Heat shock protein 70 is upregulated in the intestine of intrauterine growth retardation piglets

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Received: 3 July 2009 / Revised: 2 August 2009 / Accepted: 30 September 2009 / Published online: 15 October 2009
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Abstract The objective of this study is to investigate the expression and distribution of heat shock protein 70 (Hsp70) in the intestine of intrauterine growth retardation (IUGR) piglets. Samples from the duodenum, prejejunum, distal jejunum, ileum, and colon of IUGR and normal-body-weight (NBW) piglets were collected at birth. The results indicated that the body and intestine weight of IUGR piglets were significantly lower than NBW piglets. The villus height and villus/crypt ratio in jejunum and ileum of IUGR piglets were significantly reduced compared to NBW piglets. These results indicated that IUGR causes abnormal gastrointestinal morphologies and gastrointestinal dysfunction. The mRNA of *hsp70* was increased in prejejunum ($P<0.05$), distal jejunum ($P<0.05$), and colon in IUGR piglets. However, the *hsp70* mRNA in ileum of piglets with IUGR was decreased. Similar to *hsp70* mRNA, the protein levels of Hsp70 in prejejunum ($P<0.05$), distal jejunum, and colon ($P<0.05$) in IUGR piglets were higher than those in NBW piglets. These results indicated that the expression of Hsp70 in the intestinal piglets was upregulated by IUGR, and different intestinal sites had different responses to stress. Meanwhile, the localization of Hsp70 in the epithelial cells of the whole villi and intestinal gland rather than in the lamina propria and myenteron suggested that Hsp70 has a cytoprotective role in epithelial cell function and structure.

Keywords Intrauterine growth retardation · Heat shock protein 70 · Intestine · Piglets

Abbreviations

IUGR	Intrauterine growth retardation
Hsp	Heat shock protein
NBW	Normal body weight
NRC	National Research Council
SD	Standard deviation
OD	Optical density
HSF	Heat shock factor

Introduction

Intrauterine growth retardation (IUGR) is defined as impaired growth and development of the mammalian embryo/fetus or its organs during gestation (Wu et al. 2006). It is induced by intrauterine infection, genetic causes, congenital malformations, environmental insults, and severe malnutrition (Wu et al. 2006). Approximately 5–10% of human infants in the world (McMillen and Robinson 2005) and 15–20% of newborn pigs (Wu et al. 2006) suffer from IUGR. In animal production, IUGR may cause permanent maldevelopment, reduce the survival rate of neonates, and decrease the feed efficiency (Wu et al. 2006). In addition, it negatively affects the development and function of digestive tract (Wang et al. 2005). Studies have shown that the expression levels of growth-related proteins (Wang et al. 2005, 2008) and stress-responsive genes (Liu et al. 2008) were severely affected by IUGR. However, little is known about the effect of IUGR on the expression and function of heat shock protein 70 (Hsp70) in intestine of infants and animals.

The Hsps are a family of stress-responsive proteins found in all species. Based on their molecular weight, Hsps are classified into four major families, namely, small Hsps,

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Hsp60, Hsp70, and Hsp90 (Watanabe et al. 2004). Hsp70, one of the most abundant and best-characterized proteins in Hsps family, is a molecular chaperone involved in the folding of nascent and misfolded protein under nonstressful conditions. Hsp70 also protects the cells from various stresses (Oyake et al. 2006). Since Hsp70 is a universal cytoprotection protein, it may enhance the tolerance to environmental changes or pathogenic conditions, increase the survival rate of stressed cells, and may also play critical role in cardiovascular diseases, organism decay, or cellular aging (Njemini et al. 2007). Hsp70 has been known to protect intestinal epithelial cells from toxic agents and ulcerogenic conditions in gastrointestinal mucosa, where it is important location for digestion, absorption, and metabolism of nutrients and easily suffers injury from various stresses (Watanabe et al. 2004; Oyake et al. 2006). Over-expression of Hsp70 can accelerate ulcer healing by promoting cell proliferation, inhibiting cell apoptosis, and accelerating protein synthesis (Pierzchalski et al. 2006). In addition, expression of Hsps is induced to protect against physiological stress during development and differentiation (Kojima et al. 1996; Mao and Shelden 2006). For example, differentiation of human K562 erythroleukemia cells is accompanied by elevated expression of Hsp70 (Sistonen et al. 1992). Although there is a growing awareness regarding the protective function of Hsp70, little is known about the expression and function of Hsp70 in intestine under physiological and pathological conditions at birth. By using neonatal pigs as animal model, we investigated the expression of Hsp70 protein and mRNA in the intestine of IUGR piglets.

Materials and methods

Animals and experimental design

Pregnant sows were fed daily with a 2-kg gestating diet during the entire period of pregnancy and had free access to drinking water. The diet met the NRC (1998) requirements for nutrients. At the term of birth (day 114 of gestation), a total of ten piglets were removed from five litters and were assigned equally to two groups: normal body weight (NBW) and IUGR. A piglet was defined as IUGR when its birth weight was two standard deviations (SD) below the mean birth weight of the total population (Wang et al. 2005), while a piglet was defined as NBW when its birth weight was within one SD of the mean birth weight of the total population (Wang et al. 2005). Newborn piglets were killed with an injection of sodium pentobarbital (50-mg/kg body weight) and jugular exsanguinations within 2–4 h after birth without suckling. The experimental protocol was in accordance with the Guide for the Care and Use of

Laboratory Animals prepared by the Institutional Animal Care and Use Committee, Nanjing Agricultural University, China.

Sample collection

The body weight of all piglets was recorded at the time of birth. In neonatal pig, the small intestine was defined as the portion of the digestive tract between the pylorus and the ileocecal valve, with the first 10-cm segment being duodenum, and was divided into duodenum, jejunum, and ileum (Wang et al. 2008). The jejunum was further divided into proximal and distal segments of equal length. After the animal is dead, the duodenum, the proximal and distal jejunum, the ileum, and the colon were immediately sampled and then flushed with physiological saline to remove gut contents. One sample of ~1 cm in length from each intestinal tissue was saved for histological and immunohistochemical analyses. The sample was fixed in 4% paraformaldehyde in 100 mmol/l phosphate buffer, pH 7.4 for 24 h. The mucosa of the rest of the intestine was scraped off with a glass slide and immediately placed in liquid nitrogen then stored at -80°C for the analysis of protein and mRNA.

Morphological analyses

Paraformaldehyde-fixed and paraffin-embedded tissue sections (5 μm thick) were stained with hematoxylin and eosin. Following the descriptions of Xu et al. (1992), villus height (from the tip of the villus to the villus–crypt junction) and crypt depth (from the villus–crypt junction to the lower limit of the crypt) were analyzed under a light microscope. Only vertically oriented intact villus and crypts were measured. The measurement was performed on five tissue sections for each piglet. For each tissue section, 30 villus–crypt units in three different regions were measured.

Semiquantitative detection of Hsp70 by enzyme-linked immunosorbent assay

Frozen intestinal mucosa samples were homogenized in cold solution (200 mg tissues per milliliter) containing 0.15 M NaCl, 20 mM Tris–HCl (pH 8.0), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.1 μM E-46, 0.08 μM aprotinin, 0.1 μM leupeptin, and 0.1% NP-40 (Salokhe et al. 2006; Zhu et al. 2009) using Ultra-Turrax homogenizer on ice. The homogenate was centrifuged at $1,200\times g$, 15 min at 4°C . The supernatant fluid was collected and stored at -20°C for further analysis. The concentration of Hsp70 was determined according to the manual. Briefly, the desired number of specific antibody-coated wells (goat antiporcine Hsp70, Adlitteram Diagnos-

tic Laboratories, USA) was secured in the holder. Standards and specimens were dispensed into appropriate wells. The biotin conjugate reagent was added to the specimens' wells, and the enzyme conjugate reagent was added to each well, gently mixed for 15 s, and then incubated at $36\pm 2^{\circ}\text{C}$ for 60 min. The incubation mixture was removed, and the microtiter wells were rinsed five times with distilled water. Residual water droplets were removed by touching the wells sharply onto absorbent paper. Then, 3,3',5,5'-tetramethylbenzidine substrate solution was added to the wells, gently mixed for 5 s, and incubated at $36\pm 2^{\circ}\text{C}$ for 15 min. The reaction was stopped by adding stop solution and gently mixed for 30 s. The optical density (OD) at 450 nm was read within 30 min using a microtiter plate reader. The average absorbance values (A450) for each set of reference standards, control, and samples were measured, and a standard curve was constructed.

The data were expressed as OD units. The results were expressed in nanogram per milliliter according to the calibration curve obtained from serial dilutions of Hsp70 standard and were then normalized to the β -actin (goat antiporcine β -actin, Adlitteram Diagnostic Laboratories USA). This assay was performed in duplicate.

Real-time reverse transcription–polymerase chain reaction

Total RNA was isolated from the sample of intestine mucosa using Trizol (Invitrogen, CA, USA). RNA was quantified using absorption of light at 260 and 280 nm. RNA concentration was calculated using the following formula: RNA concentration = $A_{260} \times 40 \times \text{dilution factor}$. The RNA quality was assessed by agarose gel electrophoresis. From each sample, 2 μg RNA was used to synthesize cDNA in a 25- μl reaction mixture at 42°C for 60 min with M-MLV reverse transcriptase (Promega, San Luis Obispo, USA) using oligo dT18.

The sequences of the *hsp70* mRNA and *GAPDH* mRNA were downloaded from GenBank (accession nos. X68213, CV874334, respectively). The primer sequences were as follows: *hsp70* sense primer 5'-GCCCTGAATCCGCAGAATA-3' and antisense primer 5'-TCCCCACGGTAGGAAACG3'; *GAPDH* sense primer 5'-GAAGGTCGGAGTGAACGGAT-3' and antisense primer 5'-CATGGGTA GAATCATACTGGAACA-3'. Reverse transcription–polymerase chain reaction (RT-PCR) was performed in ABI 7300 RT-PCR system (Applied Biosystems USA) using SYBR *Premix ExTaq*TM kit (Takara, Dalian, China). The best results were obtained with 20- μl reaction mixture containing 10- μl SYBR *Premix ExTaq*TM, 0.4- μl forward primer (0.2 $\mu\text{mol/l}$), 0.4- μl reverse primer (0.2 $\mu\text{mol/l}$), 0.4- μl ROX reference dye, 2- μl cDNA template, and 6.8 μl dH₂O. The thermal profile for real-time PCR was 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for

1 min. The fluorescence output of each cycle was measured upon the completion of the entire run. Cycle threshold values were calculated by the SDS software algorithms (Applied Biosystems USA) and converted into equivalent target amount using standard curve established by the calibrator. For a standard curve, tenfold dilutions of recombinant pGEM-T plasmids were made in order to give 10^6 – 10^1 copies per milliliter.

Immunohistochemistry

Intestine sections were cut and floated onto albumin-coated slides then dried at 56°C , deparaffinized in xylene, rehydrated, and washed with phosphate-buffered saline (PBS) for 15 min at room temperature (RT). To enhance antigen retrieval, the sections were microwave-heated for 10 min in 0.01 M sodium citrate buffer, pH 6.0. Endogenous peroxidase activity was quenched with 3% H₂O₂ for 10 min at RT. Unspecific binding of the antiserum was blocked by incubating the sections for 20 min at RT in a blocking solution containing 10% normal goat serum. The sections were then incubated overnight at 4°C with 1:100 diluted mouse anti-Hsp70 monoclonal antibody (SPA-810, Stressgen, Canada). After washing in PBS, the slides were incubated for 60 min at RT with the corresponding secondary biotinylated mouse antibody (Zymed Laboratories Inc. USA) at a 1:300 dilution in 1% bovine serum albumin–PBS (pH 7.4). Subsequently, the sections were incubated for 60 min in a horseradish peroxidase–streptavidin solution (Zymed Laboratories Inc. USA). Then, the sections were washed in PBS again. Positive cells were stained in brown color using 3, 3'-diaminobenzidine tetrachloride as the enzyme substrate. After rinsing in distilled water, the slides were counterstained for 3 s by Mayer's hematoxylin. The corresponding negative control sections were prepared by omitting the primary antibody (Bao et al. 2008; San et al. 2005).

Statistical analysis

All data are expressed as mean $\pm$ standard deviation. Results were statistically analyzed by the *t* test, using the Statistical Package for Social Sciences 16.0 software. $P < 0.05$ was considered significant.

Results

Body and intestine weights of newborn piglets

In this study, IUGR piglets had a 48.9% lower ($P < 0.01$) body weight than NBW piglets (Table 1). The absolute weight of all five intestinal segments was significantly

Table 1 Body and intestine weight of newborn piglets with IUGR and with NBW

	NBW (<i>n</i> =5)	IUGR (<i>n</i> =5)
Body weight, kg	1.480±0.098	0.755±0.037**
Absolute tissue weight at birth		
Duodenum, g	2.64±0.32	0.72±0.09**
Proximal jejunum, g	13.37±1.19	5.58±0.29**
Distal jejunum, g	12.46±2.12	5.26±0.07**
Ileum, g	25.00±1.61	10.64±0.21**
Colon, g	8.31±0.69	3.37±0.45**
Percent of body weight at birth, %		
Duodenum	0.179±0.0003	0.095±0.0001*
Proximal jejunum	0.906±0.0010	0.652±0.0000*
Distal jejunum	0.844±0.0015	0.616±0.0003
Ileum	1.698±0.0020	1.2481±0.0011*
Colon	0.561±0.0002	0.395±0.0004**

All values are the mean±SD

P*<0.05; *P*<0.01 (mean differences between values for IUGR piglets and NBW piglets)

lower in IUGR piglets than in NBW piglets. Furthermore, IUGR piglets had decreased the relative weights in duodenum, proximal jejunum, and ileum compared with NBW piglets (*P*<0.05). In colon, the absolute weight of IUGR piglets was significantly decreased (*P*<0.01).

Intestinal morphology

The results of the morphometric measurements are presented in Table 2. The results showed that villus height in jejunum and ileum were both significantly lower in IUGR piglets than those in NBW piglets (*P*<0.01). Crypt depth in ileum was significantly higher in IUGR piglets than in NBW piglets. In addition, villus/crypt ratio of both jejunum

Table 2 Morphometric measurements (μm) of the small intestine in IUGR piglets and NBW piglets

	Villus height	Crypt depth	Villus/crypt ratio
Duodenum			
NBW	570.2±81.1	74.9±16.0	7.8±1.1
IUGR	439.4±18.4*	62.2±10.2	7.2±1.4
Jejunum			
NBW	725.5±79.7	57.3±24.7	16.1±4.1
IUGR	487.3±54.5**	47.6±6.1	10.3±0.2*
Ileum			
NBW	692.8±52	46.2±7.7	15.3±2.5
IUGR	394.1±27.3**	109.7±2.8**	3.6±0.3**

All values are the mean±SD

P*<0.05; *P*<0.01 (mean differences between values for IUGR piglets and NBW piglets)

(*P*<0.05) and ileum (*P*<0.01) was reduced in IUGR piglets compared with NBW piglets.

The levels of Hsp70 protein and mRNA in the intestine

Figures 1, 2, and 3 show the effects of IUGR on the concentrations of *hsp70* mRNA and proteins in the intestine of piglets, respectively. Results indicated that the *hsp70* mRNA amount in duodenum, prejejunum (*P*<0.05), distal jejunum (*P*<0.05), and colon (*P*>0.05) of IUGR piglets was higher than those of NBW piglets, but the *hsp70* mRNA in ileum of piglets with IUGR was decreased. Meanwhile, the levels of Hsp70 protein in proximal jejunum (*P*<0.05), distal jejunum, ileum, and colon (*P*<0.05) of IUGR piglets were increased at birth. However, the concentrations of Hsp70 in duodenum were reduced in IUGR piglets compared with NBW piglets. These results indicated that expression of Hsp70 is different among intestinal segments. In addition, the levels of Hsp70 protein and its corresponding *Hsp* mRNA in the duodenum and ileum of piglets differ from each other.

Immunohistochemical observation

Immunolabeling showed that the Hsp70 protein was predominantly localized in the cytoplasm of epithelial cell (Fig. 4). The signals of Hsp70 were distributed diffusely in the epithelial cells of the whole villi and intestinal gland rather than in the lamina propria and myenteron.

Discussion

Hsp70 is one of the most abundant and best-characterized proteins in Hsp family. The expression of Hsp70 is induced by several endogenous physiological or environmental stressors, such as heat, ethanol, caloric restriction, and

Fig. 1 The levels of Hsp70/β-actin in the intestines of piglets. Mean differences between values for IUGR piglets and NBW piglets are indicated by **P*<0.05 or ***P*<0.01. Values indicated are mean±SD

Fig. 2 The levels of Hsp70 in the intestines of piglets (ng/ml). Mean differences between values for IUGR piglets and NBW piglets are indicated by * $P < 0.05$ or ** $P < 0.01$. Values indicated are mean $\pm$ SD

bacterial infection in gastrointestinal epithelial cells (Musch et al. 1999; Ren et al. 2001; Wada et al. 2006). Hsp70 helps to maintain cells integrity in normal conditions and prevent and repair damages induced in cells and organs by various stresses. Our data clearly demonstrated that expressions of *hsp70* mRNA and protein were upregulated by IUGR in intestine of piglets, and different intestinal sites had different responses to stress.

The small intestine is the location for terminal digestion and absorption of nutrients (Wu 1998). Studies have indicated that natural or experimentally induced IUGR is associated with abnormal morphologies and functions of gastrointestines (Trahair et al. 1997; Wang et al. 2005). This is manifested as a reduction in intestinal mucosal mass, malabsorption of nutrients, and necrotizing enterocolitis. Consistent with previous results, the present study showed that villus height in jejunum and ileum was significantly decreased in IUGR piglets. In addition, IUGR piglets have significantly lower villus/crypt ratio in jejunum and ileum than NBW piglets. These indicated that intestinal functions and structure were impaired by IUGR and might be one of the reasons for the permanent effects of IUGR on later growth and development of animals.

The expression of heat shock protein is induced by a variety of chemical and physical stresses (Morimoto 2008). Previous studies have indicated that IUGR is associated with expression of Hsps in human placentas (Michael et al. 2005; Shah et al. 1998; Wataba et al. 2004). However, little is known about the effect of IUGR on the expression of Hsp70 in small intestine and colon of piglets after birth. In this study, we demonstrated that IUGR upregulated the expression of *hsp70* mRNA and proteins in the intestine of neonatal piglets. Several studies have shown that expressions of *hsp70* mRNA and proteins were induced by IUGR in human placenta. Liu et al. (2008) demonstrated that the expression of Hsp70 in mRNA and protein levels was

upregulated significantly in placental vascular disease induced by IUGR compared with normal pregnancies. Wataba et al. (2004) also indicated that IUGR induced expression of HSPs in avascular villi of placenta. Interestingly, we also found that the expression of Hsp70 is different among intestinal segments. A similar variation has been found in the induction of Hsp70 in pig after transportation (Sepponen and Pösö 2006). In the gastrointestinal tract of pigs, weaning has been shown to cause abnormal spatial-temporal patterns of Hsp70 expression (David et al. 2002). These imply that different intestinal sites had different responses to stress. Furthermore, animal-to-animal variation and large interspecies variation have been found in Hsp70 expression in human, rat, and pig. This may be related to two functional promoter variants of *hsp70* gene and the variation in stress susceptibility (David et al. 2002; Khassaf et al. 2001; Schwerin et al. 2001). In the present study, we also observed that the *hsp70* mRNA transcription varies with its corresponding Hsp70 protein expression in the same pattern. These phenomena also exist in the turkey lymphocytes and in the guinea pig cochlea (Miller and Qureshi 1992; Gower and Thompson 1997). The expression of Hsp70 is regulated both at the transcriptional and posttranscriptional levels (Sarge et al. 1993). At the transcriptional level, the expression of Hsp70 is primarily regulated by HSF (Ankar and Sistonen 2007). However, Hsp70 protein abundance is regulated at the posttranscriptional levels by a number of controls (Takenaka and Hightower 1993). An apparent disagreement between transcription of the messenger and Hsp70 translation has already been reported (Bruce et al. 1993; Yu et al. 2008). The mechanisms are still unknown and should be further investigated.

The increase of Hsp70 expression in the small intestine and colon of IUGR piglets may be associated with the pathological condition. As we know, neonatal piglets easily suffer from environmental stress, inadequate food intake,

Fig. 3 The levels of *hsp70* mRNA in the intestinal piglets. Mean differences between values for IUGR piglets and NBW piglets are indicated by * $P < 0.05$ or ** $P < 0.01$. Values indicated are mean $\pm$ SD

Fig. 4 Localization of Hsp70 in the intestine of IUGR piglets. Scale bar=10 μ m **a** Hsp70 (arrow) was detected predominantly in the cytoplasm of the epithelial cells of the whole villi rather than in the lamina propria and myenteron in duodenum of IUGR piglets (IHC staining) **b** Positive immunolabeling of Hsp70 (arrow) was localized

mainly in the cytoplasm of the epithelial cells of the whole villi in jejunum of IUGR piglets (IHC staining) **c** Diffuse Hsp70-positive signals (arrow) were observed, particularly in epithelial cells and gland of colon in IUGR piglets (IHC staining) **d** Lack of Hsp70 immunoreactivity when antibody was omitted (IHC staining)

and poor management. This will lead to growth retardation or even mortality. Neonatal IUGR animals have higher incidence of cardiovascular disorders (Giussani et al. 2003), hormonal imbalance (Fowden et al. 2005), metabolic disorders (Da et al. 2001; Giussani et al. 2003), organ dysfunction, and abnormal development (Da et al. 2001). Interestingly, these factors may be involved in apoptosis cascade and therefore affect the homeostasis of intestinal epithelia (Baserga et al. 2004; Jones and Gores, 1997). The apoptosis rate of intestinal epithelia inversely correlates with villus height and crypt depth and is accelerated by oxidative stress (Marshman et al. 2001). Many studies have shown that the expression of Hsp70 was induced to prevent apoptosis and necrosis by gastrointestinal lesion of stress (Oyake et al. 2006; Wada et al. 2006). In addition, it has been reported that food restriction can affect Hsp70 expression in rats (Ahmad et al. 1995). Gasbarrini et al. (1998) reported that food deprivation induced a twofold increase in *hsp70* mRNA in rat liver. This implies that inadequate nutrient supply to the fetus suffering IUGR may be one of the stimuli for higher expression of Hsp70.

The distribution of Hsp70 may be related to the protection function of molecular chaperones (Arvans et al. 2005; Yu et al. 2008). Immunolabeling showed that the Hsp70 protein was predominantly localized in the cytoplasm of epithelial cell. Goloubinoff and De Los Rios (2007) and Daugaard et al. (2007) also reported that Hsp70 primarily localized in the endoplasmic reticulum and the mitochondria that generate the high amount of adenosine triphosphate (ATP). The expression of Hsp70 is significantly associated with the amount of ATP in cells. The expression of Hsp70 is inhibited by the high levels of ATP; however, ATP depletion markedly induced the expression of Hsp70 (Arispe et al. 2002; Martin et al. 2008). Simmons et al. (2005) and Selak et al. (2003) have confirmed that mitochondrial ATP production was impaired in islets and muscle of IUGR rat, respectively. It is well known that ATP depletion has detrimental effects on cell homeostasis. These effects include protein aggregation, collapse of the cytoskeleton, and loss of ionic balance. Stress response and subsequent Hsp70 accumulation give rise to an ATP-sparing effect (Kabakov and Gabai 1997). Therefore,

Hsp70 plays a critical role in maintaining mitochondrial integrity, function, and capacity for ATP generation, which is very important for determination of cell survival undergoing harmful stress injury. In addition, our immunohistochemical results revealed that the signals of Hsp70 were predominantly distributed in the epithelial cells of the whole villi and intestinal gland rather than in the lamina propria and myenteron. These suggested that the physiological expression of Hsp70 is selective to the intestinal epithelial cells and supported that Hsp70 has a cytoprotective role in epithelial cell function and structure.

In conclusion, Hsp70 expression was upregulated by IUGR in intestinal piglets at birth. Overexpression of Hsp70 can protect epithelial cells against physiological and pathological stress, thus maintaining morphologies and functions of gastrointestines and enhancing the survival rate of IUGR piglets. However, the mechanism of Hsp70 upregulation in IUGR piglets and protective effects of Hsp70 need further investigation. Furthermore, the increase of Hsp70 expression in the intestinal mucosa of IUGR piglets may be used as a marker to determine intestinal damage induced by IUGR.

Acknowledgements This work was supported by a grant from the National Natural Science Foundation of China (30771569). We would like to thank Yuanxiao Wang and Jianjun Wang for their assistance regarding sample collection. We also thank Caiyong Chen for critical discussions and reading of the manuscript.

References

- Ahmad RH, Craig CC, Arlan R (1995) Expression of heat shock genes in hepatocytes is affected by age and food restriction in rats. *J Nutri* 125:410–418
- Anckar J, Sistonen L (2007) Heat shock factor 1 as a coordinator of stress and developmental pathways. *Adv Exp Med Biol* 594:78–88
- Arispe N, Doh M, Maio AD (2002) Lipid interaction differentiates the constitutive and stress induced heat shock proteins Hsc70 and Hsp70. *Cell Stress Chaperones* 7(4):330–338
- Arvans DL, Vavricka SR, Ren HY et al (2005) Luminal bacterial flora determines physiological expression of intestinal epithelial cytoprotective heat shock proteins 25 and 72. *Am J Physiol Gastrointest Liver Physiol* 288:G696–G704
- Bao ED, Sultan KR, Nowak B, Hartung J (2008) Localization of heat shock proteins and histopathological changes in the kidneys of transported pigs. *Livest Sci* 118(3):231–237
- Baserga M, Bertolotto C, MacLennan NK et al (2004) Uteroplacental insufficiency decreases small intestine growth and alters apoptotic homeostasis in term intrauterine growth retarded rats. *Early Hum Dev* 79:93–105
- Bruce JL, Price BD, Coleman CN, Calderwood SK (1993) Oxidative injury rapidly activates the heat shock transcription factor but fails to increase levels of heat shock proteins. *Cancer Res* 53:12–15
- Da SP, Aitken RP, Rhind SM, Racey PA, Wallace JM (2001) Influence of placental mediated foetal growth restriction on the onset of puberty in male and female lambs. *Reproduction* 122:375–383
- Daugaard M, Rohde M, Jäättelä M (2007) The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Lett* 581:3702–3710
- David JC, Grognet JF, Lalles JP (2002) Weaning affects the expression of heat shock proteins in different regions of the gastrointestinal tract of piglets. *J Nutrition* 132:2551–2561
- Fowden AL, Giussani DA, Forhead AJ (2005) Endocrine and metabolic programming during intrauterine development. *Early Hum Dev* 81:723–734
- Gasbarrini A, Esposti SD, Di CC, Di NS, Loffredo S, Abraham A, Simoncini M, Pola R, Colantoni A, Trevisani F, Bernardi M, Gasbarrini G (1998) Effect of ischemia–reperfusion on heat shock protein 70 and 90 gene expression in rat liver: relation to nutritional status. *Dig Dis Sci* 43:2601–2605
- Giussani DA, Forhead AJ, Gardner DS, Fletcher AJ, Allen WR, Fowden AL (2003) Postnatal cardiovascular function after manipulation of fetal growth by embryo transfer in the horse. *J Physiol* 547:67–76
- Goloubinoff P, De Los Rios P (2007) The mechanism of Hsp70 chaperones (entropic) pulling the models together. *Trends Biochem Sci* 32(8):372–380
- Gower VC, Thompson AM (1997) Location of inducible heat shock protein mRNA in the guinea pig cochlea with a nonradioactive in situ hybridization technique. *Laryngoscope* 107(2):228–232
- Jones B, Gores GJ (1997) Physiology and pathophysiology of apoptosis in epithelial cells of the liver, pancreas, and intestine. *Am J Physiol* 273:G1174–G1188
- Kabakov AE, Gabai VL (1997) Heat shock proteins and cytoprotection: ATP-deprived mammalian cells. RG Landes, Austin
- Khassaf M, Child RB, McArdle A, Brodie D, Esanu C, Jackson MJ (2001) Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise. *J Appl Physiol* 90:1031–1035
- Kojima M, Hoshimaru M, Aoki T, Takahashi JB, Ohtsuka T, Asahi M, Matsuura N, Kikuchi H (1996) Expression of heat shock proteins in the developing rat retina. *Neurosci Lett* 205:215–217
- Liu YX, Li NN, You L (2008) Hsp70 is associated with endothelial activation in placental vascular diseases. *Mol Med* 14(9–10):561–566
- Mao L, Shelden EA (2006) Developmentally regulated gene expression of the small heat shock protein Hsp27 in zebrafish embryos. *Gene Expr Patterns* 6:127–133
- Marshman E, Ottewill PD, Potten CS, Watson AJ (2001) Caspase activation during spontaneous and radiation induced apoptosis in the murine intestine. *J Pathol* 195:285–292
- Martin J, Masri J, Bernath A, Nishimura RN, Gera J (2008) Hsp70 associates with Rictor and is required for mTORC2 formation and activity. *Biochem and Bioph Res Co* 372:578–583
- McMillen IC, Robinson JS (2005) Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev* 85:571–633
- Michael D, Hnat JW, Meadows DE, Brochman et al (2005) Heat shock protein-70 and 4-hydroxy-2-nonenal adducts in human placental villous tissue of normotensive, preeclamptic and intrauterine growth restricted pregnancies. *Am J Obstet and Gynecol* 193:836–840
- Miller L, Qureshi MA (1992) Molecular changes associated with heat shock treatment in avian mononuclear and lymphoid lineage cells. *Poult Sci* 71:473–481
- Morimoto RI (2008) Proteotoxic stress and inducible chaperone networks in neurodegenerative disease and aging. *Genes Dev* 22:1427–1438
- Musch MW, Sugi K, Straus D, Chang EB (1999) Heat-shock protein 72 protects against oxidant-induced injury of barrier function of human colonic epithelial Caco2/bbe cells. *Gastroenterology* 117:115–122
- National Research Council (NRC) (1998) Nutrient requirements of swine, 10th edn. National Academy Press, Washington, DC revised

- Njemini R, Bautmans I, Lambert M, Demanet C, Mets T (2007) Heat shock proteins and chemokine/cytokine secretion profile in ageing and inflammation. *Mech Ageing Dev* 128:450–454
- Oyake J, Otaka M, Jin M et al (2006) Overexpression of 70-kDa heat shock protein confers protection against monochloramine-induced gastric mucosal cell injury. *Life Sci* 79:300–305
- Pierzchalski P, Krawiec A, Ptak-Belowska A, Baranska A, Konturek SJ, Pawlik WW (2006) The mechanism of heat-shock protein 70 gene expression abolition in gastric epithelium caused by *Helicobacter pylori* infection. *Helicobacter* 11:96–104
- Ren H, Musch MW, Kojima K, Boone D, Ma A, Chang EB (2001) Short-chain fatty acids induce intestinal epithelial heat shock protein 25 expression in rats and IEC 18 cells. *Gastroenterology* 121:631–639
- Salokhe S, Sarkar A, Kulkarni A, Mukherjee S, Pal JK (2006) Flufenoxuron, an acylurea insect growth regulator, alters development of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) by modulating levels of chitin, soluble protein content, and HSP70 and p34cdc2 in the larval tissues. *Pestic Biochem Physiol* 85:84–90
- San YH, Ming FT, Yu TH et al (2005) Developmental changes of heat shock proteins in porcine testis by a proteomic analysis. *Theriogenology* 64:1940–1955
- Sarge KD, Murphy SP, Morimoto RI (1993) Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. *Mol Cell Biol* 13:1392–1407
- Schwerin M, Maak S, Kalbe C, Fuerbass R (2001) Functional promoter variants of highly conserved inducible hsp70 genes significantly affect stress response. *Biochimica et Biophysica Acta* 1522:108–111
- Selak MA, Storey BT, Peterside I, Simmons R (2003) Impaired oxidative phosphorylation in skeletal muscle of intrauterine growth-retarded rats. *Am J Physiol Endocrinol Metab* 285: E130–E137
- Sepponen K, Pösö AR (2006) The inducible form of heat shock protein 70 in the serum, colon and small intestine of the pig: comparison to conventional stress markers. *Vet J* 171:519–524
- Shah M, Stanek J, Handwerger S (1998) Differential localization of heat shock proteins 90, 70, 60 and 27 in human decidua and placenta during pregnancy. *J Histochem* 30:509–518
- Simmons RA, Irena SK, Selak MA (2005) Progressive accumulation of mitochondrial DNA mutations and decline in mitochondrial function lead to cell failure β -cell failure. *J Biol Chem* 280(31):28785–28791
- Sistonen L, Sarge KD, Phillips B, Abravaya K, Morimoto RI (1992) Activation of heat shock factor 2 during hemin induced differentiation of human erythroleukemia cells. *Mol Cell Biol* 12:4104–4111
- Takenaka IM, Hightower LE (1993) Regulation of chicken Hsp70 and Hsp90 family gene expression by transforming growth factor- β 1. *J Cell Physiol* 155:54–62
- Trahair JF, DeBarro TM, Robinson JS, Owens JA (1997) Restriction of nutrition in utero selectively inhibits gastrointestinal growth in fetal sheep. *J Nutr* 127:637–641
- Wada I, Otaka M, Jin M (2006) Expression of HSP72 in the gastric mucosa is regulated by gastric acid in rats—correlation of HSP72 expression with mucosal protection. *Biochem Biophys Res Commun* 349:611–618
- Wang T, Huo YJ, Shi FX, Xu RJ, Hutz RJ (2005) Effects of intrauterine growth retardation on development of the gastrointestinal tract in neonatal pigs. *Biol Neonate* 88:66–72
- Wang JJ, Chen LX, Li DF et al (2008) Intrauterine growth restriction affects the proteomes of the small intestine, liver and skeletal muscle in newborn pigs. *J Nutr* 138:60–66
- Wataba K, Saito T, Takeuchi M et al (2004) Changed expression heat shock proteins in various pathological findings in placentas with intrauterine fetal growth restriction. *Med Electron Microsc* 37:170–176
- Watanabe D, Otaka M, Mikami K et al (2004) Expression of a 72-kDa heat shock protein, and its protective function, in gastric mucosa in cirrhotic rats. *J Gastroenterol* 39:724–733
- Wu G (1998) Intestinal mucosal amino acid catabolism. *J Nutr* 128:1249–1252
- Wu GY, Bazer FW, Wallace JM, Spencer TE (2006) Board-invited review: intrauterine growth retardation: implications for the animal sciences. *J Anim Sci* 84:2316–2337
- Xu RJ, Mellor DJ, Tungthanathanich P, Birtles MJ, Reynolds GW, Simpson HV (1992) Growth and morphological changes in the small and the large intestine in piglets during the first three days after birth. *J Dev Physiol* 18:161–172
- Yu JM, Bao ED, Yan JY, Lei L (2008) Expression and localization of Hsps in the heart and blood vessel of heat-stressed broilers. *Cell Stress Chaperones* 13:327–335
- Zhu L, Bao ED, Zhao RQ et al (2009) Expression of heat shock protein 60 in the tissues of transported piglets. *Cell Stress Chaperones* 14:61–69