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REVIEW ARTICLE

Glutamine and intestinal barrier function

Bin Wang · Guoyao Wu · Zhigang Zhou · Zhaolai Dai · Yuli Sun · Yun Ji · Wei Li · Weiwei Wang · Chuang Liu · Feng Han · Zhenlong Wu

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Abstract The intestinal barrier integrity is essential for the absorption of nutrients and health in humans and animals. Dysfunction of the mucosal barrier is associated with increased gut permeability and development of multiple gastrointestinal diseases. Recent studies highlighted a critical role for glutamine, which had been traditionally considered as a nutritionally non-essential amino acid, in activating the mammalian target of rapamycin cell signaling in enterocytes. In addition, glutamine has been reported to enhance intestinal and whole-body growth, to promote enterocyte proliferation and survival, and to regulate intestinal barrier function in injury, infection, weaning stress, and other catabolic conditions. Mechanistically, these effects were mediated by maintaining the intracellular redox status and regulating expression of genes associated with various signaling pathways. Furthermore, glutamine stimulates growth of the small intestinal mucosa in young animals and also enhances ion transport by the gut in neonates and adults. Growing evidence supports the notion that glutamine is a nutritionally essential amino acid for neonates and a conditionally essential amino acid for adults. Thus, as a functional amino acid with multiple key physiological roles, glutamine holds great promise in protecting the gut from atrophy and injury under various stress conditions in mammals and other animals.

Keywords Glutamine · Intestinal barrier function · Nutrition

Abbreviations

ASCT2	Neutral amino acid transporter type 2
ATP	Adenosine triphosphate
BSO	Buthionine sulfoximine
DP	Dipeptidase
EAA	Essential amino acids
4EBP1	Eukaryotic translation initiation factor 4E-binding protein-1
eIF4E	Eukaryotic translation initiation factor 4E
FOXO	Forkhead box O transcription factor
GA	GlutaminaseGALT gut-associated lymphatic tissue
GCL	Glutamate cysteine ligase
GS	Glutamine synthetase
GSH	Glutathione
GSS	Glutathione synthetase
GSSG	Glutathione disulfide
γ-GT	γ-Glutamyl transpeptidase
Ig	Immunoglobulin
IκB	Inhibitor of NF-κB
α-KG	α-Ketoglutarate
IUGR	Intrauterine growth restriction
MAPK	Mitogen-activated protein kinases
MS	Methionine sulfoximine
mTOR	Mammalian target of rapamycin
mTORC	mTOR complex
NADH	Reduced form of nicotinamide adenine dinucleotide

B. Wang · G. Wu · Z. Dai · Y. Sun · Y. Ji · W. Li · W. Wang · C. Liu · F. Han · Z. Wu (✉)
State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing 100193, People's Republic of China
e-mail: bio2046@hotmail.com

G. Wu
Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

Z. Zhou
Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China

NADPH	Reduced form of nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor-κB
NO	Nitric oxide
NOS	NO synthase
p70S6k	p70 Ribosomal protein S6 kinase
PI3K	Phosphatidylinositol 3-kinase
PKB/AKT	Protein kinase B
PPP	Pentose phosphate pathway
Rheb	Ras homologue enriched in brain
ROS	Reactive oxygen species
RTK	Receptor tyrosine kinase
SNAT2	Sodium coupled neutral amino acid transporter 2
TCL	Tricarboxylic acid cycle
TNF-α	Tumor necrosis factor-alpha
TPN	Total parenteral nutrition
Trx	Reduced thioredoxin
TrxSS	Oxidized thioredoxin
TSC	Tuberous sclerosis complex
ZO-1	Zonula occludens protein 1

Introduction

The intestinal mucosal barrier is a single layer of cells lining the gut that mainly consists of the enterocyte membranes and tight junctions between enterocytes (Arrieta et al. 2006). This intestinal epithelium acts as a selective barrier, allowing the transcellular transport of essential dietary nutrients, electrolytes, and water from the intestinal lumen into the circulation and preventing the passage of harmful or unwanted substances (including food antigens, bile, hydrolytic enzymes, endotoxin, microorganisms and their toxins) from entering the internal environment, thereby maintaining the intracellular homeostasis (Jacobi and Odle 2012; Ruth and Field 2013). Dysregulation of the intestinal barrier (also known as loss of intestinal barrier integrity) due to stress, invasion of pathogenic organisms, infection and immunological challenges has been reported to be associated with multiple diseases, such as food allergy, inflammatory bowel disease, celiac disease, irritable bowel syndrome, and type I diabetes through yet unknown mechanisms (Arrieta et al. 2006; Camilleri et al. 2012; Groschwitz and Hogan 2009). Based on this scenario, understanding the molecular mechanisms responsible for alterations and regulation of intestinal barrier function will have important implications for the treatment and prevention of intestinal diseases. The last decade has witnessed accumulating evidence on the beneficial effects of glutamine (Gln) on gut function and health in humans and other animals (Wu et al. 2013). Gln is oxidized by the Krebs cycle to produce ATP for rapid dividing cells (including enterocytes and lymphocytes) (Wu et al. 1995; Wu 1996). Of note, Gln activates the mTOR signaling and increases protein synthesis in enterocytes (Xi et al. 2012), promotes intestinal development, regulates tight junction protein expression and intestinal immunity, inhibits apoptosis induced by oxidative stress or other stimuli (Wu et al.

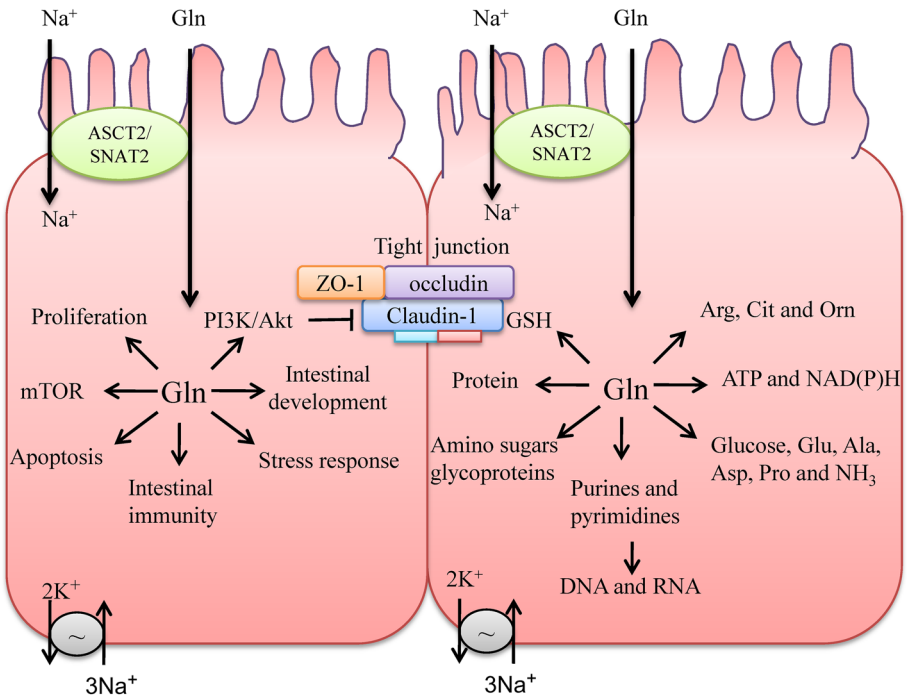

Fig. 1 Glutamine plays important roles in intestinal epithelial cells (see text for detail). *AKT* protein kinase B, *ASCT2* neutral amino acid transporter type 2, *ATP* adenosine triphosphate, *mTOR* mammalian target of rapamycin, *NADH* reduced nicotinamide adenine dinucleotide, *NADPH* reduced form of nicotinamide adenine dinucleotide phosphate, *PI3K* phosphoinositide 3 kinase, *SNAT2* sodium coupled neutral amino acid transporter 2, *ZO-1* zonula occludens protein 1

2013), which are required for gastrointestinal homeostasis (Fig. 1). Also, Gln serves as a major shuttle for the inter-organ transport of both carbon and nitrogen in animals (Curi et al. 2005) and serves as an important precursor for the synthesis of other biological active molecules, such as glutathione (the major non-enzymatic cellular antioxidant), glutamate, proline, arginine and nucleotide synthesis (Wu et al. 2011) (Fig. 1). The main objective of this review is to highlight recent advances in the understanding of the role of Gln in intestinal barrier function and health. For the synthesis, absorption, transportation, and metabolism of Gln in intestinal cells, readers are referred to the comprehensive reviews (Wu et al. 2011; Xi et al. 2011).

Gln and intestinal development

Both enterocytes and intestinal luminal bacteria degrade Gln (Dai et al. 2010, 2012; Wu and Knabe 1994). Approximately 70 % of Gln in the enteral diet is catabolized by the absorptive cells of the small intestine during the first pass (Wu 1998). However, Gln is abundant in plasma, skeletal muscle, fetal fluid and milk, due to endogenous synthesis from branched-chain amino acids and glucose (Wu et al. 2011). The concentration of free Gln in porcine milk markedly increases from <0.1 mM at day 1 of lactation to 4 mM on day 29 of lactation, becoming the most abundant amino acid in sow's milk (Wu and Knabe 1994). The predominance of Gln in sow's milk is consistent with the notion that Gln plays a vital role in the growth and development of the neonatal gastrointestinal tract (Sheard and Walker 1988). In addition, dietary Gln supplementation to early-weaned piglets prevents jejunal atrophy during the first week postweaning (Wu et al. 1996). Moreover, oral administration of Gln (0.5 g/kg of body weight, twice daily) to 7- to 21-day-old suckling piglets enhances intestinal and whole-body growth (Haynes et al. 2009). Thus, milk-borne Gln is insufficient for maximal growth of the neonate. Likewise, supplementing Gln to Gln-free total parenteral nutrition (TPN) solution prevents intestinal atrophy in humans and rats under catabolic conditions (Buchman et al. 1995; Schroder et al. 1995). Similarly, dietary supplementation with Gln or glycyl-Gln dipeptide improves growth performance, small intestinal morphology and immunity response in endotoxin-challenged weaning piglets (Jiang et al. 2009; Yi et al. 2005).

Neonates with intrauterine growth restriction (IUGR) have higher perinatal mortality and morbidity in both humans and animals, including pigs (D'Inca et al. 2011; Rezaei et al. 2011, 2013b). IUGR neonates are characterized by reduced concentrations of Gln in plasma and a relatively thin intestine in both preterm and term neonates of many species (D'Inca et al. 2011; Wu et al. 2011). Alterations in intestinal development impair nutrient absorption and utilization and contribute to IUGR-related high morbidity and mortality (D'Inca et al. 2010, 2011; Wang et al. 2008). Interestingly, oral administration of Gln (1.0 g/kg of body weight per day) between day 0 and day 21 of age enhances the growth of IUGR piglets and reduces post-weaning mortality (Wu et al. 2011). Similar observations have been reported in low-birth-weight infants (Neu et al. 1999), suggesting Gln provision as an effective nutritional strategy to enhance intestinal development in IUGR neonates. All these results indicate a beneficial effect of Gln on postnatal intestinal growth and development.

Gln and intestinal cell proliferation

The epithelium of the mammalian intestine undergoes rapid renewing and turnover every 3–5 days (Camilleri et al. 2012). This process is fulfilled by the fine-tune balance between cell proliferation and loss, thus constituting a homeostasis which is required for the physiological function of the intestinal mucosal barrier (Bach et al. 2000). The underlying regulatory mechanisms may involve multiple signaling and growth factors. It is generally accepted that intestinal stem cells located near the base of the crypts divide rapidly and migrate along the crypt/villus axis and differentiate into absorptive enterocytes, enteroendocrine cells, mucous-secreting goblet cells, tuft cells, and Paneth cells (Barker et al. 2008; Shaker and Rubin 2010).

Gln enhances intestinal cell proliferation through a number of mechanisms. First, oxidation of Gln provides ATP to support intestinal ion transport, cell growth and migration and to maintain intestinal integrity (Curi et al. 2005). Second, Gln is a precursor for the synthesis of purine and pyrimidine nucleotides, which are essential for DNA synthesis and the proliferation of cells (Wu 1998). Third, Gln is a major substrate used to produce glutathione, an antioxidant in cellular environment (Reeds et al. 1997). Fourth, Gln up-regulates the expression of ornithine decarboxylase, a key enzyme for converting ornithine into polyamines, which are required for DNA and protein synthesis (Wu 2013). Fifth, Gln stimulates expression of heat shock proteins (Marc Rhoads and Wu 2009) to promote cell survival. Sixth, Gln regulates cellular signaling pathways for cell proliferation. For example, addition of physiological levels of Gln (0.5 and 2 mM) increases protein synthesis and inhibits protein degradation in enterocytes, resulting in enhanced cell proliferation (Xi et al. 2012). Similarly, Gln has been reported to stimulate protein synthesis in the small intestinal mucosa of humans and rats (Deniel et al. 2007; Rhoads et al. 1997; Ziegler 1994). Finally, Gln enhances expression of genes for mitogen-

activated protein kinases, including both ERK1/2 and JNK, resulting in activation of AP1-dependent gene transcription, thereby contributing to cell proliferation (Rhoads et al. 1997).

Growing evidence shows interactions between Gln and growth factors in intestinal cells. Of note, several growth factors, such as epidermal growth factor (EGF), transforming growth factor, and insulin-like growth factor 1 (IGF-1), may regulate proliferation and differentiation in various cell types, including enterocytes (Booth et al. 1995). It is known that the intestinal epithelium contains receptors for IGFs, including IGF-1 (Rhoads et al. 1997). Gln supplementation may increase protein abundances of these growth factors by modulating rates of protein turnover, and consequently promotes cell proliferation by activating downstream signaling cascades. It has also been reported that EGF up-regulates the activity of Gln transporter B^0/ASCT2 and system $B^{0,+}$ and ASCT2 expression in a multiple kinases (MAPK, PI3K, Rho)-dependent manner in human enterocytes (Avissar et al. 2008), indicating a feedback loop between growth factors and Gln-induced cell signaling. These signaling pathways contribute to Gln-mediated enhancement of intestinal cell proliferation (Rhoads 1999).

Gln and tight junction

Intestinal epithelial cells are tightly bound together by intercellular junctional complexes (Fig. 1) that regulate the paracellular permeability and are crucial for the integrity of the epithelial barrier (de Santa Barbara et al. 2003). To date, four types of junctional complexes have been identified, including tight junctions, adherens junctions, gap junctions, and desmosomes. Tight junctions are the most apical structure of the apical complex demarcating the border between apical and basolateral membrane domains, selectively regulate the passage of molecules and ions via the paracellular pathway, and also restrict the lateral movement of molecules across the cell membrane (Prasad et al. 2005). Adherens junctions are located beneath the tight junctions and are involved in cell–cell adhesion and intracellular signaling (Farhadi et al. 2003; Matter and Balda 2003). Desmosomes and gap junctions contribute to cell–cell adhesion and intracellular communication, respectively. Among the junctional complexes, tight junctions are extensively studied due to its critical role in regulating barrier function and preventing relocation of toxins and pathogens from the gut lumen into mucosal tissue and circulation (Chen et al. 2014; Zhang et al. 2013).

Three types of structural transmembrane components that are enriched at tight junctions include the IgG-like family of junctional adhesion molecules, the claudin, and occludin families of transmembrane proteins (Furuse and Tsukita 2006; Schneeberger and Lynch 2004). Regulation of the assembly, disassembly, and maintenance of tight junction structure are influenced by various physiological and pathological stimuli that activate several kinases, including protein kinase C, mitogen-activated protein kinases (MAPK), myosin light chain kinase, and the Rho family of small GTPases (Ulluwishewa et al. 2011). In general, the activation of protein kinase phosphorylates tight junction protein, leading to alterations of barrier integrity and permeability. It should be noted that the results from different research groups are not always consistent and need further clarification (Findley and Koval 2009). Although the beneficial effect of Gln on intestinal health was documented in the 1990s (Deniel et al. 2007), the regulation of tight junction protein by Gln was reported only in recent years. Gln deprivation or inhibition of Gln synthetase led to significant decreases in transepithelial resistance, an increased permeability, as well as reduced tight junction proteins, claudin-1 and occludin in Caco-2 cells, a classic human cell line model for gut barrier function (DeMarco et al. 2003; Li et al. 2004). Importantly, the dysfunction of gut barrier function can be rescued by Gln addition (DeMarco et al. 2003). Mechanistically, Gln deprivation up-regulates the PI3K/AKT pathway, which, in turn, reduces the abundance of tight junction protein claudin-1, resulting in barrier function breakdown (Li and Neu 2009). It is unknown whether rates of claudin-1 synthesis, degradation, or both processes are affected in the gut. These observations suggest that Gln can directly or indirectly function as regulator of signal pathways associated with gut function. In addition, the protective effect of Gln on tight junction protein is also observed under stress response. For example, it has been reported that Gln prevented the acetaldehyde (a carcinogenic metabolite of ethanol)-induced increase in permeability to endotoxin by ameliorating the disruption of tight junction, adherence junction, and redistributing the tight junction proteins, ZO-1, occludin, E-cadherin, and β-catenin from the intracellular junction complex in Caco-2 cell monolayer (Basuroy et al. 2005; Seth et al. 2004), indicating a therapeutic potential of Gln on carcinoma progression. It should be noted that several lines of studies indicated that endogenous Gln synthesis by Gln synthetase is critical for the beneficial effect on protein expression of tight junction in human Caco-2 monolayer (DeMarco et al. 2003; Li and Neu 2009). However, in our recent study, we found that deprivation of Gln decreased monolayer transepithelial resistance which can be reversed by the provision of Gln to the culture medium in the absence of the Gln synthetase inhibitor in intestinal porcine epithelial cells. This

suggests that the amount of endogenous Gln synthesized by Gln synthetase is much less than that of the Caco-2 cells used in the previous study (DeMarco et al. 2003; Li and Neu 2009). Indeed, Gln synthetase activity is negligible in enterocytes of neonatal pigs (Haynes et al. 2009) and lactating sows (Li et al. 2009). Another reason for this discrepancy might be due to metabolic, genetic, or epigenetic differences between cancer cells and normal intestinal epithelial cells (Meadows et al. 2008). In a recent study, Noth and his colleague demonstrated that oral Gln supplementation ameliorated intestinal permeability dysfunction as shown by increased occludin expression, reduced gastrointestinal permeability and reduced apoptotic cells in the crypt (Noth et al. 2013). All these in vivo and in vitro data suggest an important role for tight junction in the maintenance of gut function. Regulation of tight junction proteins by Gln is responsible, at least partially, for the beneficial effect of Gln on gut barrier function in humans and animals.

Gln and stress response

It has become increasingly clear that environmental factors, including stress and diets, play a crucial role in gastrointestinal health and defense against important intestinal disorders in both humans and animals (Arrieta et al. 2006; Wijtten et al. 2011). Several lines of evidence from epidemiological and basic animal research studies showed an important role of stress in disruption of intestinal barrier function, as well as the development and clinical onset of many gastrointestinal disorders (Smith et al. 2010; Wijtten et al. 2011). The inverse correlation between plasma concentration of Gln and intestinal barrier function upon weaning stress (Wang et al. 2008) or endotoxin challenge (Haynes et al. 2009) suggests a beneficial effect of Gln on improving intestinal mucosal integrity. Moreover, stresses occurring early life can have a long-lasting effect on gastrointestinal health in adult life (Gareau et al. 2007; Moeser et al. 2007; Soderholm et al. 2002). For example, the

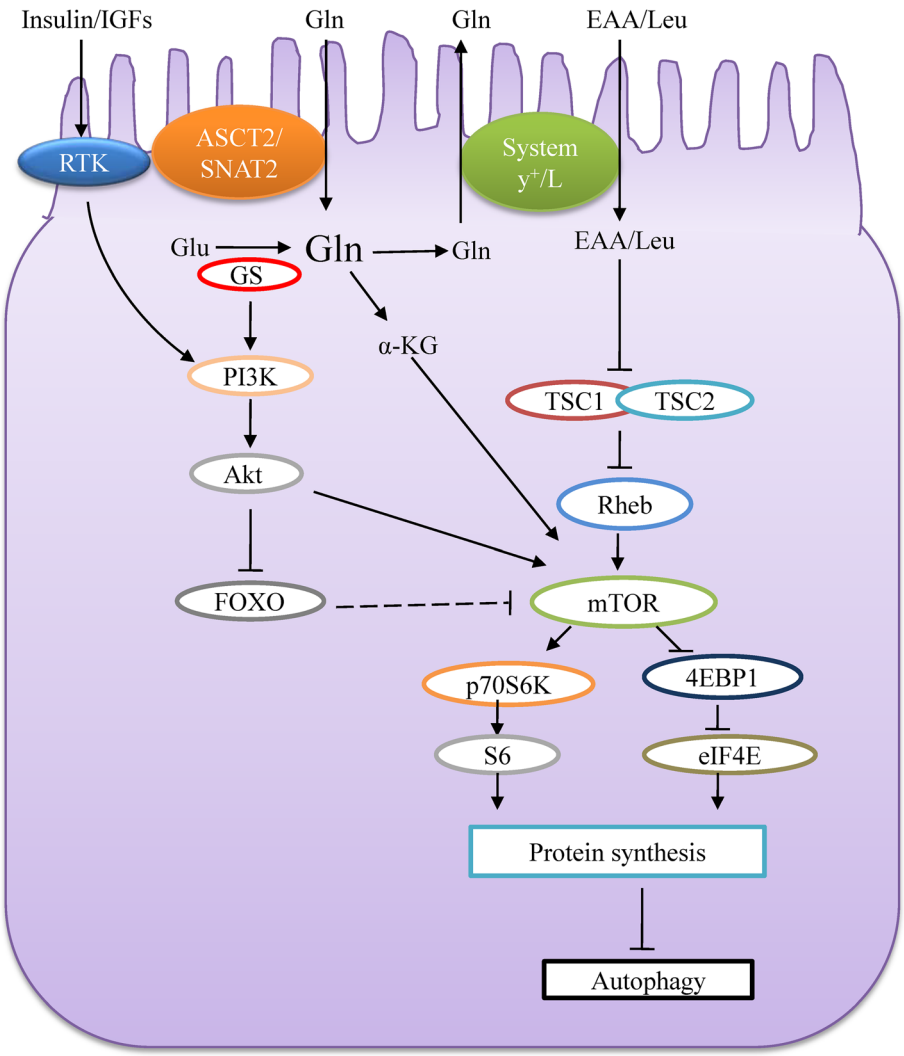

Fig. 2 Proposed signaling pathway of glutamine-mediated cell growth and autophagy by mTOR pathway (see text for details). *α-KG* α-ketoglutarate, *ASCT2* neutral amino acid transporter type 2, *SNAT2* sodium coupled neutral amino acid transporter 2, *EAA* essential amino acids, *4EBP1* eukaryotic translation initiation factor 4E-binding protein-1, *eIF4E* eukaryotic translation initiation factor 4E, *FOXO* forkhead box O transcription factor, *GS* glutamine synthetase, *IGFs* insulin-like growth factors, *mTORC1* mammalian target of rapamycin complex 1, *mTORC2* mammalian target of rapamycin complex 2, *p70S6K* p70 ribosomal protein S6 kinase, *PKB* protein kinase B, *PI3K* phosphoinositide 3 kinase, *Rheb* Ras homologue enriched in brain, *RTK* receptor tyrosine kinase, *TSC* tuberous sclerosis comple

process of weaning is one of the most stressful events in the life of pigs, which contributes to intestinal and immune system dysfunction (Campbell et al. 2013; Wijtten et al. 2011). Early-weaned pigs commonly experience diarrhea, reduced food intake during the first 7–14 day postweaning, and impaired mucosal barrier function, as indicated by reductions in jejunal transepithelial electrical resistance and elevations in paracellular permeability (Campbell et al. 2013). The intestinal dysfunction is associated with increased concentrations of corticotrophin-releasing factor and adrenal cortisol (the major glucocorticoid in swine) in plasma (Smith et al. 2010), as well as abnormal activation of mast cells in the intestinal mucosa (Crowe and Perdue 1992). Appropriate activation of mast cell contributes to innate and acquired immunity and is important for host defense, by releasing such mediators as histamine, cytokines, proteoglycans, and proteases (Ramsay et al. 2010). However, over-activation of mast cells and related bioactive mediators exerts a profound influence on intestinal function, including increased intestinal permeability and visceral hypersensitivity (Ramsay et al. 2010). Further studies have identified mast cell-derived proteases Mcpt4 as a critical factor that regulate intestinal epithelial migration and barrier function (Groschwitz et al. 2009). Gln supplementation reduces the production of cortisol and ameliorates mucosal barrier dysfunction in weaning pigs (Wu et al. 2011). Moreover, cells have evolved a cellular mechanism of "self-eating" process (hereafter referred to as autophagy) for surviving under nutrient shortage in extracellular environment. Under normal conditions, autophagy allows cells to break down long-lived proteins for homeostasis. Autophagy is rapidly up-regulated and functions as a pro-survival process in response to different forms of stresses such as nutrient starvation, growth factor depletion, and endoplasmic reticulum (ER) stress (Kourtis and Tavernarakis 2009). Autophagy is negatively regulated by mTOR, a central regulator of cell growth and survival in responses to extracellular amino acids and growth factors. In an amino acid-rich environment, mTOR is active and regulates protein translation but also inhibits autophagy (Fig. 2). When extracellular amino acids are limited, autophagy recycles intracellular constituents to provide an alternative source of amino acids (Nicklin et al. 2009). Considering that amino acid is an activator of mTOR signaling, it is plausible that intracellular Gln status might be potential regulator of autophagy. Consistent with the hypothesis, it has been reported that Gln can inhibit the mTOR and p38 MAP kinase pathways under basal and stressed conditions, thus inducing autophagy which can contribute to cell survival during physiologic stress in intestinal epithelial cells (Sakiyama et al. 2009). Importantly, Kristan and colleagues recently demonstrated that transcription factor FOXOs can induce autophagy by transactivation of Gln synthetase activity under conditions of growth factor deprivation (Sandri 2012; van der Vos et al. 2012). The FOXO family (FOXO1, 3, 4 and 6) is downstream of the insulin pathway and is negatively regulated by PI3K/AKT signaling. In the absence of growth factors,

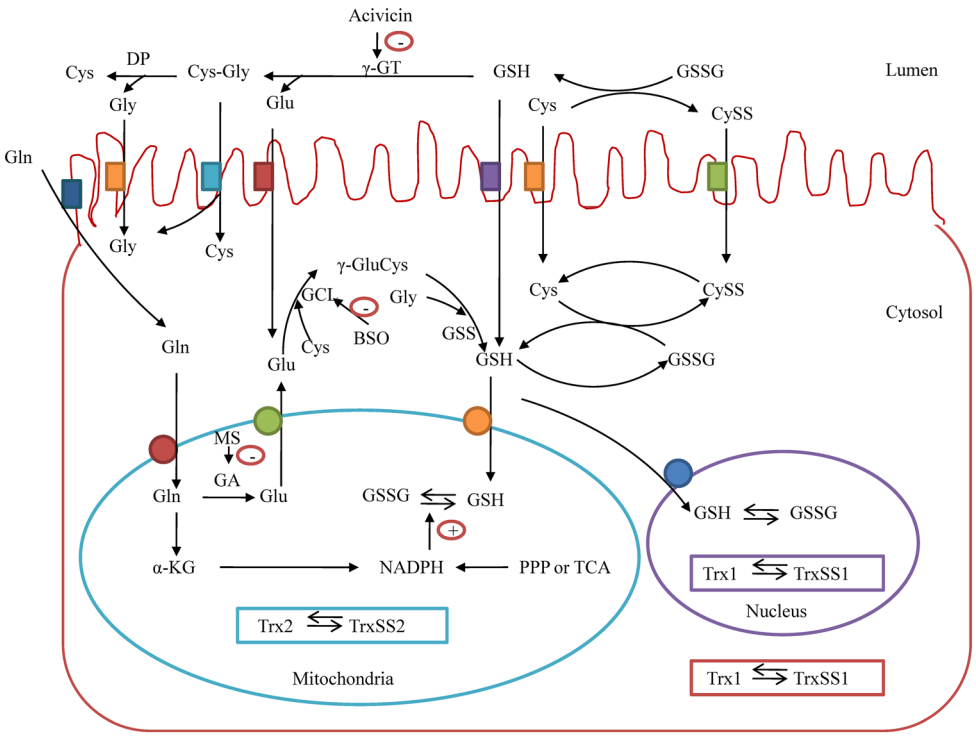

Fig. 3 Glutamine and intracellular redox homeostasis (see text for details). *α-KG* α-ketoglutarate, *acivicin* an irreversible inhibitor of γ-glutamyl transpeptidase, *BSO* buthionine sulfoximine, an inhibitor of glutamate cysteine ligase, *DP* dipeptidase, *GSH* glutathione, *GSSG* glutathione disulfide, *γ-GT* γ-glutamyl transpeptidase, *NO* nitric oxide, *NOS* NO synthase, *GA* glutaminase, *GCL* glutamate cysteine ligase, *GS* glutamine synthetase, *GSS* glutathione synthetase, *MS* methionine sulfoximine, *PPP* pentose phosphate pathway, *TCA* tricarboxylic acid cycle, *Trx* reduced thioredoxin, *TrxSS* oxidized thioredoxin

PI3K/AKT is inactived and FOXOs translocate into the nucleus and turn on the expression of Gln synthetase, a rate-limiting enzyme for Gln synthesis, thus leads to an increased level of Gln production (Sandri 2012; van der Vos et al. 2012). Interestingly, the FOXO-induced expression of Gln synthetase results in autophagy due to the inhibition of mTORC1 activity as evidenced by the lack of phosphorylation of the S6K1 kinase, and re-localization of mTOR from the lysosomes to the cytoplasm. These findings provide an intriguing link between FOXO transcription factors and autophagy, in which Gln production is a key mediator between these two processes (Sandri 2012). The autophagic effect induced by Gln is a critical survival mechanism for cells in response to growth factors or nutrition deprivation; thereby enhancing stress resistance.

Preventive effects of Gln against apoptosis

The main function of the intestinal epithelial barrier is to separate the hostile external environment from the internal milieu. As the first line defensive system, the intestinal epithelial is exposed to various pathogens (commensal bacteria or pathogenic bacteria), toxin, damaged cells, and nutrient metabolites. The physical and chemical barriers created by the intestinal epithelium protect the intestinal mucosa from attack by potentially harmful enteric microorganisms (Ren et al. 2013a, b). To function properly, the epithelium has evolved to possess a relatively short life of 3–5 days. This rapid turnover of the enterocytes is maintained by the well-controlled balance between cell proliferation and apoptosis (Bach et al. 2000; Mates et al. 2006; Rhoads et al. 1997). Breakdown of this balance will lead to dysfunction of intestinal barrier which is associated with the development of diseases or an inflammatory response (Radtke and Clevers 2005). Various stresses, such as nutrient and growth factor deprivation, pathogenic bacteria toxins (e.g., endotoxin), and inflammatory cytokines, have been reported to induce apoptosis in intestinal epithelial cells, thus resulting in abnormal structure and function of the gut (Mates et al. 2006). It has been reported that Gln starvation-induced apoptosis is caspase 3-dependent, which can be prevented by Gln supplementation in small intestinal epithelial cells in which the activation of MAP kinase ERK and PI3K/AKT signaling plays an important role in limiting apoptosis (Larson et al. 2007; Papaconstantinou et al. 1998). In addition to intestinal cells, the apoptotic effect induced by Gln deprivation was observed in other cell types, including both normal and cancerous cells through mitochondrial or death receptor-mediated apoptotic pathway (Mates et al. 2006). The anti-apoptotic effect of Gln is also found in cytokine-treated cells. For example, Gln protects against apoptosis induced by the TNF-α related apoptosis inducing ligand (known as TRAIL) through a mechanism involving the pyrimidine pathway in human intestinal cells (Evans et al. 2003, 2005). Gln or alanyl-Gln also prevents *Clostridium difficile* toxin A-induced caspase 8 activation, and thus attenuates apoptosis in human intestinal epithelial T84 cells (Carneiro et al. 2006). Also, Gln significantly suppressed the release of cytochrome c from mitochondria [an indicator of apoptosis (Yang et al. 2013)] and diminished activities of caspases induced by sodium laurate, thus protected cell from undergoing apoptosis (Takayama et al. 2009). Comparative functional proteomics demonstrated that Gln significantly influences the protein expression profile in response to the mouse agonistic Fas antibody treatment in human epithelial HCT-8 cells (Deniel et al. 2007). Of the proteins, they noted that pro-apoptotic proteins, such as caspase 3 was down-regulated, whereas the cell death regulator Aven, a novel anti-apoptotic member, was up-regulated, suggesting the existence of other regulators that contribute to the anti-apoptotic effect of Gln (Deniel et al. 2007). In addition to the inactivation of caspase involved in apoptosis cascade, regulation of redox status can also contribute to anti-apoptosis. The cellular reducing environment is provided by two mutually interconnected systems; the TRX system and the glutathione (GSH) system (Fig. 3). Under physiological conditions, the intracellular reducing environment is maintained by the disulfide/dithiol-reducing activity of the GSH and TRX systems which is required for cellular homeostasis and cell survival (Circu and Aw 2011; Franco and Cidlowski 2009). Studies have shown a correlation between GSH depletion and the induction of apoptosis triggered by stimuli that activate the mitochondrial or death receptor-mediated apoptosis in various cell types (Franco and Cidlowski 2009; Kern and Kehrer 2005). Consistently, Gln has been reported to be responsible for the anti-apoptotic effect in epithelial cells due to its implication in the production of intracellular GSH, one of the potent antioxidants that contribute to the elimination of intracellular reactive oxygen species (ROS) and maintenance of redox status (Circu and Aw 2011, 2012). Thirdly, Gln treatment can induce heat shock protein 72 at transcriptional levels, thus contributing to the anti-apoptotic effect and reduced cellular damage (Musch et al. 1998; Ropeleski et al. 2005; Wischmeyer 2002). We recently found that addition of 0.5 mM H_2O_2 to culture media induced apoptosis in porcine intestinal epithelial cells and an increase in permeability, which could be attenuated by Gln supplementation (Data not shown).The anti-apoptotic effect of Gln on oxidative damage might involve several signaling pathways, such as reduced expression of Toll-like receptor and caspase 3 in neonatal pig enterocytes (Haynes et al. 2009), attenuated synthesis of NO by inducible NOS (Wu et al. 2011), enhanced

abundance of anti-oxidative proteins including glutathione S-transferase (Mates et al. 2002; Wang et al. 2008), and increased expression of heme oxygenase-1 (Coeffier et al. 2002). In a recent study, Gln supplementation via rectal route has been reported to reduce endoplasmic reticulum stress markers such as, CHOP, BIP, ATF6, and apoptotic markers, including cytochrome c release, caspase activation, further expanding our understanding of the anti-apoptotic function of Gln in response to stresses (Crespo et al. 2012). It should be noted that most of the cell lines used on the anti-apoptotic effect of Gln are transformed or carcinoma cells with multiple genetic or epigenetic alterations which are associated with high rates of cell proliferation and apoptosis resistance. Cautions should be taken in interpreting these results from in vitro studies (Mates et al. 2006).

Gln, bacterial translocation and intestinal immunity

In addition to its role in digestion and absorption of nutrients, the small intestine is also viewed as the largest immune organ in the body to protect the internal milieu from potentially hostile pathogens which is required for the maintenance of normal intestinal epithelial barrier function (Menard et al. 2010; Veldhoen and Brucklacher-Waldert 2012). The normal intestinal barrier to bacteria invasion mainly depends on specific IgA antibody secreted from the gut-associated lymphatic tissue (GALT) with which several types of specialized cells, such as macrophages, natural killer cells, mast cells, and intraepithelial lymphocytes, are involved (Ruth and Field 2013). Under physiological conditions, IgA is secreted into the intestinal tract and has an ability to prevent the adherence of bacteria to the mucosal cell, which is the initiating and prerequisite step for colonization and invasion of bacteria to the deeper layers of the intestine (Artis 2008; Jacobi and Odle 2012). Abnormal regulation of secretory lgA (s-IgA) production due to pathologic invasion, stress exposure, or perturbation of the components of GALT, will impair the mucosal immune system, while leading to bacterial translocation and defective barrier integrity (Alverdy et al. 1988; Mestecky et al. 1986). The beneficial effect of Gln addition on mucosal during provision of standard total parenteral nutrition (Grant and Snyder 1988) promoted Burke and colleagues to elucidate immune function of Gln on the gastrointestine (Burke et al. 1989). These authors reported that addition of Gln to TPN reversed the decrease of s-IgA production, bacterial adherence to the intestinal mucosa, and bacterial translocation, suggesting a critical role of Gln in gut immune function (Alverdy 1990; Li et al. 2007). Experimental studies support the role of intestinal s-IgA as a significant component of the intestinal barrier function and that impaired production of s-IgA is associated with increased adherence of bacteria to the intestinal mucosa and breakdown of barrier function (Spitz et al. 1995). To demonstrate the role for Gln in bacterial translocation, rats exposed to a single dose of abdominal radiation were supplemented with Gln or isonitrogenous control in their drinking water for 4 days. The results indicated that provision of Gln blunts mucosal injury and the incidence of bacterial translocation (Souba et al. 1990). It has also been demonstrated that administration of Gln improves gut barrier function and reduces bacterial translocation in both in vivo (Chen et al. 1994; Souba et al. 1990) and in vitro (Scheppach et al. 1996) models of gut disorder. Furthermore, Gln deprivation facilitates TNF-α-induced bacterial translocation in Caco-2 cells, and the sensitization of TNF-α-induced bacterial translocation was blocked by an inhibition of the conversion of Gln to α-ketoglutarate (α-KG), a key step in oxidative metabolism of Gln (Clark et al. 2003). α-KG can activate mTOR and stimulate protein synthesis in enterocytes (Yao et al. 2012), thereby improving intestinal function (Hou et al. 2011). ATP depletion following Gln deprivation may be responsible, in part, for the sensitization effect of Gln on bacterial translocation (Clark et al. 2003). This view is supported by the observation showing that Gln is the major fuel energy for fast-dividing cells, including intestinal epithelial cell and lymphoma cells (Mates et al. 2006; Wu 1998).

Growing evidence shows that Gln inhibits intestinal expression and activation of nuclear factor-κB (NF-κB) (Haynes et al. 2009; Mondello et al. 2010) which is a pleiotropic transcription factor present in almost all cell types (Pasparakis 2012). NF-κB has been recognized as a critical regulator of immune responses and implications in the pathogenesis of diverse inflammatory diseases (Pasparakis 2009). While early studies mainly focused on the role of NF-κB in the development and function of immune cells, accumulating experimental evidence indicates that NF-κB signaling in epithelial cells is important for the maintenance of immune homeostasis in barrier tissues such as the skin and the intestine (Pasparakis 2012). Under normal conditions, NF-κB is located in the cytosol and complexed with the inhibitory protein IκB-α, and thus exists in an inactive state. Extracellular signals, such as cell stress and inflammatory signal, can activate the enzyme IκB kinase (IKK). IKK, in turn, phosphorylates the IκBα protein, which results in ubiquitination, dissociation of IκB-α from NF-κB, and eventual degradation of IκB-α by the proteasome. The activated NF-κB is then translocated into the nucleus where it binds to the promoter region of specific genes and results in their functional change (Li and Verma 2002). It becomes clear that the immune homeostasis of intestine depends on the interactions of the bacteria with the mucosal immune

system. Activation of NF-κB has been observed in stress-induced or bacteria-induced disruption of barrier integrity (Banan et al. 2007; Han et al. 2009), suggesting that NF-κB signaling might contribute to disease pathogenesis. Pharmacological inhibition of NF-κB had protective effects in gut disease, including colitis, supporting a pathogenic role for NF-κB in intestinal inflammation. Commensal bacteria are believed to activate NF-κB in epithelial cells by stimulating pattern recognition receptors including Toll-like receptors and NOD-like receptors, which are expressed to allow sensing of commensal bacteria in intestinal epithelial cells (Abreu 2010). In additional to the classic activation of NF-κB signaling, including IκB degradation and direct modifications on NF-κB protein (Li and Verma 2002), the activity of NF-κB can also be regulated by Gln under stress condition or critical illness. Experimental in vitro studies show that Gln deprivation modulates endotoxin-induced IL-8 production via decreasing IκB protein in both human fetal and adult enterocytes, with the immature intestine showing the greatest response (Liboni et al. 2005). A recent study also demonstrated that, in addition to modulating IKK activity, Gln can induce nuclear degradation of the NF-κB p65 subunit via the ubiquitin–proteasome pathway, thus diminishing inflammatory response (Lesueur et al. 2012). These results clearly indicate a repressing effect of Gln on intestinal inflammation, which might be another mechanism responsible for its beneficial effect on barrier function as observed from the in vivo studies (Wu et al. 1996).

Conclusion and perspectives

Gln is a truly functional amino acid in nutrition (Wu et al. 2013). Dietary Gln has been shown to be important for maintenance of the intestinal mucosal barrier by regulating expression of genes and proteins involved in cell proliferation, differentiation and apoptosis, protein turnover, anti-oxidative property, and immunity responses. The critical dependence of gut function on the provision of dietary Gln is observed under physiological and stress conditions, such as weaning, lactation, gestation, and various gastrointestinal disorders (Wu 2014). This beneficial effect of Gln continues to stimulate research on basic and clinical studies involving animal models and humans. Compelling evidence showed that Gln is a nutritionally essential amino acid for neonates and a conditionally essential nutrient for adults (Rezaei et al. 2013a; Wu et al. 2014). Despite much progress in this research area, the underlying mechanisms for the actions of Gln remain largely unknown and should be addressed in future studies involving in vitro and in vivo experiments. It should be borne in mind that in vitro studies can provide useful information about the biochemistry, nutrition and physiology of Gln, but cannot fully mimic conditions in intact animals which require coordination of multiple systems for maintenance of homeostasis. For example, Gln regulates metabolism of intestinal bacteria (Dai et al. 2013), which is expected to have profound impacts on the health of the gut and the whole body. Cautions should be taken to interpret and extrapolate in vitro data to in vivo models, particularly with regard to concentrations of Gln and other amino acids in cell culture medium. Integration of results from all studies is necessary to better understand how Gln functions in animals and humans and how to develop new means to improve Gln nutrition in the organisms.

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