestinal barrier dysfunction are closely associated with ERS-related PERK-eIF2α-ATF4-CHOP signaling pathway in vitro (Pan et al., 2023; Song et al., 2022b) and in vivo (Qiao et al., 2022). A mouse model with eIF2α mutations in IECs showed increased susceptibility to dextran sulfate sodium (DSS) induced and *Salmonella* infection-associated colitis (Hooper et al., 2019). In addition, the intestinal epithelium of patients with inflammatory bowel diseases has been shown to express CHOP at a higher level than that of the normal epithelium (Rodrigues et al., 2022). Colitis may occur as a result of elevated CHOP expression, which might encourage the invasion of macrophages, leading to the production of reactive oxygen species and interleukin-16 or increased death of epithelial cells (Ma et al., 2017). Similarly, upregulation of CHOP leads to dysfunction of the intestinal epithelial barrier induced by brefeldin A in intestinal porcine epithelial cell line-1 cells (Yang et al., 2022), and inhibiting the expression level of CHOP can relieve ER stress and exert the protective effect of chlorogenic acid on the intestinal barrier (Song et al., 2022a). These findings highlight the importance of the PERK-eIF2α-ATF4-CHOP signaling pathway in regulating intestinal barrier function.

2.3. ATF6 signaling

After disassociation from Bip, ATF6 translocates from the ER to the Golgi apparatus, where it is cleaved by site-1 protease (S1P) and site-2 protease (S2P). The released ATF6 fragment (ATF6f) then translocates to the nucleus and regulates the expression of XBP1 and CHOP. ATF6 has also been implicated in mechanisms that control autophagy. ATF6 activates AKT, resulting in the inhibition of mTORC1 (Benedetti et al., 2022; Hooper et al., 2019) (Fig. 1). Studies have demonstrated that Bip, ATF4, CHOP, and spliced XBP1 levels are increased in ATF6-deficient mice, indicating increased ER stress, which increases the susceptibility to DSS-induced colitis (Hooper et al., 2019). ATF6α-knockout ($ATF6\alpha^{-/-}$) mice display reduced expression of ER chaperone genes (e.g., $P58^{IPK}$) and increased expression of CHOP in the colonic epithelium (Cao et al., 2013). This may lead to a reduction in the number of goblet cells, increased inflammatory cell infiltration, and more severe mucosal damage following DSS challenge (Benedetti et al., 2022; Hooper et al., 2019). Strategies to inhibit the ATF6 signaling pathway may be developed for the treatment of inflammatory bowel diseases (Stengel et al., 2020). The ATF6/CHOP pathway may be associated with intestinal barrier dysfunction during sepsis (Wang et al., 2022). Many studies have shown that inhibition of ATF6 expression can relieve ER stress and exert protective effects on the intestinal barrier function of SeNPs (Pan et al., 2023), chlorogenic acid (Song et al., 2022a), and berberine (Gong et al., 2022). These findings underscore the importance of ATF6 signaling in intestinal barrier function.

In pigs, absorbent enterocytes are primarily responsible for nutritional absorption, whereas secretory cells, such as Paneth and goblet cells, play critical roles in regulating intestinal homeostasis and mucosal immunity (Cai et al., 2019). Goblet cells synthesize and secrete MUC2, a major component of the intestinal mucus layer that protects epithelial cells from bacterial infections (Mccauley and Guasch, 2015). Goblet cells contain abundant rough endoplasmic reticula at the cellular level to adapt to the high demand for synthesis, folding, modification, and secretion of proteins. Mucin secretion may be maintained at equilibrium under physiological conditions because of adequate ER activity in the intestinal epithelium. In pathogenic bacterial infection, the production of MUC2 can be stimulated in goblet cells, thus exerting a significant protein folding and modification burden on the ER (Ma et al., 2017).

In goblet cells, if ER homeostasis cannot be restored, this burden may provide specific hurdles for the ability of proteins to fold, leading to ER stress, activation of UPR survival signaling, or induction of cell death (Cai et al., 2019). Therefore, goblet cells, which synthesize and secrete large quantities of proteins, are highly dependent on normal UPR function, and disturbances in these functions lead to reduced capacity or even loss of secretion in goblet cells, resulting in impaired intestinal barrier function and imbalanced intestinal homeostasis.

Based on the above research, the following important points were proposed: 1) under stressful weaning conditions, piglets consume a limited quantity of feed, and the intestinal epithelium is deprived of nutrients and undergoes a catabolic process that might trigger ER stress and UPR signaling for survival; 2) weaning stress has been linked to oxidative stress and increased inflammatory cytokine production in the small intestine, which are also associated with ER stress and UPR signaling in piglets; and 3) goblet cell autophagy and apoptosis can be activated by ER stress, suggesting a potential role for UPR signaling in weaning stress-related intestinal. Therefore, elucidating the underlying mechanisms between the UPR and MUC2 secretion in goblet cells is crucial, especially for the development of novel research ideas on intestinal barrier function in early weaned piglets.

3. AGR2 has the potential to function as a critical regulator in the maintenance of intestinal barrier function in weaned piglets

AGR2, a member of the protein disulfide isomerase family, is an ER-resident secretory protein highly expressed in goblet cells (Ye et al., 2021). When the UPR is triggered, AGR2 can rearrange incorrect disulfide bonds in misfolded proteins using an active cysteine residue (CXXS motif) in the pseudo thioredoxin domain, thereby assisting in the maintenance of goblet cell ER homeostasis (Delom et al., 2020; Higa et al., 2011; Park et al., 2009) (Fig. 1). Previous studies have reported that AGR2 regulates tight junction protein expression and protects the intestinal mucosal barrier from inflammatory factor-mediated injury (Ye et al., 2021). Furthermore, loss of intestinal mucus and increased susceptibility to DSS-induced colitis have been observed in *AGR2* knockout mice (Park et al., 2009; Viladomiu et al., 2022; Zhao et al., 2010). Recent studies demonstrated the crucial role of AGR2 in preserving goblet cell health, function, and mucus barrier integrity to maintain gut homeostasis (Al-Shaibi et al., 2021). AGR2 deficiency makes goblet cells susceptible to ER stress, and *AGR2* knockout mice show dysfunction and depletion of goblet cells (Al-Shaibi et al., 2021; Ye et al., 2021). These results suggested that AGR2 is crucial for mucus production and mucosal homeostasis, particularly in goblet cells.

3.1. AGR2 has a potentially crucial role in goblet cell differentiation and maturation

Over the past decade, intestinal goblet cells have emerged as central players in the regulation of intestinal health because of their role in providing a protective mucus layer that covers the intestine, sensing changes in the local environment, and shaping gut immunity (Gustafsson and Johansson, 2022). Goblet cells are transient cells that move along the axes of the crypt and crypt villi. The goblet cell population in mice is replaced in approximately 7 d owing to rapid epithelial turnover (Gustafsson and Johansson,

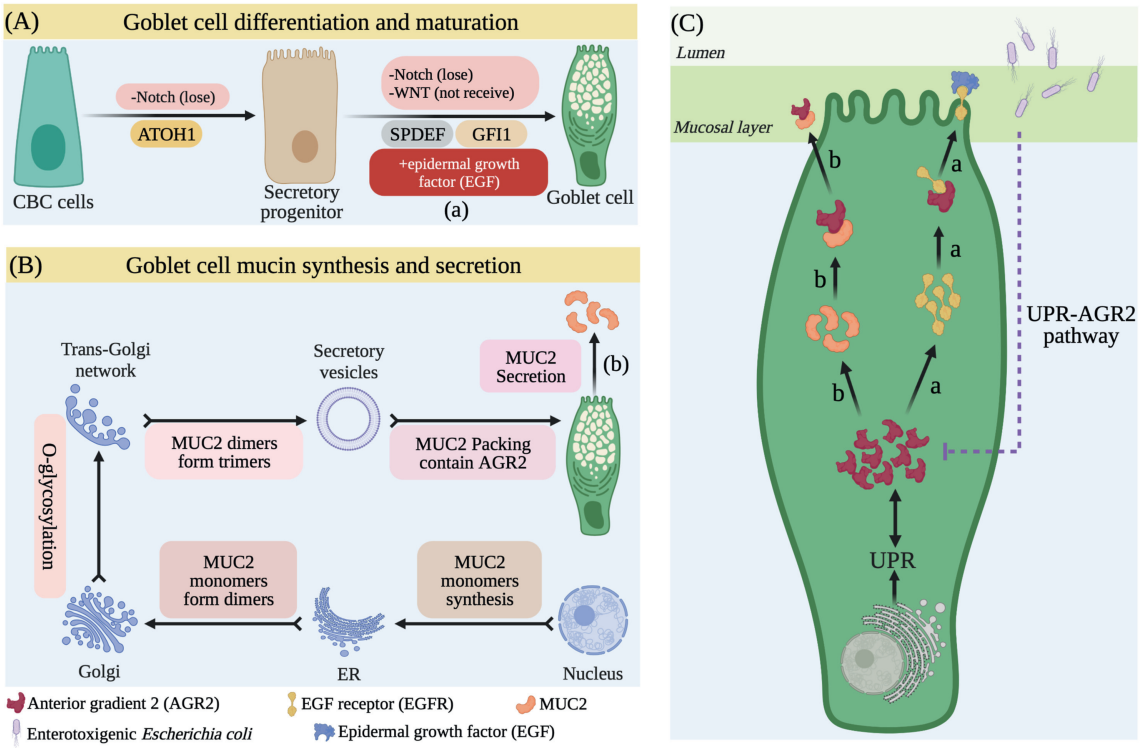

Fig. 2. Potential role of AGR2 as a critical regulator of maintaining intestinal barrier function by affecting the intestinal goblet cell differentiation, maturation, and secretion of MUC2. (A) Differentiation and maturation of goblet cells. (B) Mucin synthesis and secretion by goblet cells. (C) EGF is a key growth factor that binds to its receptor (EGFR) at the plasma membrane to regulate goblet cell differentiation. AGR2 interacts physically (through a CXXS motif) with EGFR within the ER before the receptor can progress to the plasma membrane (a). Physical interaction between AGR2 and MUC2 is a critical step in mucin synthesis and secretion. AGR2 forms disulfide bonds with the N- and C-terminal cysteine-rich sections of MUC2, thereby contributing to mucin synthesis (b). Under ER stress, such as ETEC infection, the UPR-AGR2 signaling pathway is affected, which in turn influences the interaction of AGR2 with EGFR and MUC2. AGR2 = anterior gradient 2; ATOH1 = atonal homolog 1; CBCs = crypt-based columnar cells; GFI1 = growth factor independent 1; SPDEF = sterile alpha motif-pointed domain ETS factor; UPR = unfolded protein response.

2022). AGR2 may protect the mucosa by promoting intestinal epithelial cell proliferation (Ye et al., 2021). Suppression of the Notch and Wnt signaling pathways regulates goblet cell differentiation, resulting in fully differentiated cells in tiny intestinal villi (Beumer and Clevers, 2020; Gustafsson and Johansson, 2022; Koo et al., 2015). The transcription factor atonal homolog 1 (ATOH1) regulates the transcriptional control of crypt-base columnar cells (CBCs) toward secretory progenitor cells, and subsequent goblet cell differentiation is mediated by several transcription factors, including growth factor independent 1 (GFI1) and sterile alpha motif pointed domain ETS factor (SPDEF); in particular, epidermal growth factor (EGF) is very important (Beumer and Clevers, 2020; Gustafsson and Johansson, 2022; Lo et al., 2017; Noah et al., 2010; Yang and Yu, 2021) (Fig. 2A). EGF, which is abundant in maternal milk during the first four weeks after birth, stimulates both goblet cell proliferation and differentiation in pigs (Gustafsson and Johansson, 2022; Wang et al., 2020). EGF plays an important role by binding to its receptor (EGFR) on the plasma membrane (Delom et al., 2020). Recent studies have shown that the delivery of EGFR to the plasma membrane requires AGR2 expression (Dong et al., 2015). It has been established that AGR2 interacts physically (through a CXXS motif) with EGFR within the ER and that this interaction is necessary before the receptor can progress to the plasma membrane (Delom et al., 2020; Dong et al., 2015; Gustafsson and Johansson, 2022; Wodziak et al., 2016) (Fig. 1). Studies have also shown that *AGR2* knockout mice exhibit a decreased number of goblet cells (Hooper et al., 2019; Ma et al., 2017). Overall, these results suggest that AGR2 availability is advantageous for goblet cell differentiation and maturation. However, the underlying mechanism remains unclear.

3.2. *AGR2 is essential for the secretion of the mucin MUC2 in intestinal goblet cells*

The creation of a mucus barrier is a crucial defense that the gastrointestinal epithelium employs to prevent bacterial entry. MUC2, a highly glycosylated gel-forming mucin, is generated in the intestine by goblet cells and forms a multilayered matrix of the mucus barrier (Al-Shaibi et al., 2021; Johansson and Hansson, 2022). MUC2 is a key component of the mucosal layer that covers the surface of gastrointestinal epithelial cells and is the initial line of defense against both symbiotic and pathogenic bacteria (Ye et al., 2021). Mucus production, secretion, and expansion in the lumen of

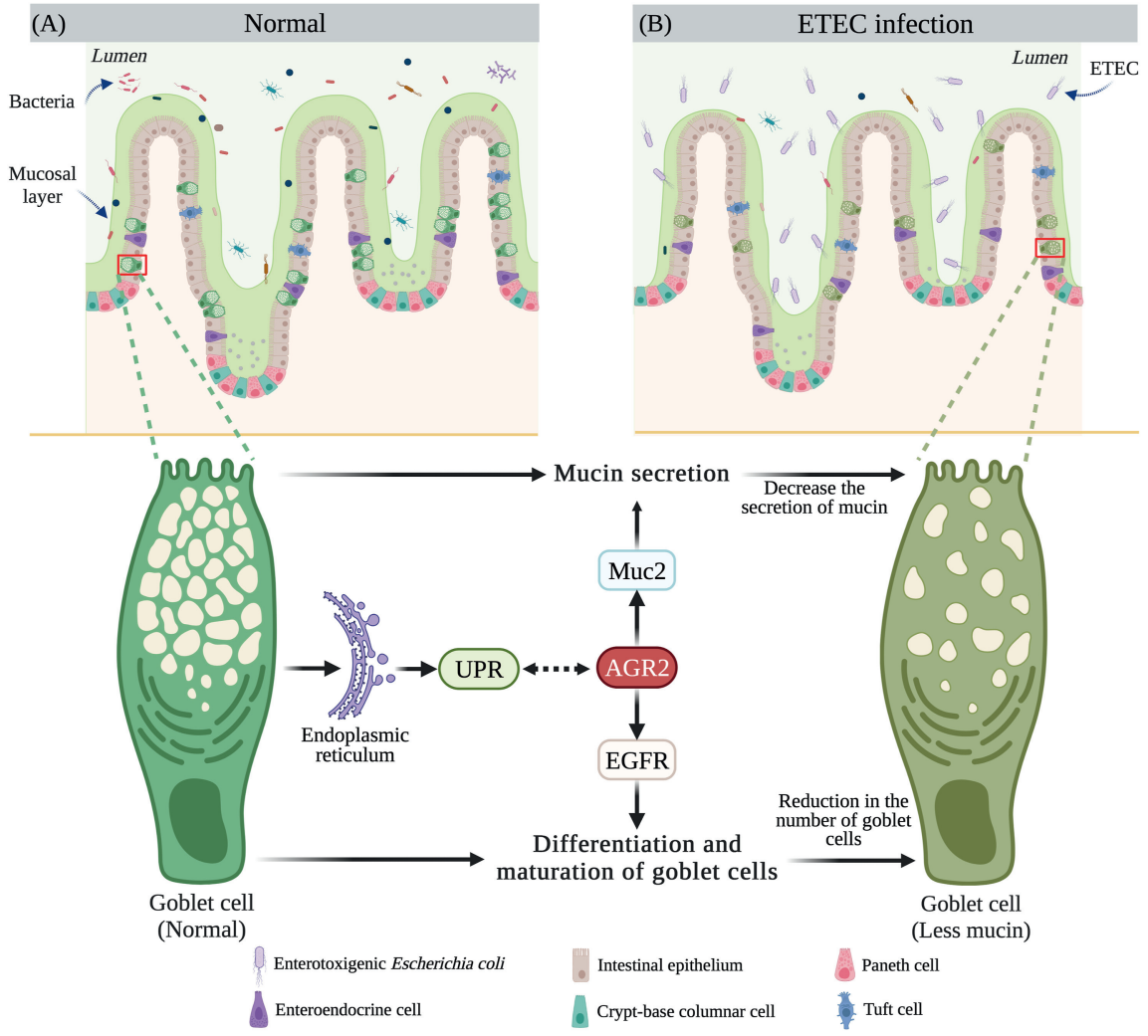

Fig. 3. Potential effect of UPR-AGR2 signaling pathways on goblet cells during ETEC infection. Compared with the normal intestinal environment (A), during ETEC infection (B), the most significant changes were the decreased secretion of mucin and reduced numbers of goblet cells. The UPR-AGR2 signaling pathway could be the chief regulator of these changes during ETEC infection in the intestines of weaned piglets. AGR2 = anterior gradient 2; ETEC = enterotoxigenic *Escherichia coli*; EGFR = epidermal growth factor receptor; MUC2 = mucin 2; UPR = unfolded protein response.

goblet cells involve multiple proteins. Through its cysteine residues, AGR2 forms disulfide bonds with the N- and C-terminal cysteine-rich sections of MUC2, thereby contributing to mucin synthesis (Al-Shaibi et al., 2021; Ye et al., 2021). While migrating from the bottom of the crypt, goblet cells fill their secretory vesicles with MUC2 and other components including AGR2 (Al-Shaibi et al., 2021; Delom et al., 2020; Gustafsson and Johansson, 2022; Paone and Cani, 2020) (Fig. 2B). The deletion of AGR2 has been consistently shown to be associated with decreased MUC2 expression in the intestine (Hooper et al., 2019; Johansson and Hansson, 2022; Ma et al., 2017), but the specific potential molecular mechanism has not been elucidated.

Overall, AGR2 has regulatory roles in goblet cell proliferation and plays a key role in producing sufficient levels of mucus (Fig. 2C). Therefore, the underlying molecular mechanisms by which AGR2 promotes intestinal barrier dysfunction caused by nutrient deprivation and bacterial infection in the intestinal epithelium after weaning of piglets have not yet been elucidated and warrant further investigation.

4. Activation of the UPR-AGR2 pathway may play a role in relieving functional impairment of the intestinal barrier in weaned piglets

In piglets, the intestinal mucosal layer is the first line of defence against commensal bacteria that invade enteric pathogens and natural toxins (Gustafsson and Johansson, 2022). Mucus layer integrity is critical for the gastrointestinal health and function of weaned piglets (Rogers et al., 2023). Therefore, the number of goblet cells and mucin synthesis and secretion are key factors in the maintenance of intestinal barrier mucosal function in weaned piglets, particularly during pathogen infection (Cortez et al., 2020). When goblet cells are infected with ETEC, the ER is in a state of stress and the probability of misfolded mucin increases, resulting in a significant decrease in the synthesis and secretion of MUC2 (Zhang et al., 2022b), which in turn influences the formation of the mucus layer in the intestine (Hansson, 2020; Xiong et al., 2020). Altered intestinal microflora are always present following ETEC infection in weaned piglets (Ren et al., 2020); significant reductions in goblet cell numbers and secreted MUC2 have been observed (Peng et al., 2019; Zhang et al., 2017), as well as marked thinning of the mucus layer (Zhang et al., 2017) (Fig. 3). These findings suggest that ETEC infection impairs the intestinal mucosal barrier in weaned piglets.

Based on the results of the studies summarized above, we propose the following research hypothesis: AGR2 is involved in UPR signaling cascades, and the UPR-AGR2 pathway affects goblet cell differentiation, maturation, and mucin synthesis and secretion through the regulation of EGFR and MUC2. This, in turn, affects the number of goblet cells and integrity of the mucosal layer (Fig. 3). The UPR-AGR2 pathway is a key signaling pathway that maintains the intestinal barrier function in weaned piglets. However, the underlying mechanisms remain unclear and related studies have rarely been reported.

5. Conclusions and perspectives

Over the past decade, intestinal goblet cells have become key participants in controlling intestinal health. Early weaning of piglets susceptible to ETEC infection may result in the dysregulation of goblet cells, and perturbations in mucus barrier properties may contribute to severe PWD in post-weaned piglets. Previous studies have demonstrated that the UPR triggered by ERS in goblet cells has a potential regulatory role in weaning stress-induced intestinal mucosal dysfunction in piglets. AGR2 is involved in the regulation of goblet cell differentiation, maturation, and mucin synthesis and secretion by specifically binding to EGFR and MUC2. The UPR-AGR2 pathway may play a role in relieving functional impairment of the intestinal barrier in weaned piglets.

China banned the addition of antibiotics in livestock feed on July 1, 2020. Determining the types of antibiotic feed substitutes that can improve intestinal mucosal barrier damage in early-weaned piglets has become an urgent issue in weaned piglet production. Natural plants have become a popular topic in the research and application of substitutes for antibiotic growth promoters because of their safety, efficiency, and availability. Exporting the potential mechanisms of bioactive components from natural plants involved in the regulation of the UPR-AGR2 pathway in the maintenance of intestinal barrier function will help improve the intestinal health of weaned piglets and drive the healthy development of the pig industry.

Author contributions

Feng Zhang: conceptualization, writing—original draft preparation, revision, visualization, investigation, and funding acquisition. **Mengxian Chen:** writing, reviewing, editing, and investigation. **Xiaodan Liu:** visualization and investigation. **Xu Ji:** Software, visualization, and investigation. **Shenghe Li:** oversight and leadership responsibility for research activity planning and execution, including mentorship outside the core team. **Erhui Jin:** investigation, management, and responsibility for coordinating research activities, planning, and execution. All authors have read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (32172816), the National Natural Science of Anhui Province (2208085MC77), Excellent Research Innovation Team in Universities in Anhui Province (2022AH010088), and the College Student Innovation and Entrepreneurship Project (202110879058).

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