Research Advances on Differential Protein Expression in Porcine Intestine under Stress and Certain Disease Conditions: Postprint

Authors: Lü Dinghong, Xiong Xia, Wang Xiaocheng, Yang Huansheng, Li Jianzhong, Yin Yulong

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Abstract

The intestine serves not only as the primary organ for nutrient digestion and absorption, but also as the largest immune organ of the organism. Proteins are the executors of life activities, and alterations in intestinal function are typically accompanied by changes in protein expression, modification, or stability. In recent years, with the vigorous development of proteomics technologies, proteomics has been extensively applied in the field of animal science. This article primarily provides a brief overview of research advances on differential protein expression in porcine intestine under stress and certain disease conditions.

Full Text

Research Advances on Intestinal Differential Protein Expression in Pigs Under Stress and Certain Disease Conditions

LÜ Dinghong[1],[2], XIONG Xia[2],, *WANG Xiaocheng[2], YANG Huansheng[1], LI Jianzhong[1], YIN Yulong[1],[2]*

[1]College of Life Sciences, Hunan Normal University, Laboratory of Animal Nutrition and Human Health, Changsha 410006, China
[2]Key Laboratory of Agro-Ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China

Abstract: The intestine is not only the primary organ for nutrient digestion and absorption but also the body' s largest immune organ. Proteins are the principal executors of life processes, and alterations in intestinal function are

typically accompanied by changes in protein expression, modification, or stability. In recent years, with the rapid development of proteomics technology, its application in animal science has become widespread. This review summarizes recent research advances on differential protein expression in the pig intestine under stress and certain disease conditions.

Keywords: pigs; proteomics; intestine; stress; diseases

Proteomics is the study of dynamic changes in all proteins (including expression levels, post-translational modifications, and protein-protein interactions) within a species, individual, organ, tissue, or cell [1-2]. Protein separation and identification represent two critical steps in proteomics research. Protein separation techniques primarily include two-dimensional chromatography, two-dimensional capillary electrophoresis, and liquid chromatography-capillary electrophoresis [3]. In addition to traditional Edman degradation and amino acid composition analysis for protein identification, mass spectrometry has become the cornerstone of current proteomics technology due to its sensitivity, accuracy, high throughput, and automation [4]. Isotope-coded affinity tag technology has also emerged as a core proteomics approach because of its high sensitivity and precision [5].

Proteins are the executors of physiological functions, and high-throughput proteomic screening in organisms serves as an important method for rapidly identifying protein profiles [6]. Currently, proteomics technology has been extensively applied in life science research. The intestine is the primary organ for digestion and absorption and the largest immune organ, playing crucial roles in nutrient absorption and defense against foreign pathogens [7]. Moreover, the intestine itself participates in the metabolism of dietary nutrients [8]. Therefore, intestinal health directly affects not only nutrient uptake in animals but also the utilization of dietary nutrients by extra-intestinal tissues and organs. In modern animal production, numerous factors can induce stress and disease, generating large quantities of toxic reactive oxygen metabolites in intestinal epithelial cells and causing intestinal mucosal damage [9]. Damage to intestinal structural integrity and function subsequently threatens animal performance and health [10]. Consequently, alleviating stress responses and maintaining intestinal health are critical issues in animal production research. Applying proteomics to obtain information on intestinal protein changes (in expression, modification, or stability) in animals under stress or disease states can provide deep insights into alterations in intestinal structure and metabolism, offering a theoretical basis for subsequent regulatory studies. Pigs are not only a major source of protein nutrition for humans but also important model animals. This review focuses on recent research advances in differential protein expression in the pig intestine under stress and certain disease conditions.

1. Changes in Intestinal Protein Expression in Pigs Under Heat Stress (HS)

HS causes significant increases in respiratory rate and body temperature, inhibits growth, and reduces growth performance [11]. The intestine is one of the organs most sensitive to HS in animals; HS damages intestinal integrity and causes functional disorders, thereby affecting growth performance and leading to high morbidity and mortality [12]. The effects of HS on the intestine involve changes in gene and protein expression at the molecular level. Therefore, analyzing differential protein expression in intestinal tissue under HS can reveal the physiological mechanisms underlying HS-induced intestinal damage and dysfunction, providing a theoretical basis for improving management practices under HS conditions.

1.1. Effects of HS on Intestinal Structural Integrity-Related Protein Expression

HS damages pig intestinal structure [13]. Pearce et al. [14] demonstrated that under HS conditions, although tight junction-related protein expression was unaffected, the abundance of numerous proteins related to cell structural integrity and signaling pathways changed. Vimentin is an intermediate filament protein associated with cell migration that forms the cytoskeleton together with other microfilaments [15]. Studies have reported that HS upregulates vimentin expression, potentially altering intestinal integrity [16]. Additionally, cofilin is an actin-depolymerizing factor that regulates actin polymerization and causes microfilament disassembly. Nagumo et al. [17] found that compared with thermal neutral (TN) conditions, cofilin in the ileum was dephosphorylated under HS, leading to reduced content, and cofilin dephosphorylation damages intestinal integrity. Under HS, the expression of the cytoskeletal protein calponin also changed, with reduced calponin-1 abundance. Calponin is a calcium-binding inhibitor of myosin ATPase activity that reduces smooth muscle binding capacity. Therefore, reduced calponin can promote smooth muscle contraction while inhibiting myosin ATPase activity [18]. Furthermore, HS alters intestinal permeability. Pearce et al. [18] compared protein expression changes in pig intestine after 24 h under HS and TN conditions, finding that transmembrane resistance significantly decreased ($P < 0.05$) in the ileum and colon under HS, indicating damaged intestinal integrity. Intestinal permeability also increased with elevated expression of myosin light chain kinase and casein kinase II-α. Subsequently, Cui et al. [10] performed proteomic analysis on jejunal mucosa of pigs after 3 weeks of HS, detecting 992 protein spots by two-dimensional gel electrophoresis (2-DE) and identifying 53 differentially expressed proteins by matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI-TOF/TOF MS), of which 18 were related to changes in cell structure and motility.

1.2. Effects of HS on Intestinal Metabolism-Related Protein Expression

HS causes downregulation of multiple metabolism-related proteins. Isocitrate dehydrogenase (IDH) is a key enzyme in the tricarboxylic acid (TCA) cycle. Besides its role in the TCA cycle, IDH also produces 2-oxoglutarate necessary for HIF-1α hydroxylation and activation [19]. HS causes a sharp decline in IDH content and activity. Meanwhile, HS leads to downregulation of glycolysis-related enzymes, such as fructose-bisphosphate aldolase, enolase, and glyceraldehyde-3-phosphate dehydrogenase. Pearce et al. [14] found that compared with TN, HS caused significant changes ($P < 0.05$) in metabolic enzyme abundance, with mitochondrial isocitrate dehydrogenase decreasing by 200%, glyceraldehyde-3-phosphate dehydrogenase by 4%, and fructose-1,6-bisphosphate aldolase by 11%. Similarly, Cui et al. [10] showed that HS downregulated proteins related to the TCA cycle, electron transport chain, and oxidative phosphorylation, indicating that HS disrupts energy metabolism. Additionally, Pearce et al. [18] analyzed glucose transport and blood glucose levels in the intestine under HS, finding elevated blood glucose levels in the ileum under HS, increased Na+/K+ ATPase activity, unchanged protein abundance of sodium-glucose cotransporter-1 (SGLT-1), but increased protein abundance of glucose transporter-2 (GLUT-2) in the ileum, which may be related to accelerated glucose transport under HS. Thus, HS causes changes in the content and activity of many enzymes in the intestine, thereby affecting intestinal metabolism.

1.3. Effects of HS on Oxidative Stress-Related Protein Expression

Heat shock proteins (HSPs) play important roles in inhibiting apoptosis, controlling cell proliferation and differentiation, signal transduction, and thermotolerance, and are crucial proteins in stress responses [20]. Under HS, the abundance of HSP70 in pig ileum and colon increases [7]. Pearce et al. [14] also showed that compared with TN, HS upregulates HSP27, HSP65, HSP70, and HSP90-α in the ileum. Yu et al. [21] analyzed protein expression changes in pig jejunum under HS, finding increased abundance of HSP27, HSP70, and HSP90. Similar studies have also confirmed that HS increases HSP70 protein abundance [7]. Additionally, antioxidation plays an important role in disease and defense against invading pathogens, and HS induces oxidative stress [22]. Studies have shown that HS downregulates peroxiredoxin-1 expression [23], while peroxiredoxins control and reduce cytokine-induced hydrogen peroxide and lipid hydroperoxide production [24]. Furthermore, HS causes intestinal hypoxia [25]. Hypoxia-inducible factors (HIFs) are transcriptional regulators including HIF-1α, HIF-2α, and HIF-3α that play key roles in maintaining oxygen homeostasis. Current studies show that HIF-1α is upregulated in the intestine under HS [18].

HS increases HSP expression in piglet intestine, induces oxidative stress responses, and causes intestinal hypoxia. Under HS, key metabolic enzymes involved in glycolysis and the TCA cycle, as well as proteins related to maintaining cell structural integrity and cell migration, show decreased expression in piglet

intestine. Additionally, short-term exposure to high temperature also increases intestinal permeability and the activity of key kinases regulating tight junction protein complex expression [18]. Therefore, osmotic stress, tissue hypoxia, and inflammation are the main factors causing intestinal pathological states under HS.

2. Changes in Intestinal Protein Expression During Piglet Weaning Stress

Weaning causes significant changes in nutrition and environment, leading to decreased feed intake and reduced growth rate in piglets. Weaning damages intestinal structure and function, thereby inducing stress responses. Weaning stress involves changes in pathways related to energy metabolism, oxidative reactions, and apoptosis [26]. Ren et al. [27] added free amino acids to protein-restricted diets and performed proteomic analysis on weaned piglet jejunum using 2-DE and mass spectrometry (MS), identifying 16 differentially expressed proteins mainly involved in stress, immunity, and carbohydrate metabolism. Supplementing free amino acids downregulated HSP60 expression in jejunum. Therefore, supplementing amino acids in low-protein diets can improve intestinal nutrient absorption and transport, intestinal health, and mucosal immunity, thereby promoting daily weight gain in weaned piglets. Glutamine, as a common free amino acid, plays an important role in maintaining normal intestinal mucosal structure and function and signal transduction. Lin et al. [28] studied the effects of exogenous glutamine supplementation on small intestinal structure and expression of amino acid receptors and transporters in jejunal mucosa of weaned piglets. The results showed that glutamine increased plasma concentrations of multiple amino acids and promoted expression of glutamate metabolic receptors in jejunal mucosa. Wang et al. [29] used differential proteomics to reveal the relationship between glutathione metabolism and apoptosis in small intestinal tissue of piglets supplemented with zinc oxide. The results showed that zinc oxide supplementation improved the redox state of weaned piglet jejunum and prevented apoptosis, alleviating intestinal dysfunction caused by weaning.

Intestinal epithelial cells are the main executors of intestinal function, and the crypt-villus axis is a unique structural and functional unit of small intestinal epithelium. Differentiation of crypt-villus axis epithelial cells is crucial for damage repair after intestinal stress, intestinal barrier function, and normal intestinal function [30]. Xiong et al. [31] used isobaric tags for relative and absolute quantitation (iTRAQ) to analyze protein expression in jejunal crypt-villus axis epithelial cells of 21-day-old weaned and suckling piglets, finding that weaning significantly downregulated proteins related to the TCA cycle, β-oxidation, and glycolysis in villus tips and mid-villus, affecting energy metabolism. Yang et al. [32] studied developmental changes in upper villus epithelial cells of jejunal crypt-villus axis in weaned piglets by proteomics. The results showed that proteins related to energy metabolism, Golgi vesicle transport, protein amino

acid glycosylation, ion transport, epithelial cell differentiation, and metabolism were downregulated with weaning, and these changes may be regulated by the mammalian target of rapamycin (mTOR) signaling pathway.

3. Changes in Intestinal Protein Expression in Pigs Under Certain Disease States

The intestine is an important digestive and immune organ. Due to its structural complexity, it is susceptible to invasion by various pathogens [33]. Because of the similarity of their organs in shape and physiology to humans, pigs are used as major model organisms for many human disease studies [34].

3.1. Necrotizing Enterocolitis of Newborn (NEC)

Necrotizing enterocolitis of newborn (NEC) is the most severe gastrointestinal complication in premature piglets [35]. Differential protein expression analysis of intestine in NEC pigs helps characterize disease pathology and identify new biomarkers and therapeutic targets. Jiang et al. [36] analyzed intestine of NEC piglets. Thirty specifically differentially expressed proteins were identified in necrotic versus normal small intestine, and 23 in colon. Histamine receptor, actin, globulin, immunoglobulin, and antitrypsin had the same effects on small intestine and colon, while HSPA5 and HSP27 were only differentially expressed in small intestine. Proteins involved in antioxidation, angiogenesis, cytoskeleton formation, and metabolism were all affected. Additionally, Jiang et al. [37] used proteomics to analyze the effects of three key NEC-inducing factors (prematurity, intestinal microbiota, and enteral nutrition) on intestinal protein expression. The results showed different numbers of differentially expressed proteins after induction of NEC by prematurity, intestinal microbiota, and enteral nutrition. Intestinal microbiota colonization affected expression of proteins related to tissue stress response, while enteral nutrition affected expression of proteins related to carbohydrate metabolism. Jiang et al. [38] used 2-DE to analyze cellular dynamic responses in gastrointestinal tract of piglets receiving early enteral nutrition. The results showed 25 differentially expressed proteins between parenteral and enteral nutrition groups. Upregulation of prohibitin (PHB) and glucose-regulated protein 78 (GRP78) indicated that NEC caused stress, while increased expression of GDP dissociation inhibitor 1 (GDI1) and GDP dissociation inhibitor 2 (GDI2) indicated that NEC affected cell motility, actin assembly, and cell fluidity [34]. Although differential protein expression analysis of NEC piglet intestine provides some guidance for disease treatment, we still need to further identify specific marker proteins in NEC piglet tissue or plasma for early disease recognition.

3.2. Short Bowel Syndrome (SBS)

Short bowel syndrome (SBS) in newborn piglets usually occurs as a preventive measure for NEC, intestinal atresia or aganglionosis, and results from intestinal

resection that reduces intestinal length. After resection, intestinal epithelial cells proliferate rapidly and nutrient absorption capacity is greatly enhanced [38,39-40]. Stephens et al. [41] used proteomics to analyze intestinal protein expression differences in three groups of pigs (75% small bowel resection group, resection with anastomosis group, and control group). After 6 weeks of feeding, ileal tissue proteins were separated by two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) and identified by MALDI-TOF-MS/MS. Compared with the control group, 71 and 53 differential proteins were identified in the two treatment groups, respectively. Differential proteins including liver fatty acid binding protein (L-FABP), intestinal fatty acid binding protein (IL-FABP/FABP-6) were selected for further validation. The results showed that L-FABP expression increased in the 75% small bowel resection group, accompanied by increased villus area and number of L-FABP-positive cells on the crypt-villus axis. These findings suggest that fatty acid binding proteins may serve as clinical markers for the intestinal adaptation process after SBS resection [41].

3.3. Intrauterine Growth Retardation (IUGR)

Intrauterine growth retardation (IUGR) impairs small intestinal development, thereby affecting nutrient absorption [42]. Studies have shown that IUGR affects expression of proteins in piglet small intestine related to intracellular signaling, redox balance, protein synthesis, and proteolysis [43]. Wang et al. [44] studied dynamic changes in small intestinal mucosal proteins in IUGR piglets. The results showed 56 differentially expressed proteins between IUGR and normal birth weight (NBW) piglets. Proteins related to oxidative stress and apoptosis were upregulated, while proteins related to digestion, absorption, and metabolism of nutrients (including glucose, lipids, amino acids, vitamins, and minerals) were downregulated. These differential proteins may be the main causes of growth retardation, atrophy, and dysfunction in IUGR piglet intestine. This study reveals the continuous impairment of intestinal development in neonatal piglets with IUGR, provides theoretical basis for understanding metabolic defects in IUGR newborn piglet intestine, and may offer new strategies for improving piglet growth.

Currently, proteomic analysis of pig intestine related to stress and disease has yielded substantial information, laying the foundation for further research and providing theoretical basis for understanding intestinal structure, function, metabolic regulation, and disease pathogenesis. However, since intestinal stress or disease involves multiple levels from whole organism to organ, tissue, cell, and molecule, and is a dynamic process, we cannot limit ourselves to gene or protein levels alone. We also need to analyze cellular signaling and gene regulatory networks and their interrelationships at the whole organism level through "bottom-up" or "top-down" approaches to ultimately determine the patterns of animal nutritional and physiological changes.

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