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ORIGINAL PAPER

Integrating miRNA and mRNA expression profiles in response to heat stress-induced injury in rat small intestine

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Abstract The molecular mechanisms underlying the pathophysiology of heat stress in the small intestine remain undefined. Furthermore, little information is available concerning changes in microRNA (miRNA) expression following heat stress. The present study sought to evaluate miRNA and mRNA expression profiles in the rat small intestine in response to heat stress. Male Sprague–Dawley rats were subjected to 2 h of heat stress daily for ten consecutive days. Rats were sacrificed at specific time points immediately following heat treatment, and morphological changes in the small intestine were determined. The miRNA and mRNA expression profiles from sample of small intestine were evaluated by microarray analysis. Heat stress caused pronounced morphological damage in the rat small intestine, most severe within the jejunum after 3 days of heat treatment. A mRNA microarray analysis found 270 genes to be up-regulated and 122 genes down-regulated ($P \leq 0.01$, ≥ 2.0-fold change) in the jejunum after heat treatment. A miRNA microarray analysis found 18 miRNAs to be up-regulated and 11 down-regulated in the jejunum after heat treatment ($P \leq 0.05$). Subsequent bioinformatic analyses of the differentially expressed mRNAs and miRNAs were carried out to integrate miRNA and mRNA expression and revealed that alterations in mRNA following heat stress were negatively correlated with miRNA expression. These findings significantly advance our understanding of the regulatory mechanisms underlying the pathophysiology of heat stress-induced injury in the small intestine, specifically with regard to miRNAs.

Keywords Heat stress · Damage · miRNAs · mRNA · Small intestine · Microarray · Rat

Fenghua Liu contributed equally to this work.

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Introduction

The intestinal epithelium is directly exposed to a large and diverse assortment of nutrients, microbes, and exogenous toxins, providing both a protective barrier and absorptive layer within the intestine (Turner 2009). Due to its direct exposure, the intestinal epithelium is subjected to many harmful effects including radiation, lipopolysaccharides (LPS), pharmacological drugs, endotoxins, and heat stress, all of which capable of causing significant injury (Shen et al. 2009). Ambient temperature is one of the most important environmental factors, as it alters behavior and physiological responses of animals (Harikai et al. 2004). Mammals exposed to environmental temperatures greater than their

thermoneutral temperature increase their peripheral blood flow to dissipate internal body heat, concomitantly resulting in a significant reduction in blood flow to the small intestine. During this time, the epithelium of the small intestine can experience ischemic and hypoxic conditions, resulting in tissue damage, potentially exacerbated following reperfusion (Kregel et al. 1988). Epithelial cells of the intestine are replenished rapidly as mature differentiated epithelial cells are shed into the lumen and are continuously replaced by a progeny of stem cells, crucial in maintaining proficient intestinal function. Our previous studies have suggested that ERK1/2 signaling, EGFR signaling, and growth factors are all involved in the response to heat stress-induced damage in small intestinal epithelial cells, regulating cellular proliferation, growth, differentiation, and migration of crypt stem cells (Liu et al. 2009; Yu et al. 2010a, b).

Heat stress-induced damage within the small intestine is a complex process stimulating a multifaceted cellular response, including rapidly altering the expression of a number of genes. These heat-induced alterations in gene expression regulate both intra- and extracellular signaling, coordinating a cellular response to maintain physiological homeostasis during stress. Changes in mRNA expression in response to heat stress primarily include: (1) increased expression of heat shock proteins; (2) altered expression of metabolic-related genes; (3) increased expression of redox genes; (4) altered expression of transcription-regulation genes; (5) altered expression of genes regulating apoptosis, proliferation, and differentiation; and (6) altered expression of immunological genes (Hazen et al. 2003; Rajan and D'Silva 2009; Rensink et al. 2005; Sonna et al. 2002; Soos et al. 2009).

MicroRNAs (miRNAs) are non-coding small RNA molecules of 21 to 24 nucleotides, capable of regulating the expression of hundreds of target genes. A number of investigations have demonstrated that miRNAs serve as guide molecules, base pairing with partial or full complementary sequences of target mRNAs, leading to translational repression and/or mRNA cleavage. Recent advances in the understanding of miRNA mechanisms of activity have reported that miRNA can affect stem cell differentiation, organ development, cell death, phase change of the cell cycle, signal transduction, and several diseases including cancer (Arvey et al. 2010; Lionetti et al. 2009). Interestingly, environmental stressors have been shown to alter miRNA expression. Moreover, miRNAs have recently been implicated in cellular responses to oxidative stress, DNA damage, UV irradiation, hypoxic stress, oncogenic stress, and heat stress where genetic knockdown of specific miRNAs renders the mutant animals incapable of coping with these stressors (Pothof et al. 2009; Rinaldi et al. 2010; Wilmink et al. 2010).

Although heat stress-induced damage and repair within the small intestine has been intensively studied, the molecular mechanisms underlying the pathophysiological changes induced by stress remain to be defined. Moreover, minimal information is available concerning changes in miRNA expression within the small intestine during heat stress. Thus, the present study integrated microRNA and mRNA expression profiles to observe and compare the effect of heat stress on the rat small intestine using Agilent microRNA and mRNA microarrays. Furthermore, bioinformatic analyses (including molecular function, biological processes, cellular components, KEGG pathway, and predicted target genes) were performed on mRNA or microRNA that displayed altered expression following heat treatment. The findings of the current study provide novel insight into the molecular mechanisms mediating heat stress-induced damage and repair in the rat small intestine.

Materials and methods

Animal experimental groups

All experimental protocols were approved by the Committee for the Care and Use of Experimental Animals, China Agricultural University. Forty-eight male Sprague–Dawley rats weighing 200±20 g (obtained from Beijing Vital River Laboratory, Animal Technology Co., Beijing, China) were acclimatized to 25°C, 60% relative humidity (RH), and maintained under a 12:12 h light:dark cycle. Food and water were provided ad libitum for 7 days. On day 8, rats were randomly assorted into control or heat-treatment groups. Each group consisted of 24 rats which were housed in plastic cages (400×300×180 mm) with a carpet of soft woodchips. Feed (200 g) and water (400 mL) were provided daily.

Treatment

Rats in the control group were housed under controlled conditions (25°C, 60% RH) throughout the treatment period. Heat-stressed rats were housed under control group conditions, but additionally exposed to 40°C and 60% RH between 11:00 am and 1:00 pm daily for ten consecutive days. On the first, third, sixth, and tenth days, six rats from each group were sacrificed immediately following the 2-h heat exposure period.

Sampling

Rat body temperature was recorded daily before and after heat exposure using a thermistor probe connected to a digital thermometer. Body surface temperature was also recorded daily before and after heat exposure using both an

infrared and contact thermometer (Fluke 561, Evered, Washington, USA). Following sacrifice, blood samples were collected and centrifuged at 3,000×*g* for 10 min; sera was collected and stored at −80°C until required. The intestine was removed and immediately irrigated with physiological saline to remove all intestinal contents before being sectioned into the duodenum (15 mm from the pylorus), the distal jejunum–ileum (half of the remaining small intestine up to the cecum), and the ileum (20 mm proximal to the cecum). Each intestinal section was divided into three pieces: one fixed in 10% buffered formalin phosphate for histological analysis; one for microarray analysis; and one stored at −80°C. Total serum cortisol concentration was determined using an I^{125} cortisol radioimmunoassay kit, performed according to the manufacturer's instructions (Beijing Chemclin Biotech Co., Ltd, Beijing, China).

Fixing intestinal sections and staining

Following fixation of intestinal sections in 10% buffered formalin phosphate, the formalin-fixed samples were embedded in paraffin and transversely sectioned (5 μm thick). After deparaffinization and dehydration, the sections of duodenum, jejunum, and ileum were stained with hematoxylin and eosin (Sigma, St. Louis, MO, USA). Microstructures of the small intestine were observed using a BH2 Olympus microscope (DP71, Olympus, Tokyo, Japan).

mRNA microarray experimental setup and initial data analysis

RNA extraction and target labeling

Total RNA was isolated from small intestinal tissue collected after 3 days of treatment using a phenol and guanidine isothiocyanate-based Trizol reagent in accordance with manufacturer's instructions (p/n 15596-026, Invitrogen, Carlsbad, CA, USA). RNA quality of each sample was determined and recorded using an RNA 6000 LabChip Kit and the 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Isolated RNA consistently had a 28S/18S ratio of ~1.8. RNA was purified using a QIAGEN RNeasy® Mini Kit (#74106, QIAGEN, Chatsworth, CA, USA) and amplified using a low RNA input linear amplification kit (#5184-3523, Agilent Technologies). Each RNA sample was annealed with a primer containing a poly-dT and a T7 polymerase promoter. Reverse transcriptase produced primary and secondary cDNA strands. T7 RNA polymerase was then used to create cRNA from the double-stranded cDNA by incorporating cyanine-3-labeled cytidine 5-triphosphate. The quality of the labeled cRNA was again verified, and the absolute concentration was measured using a spectrophotometer (Nanodrop ND1000, NanoDrop Technologies, Wilmington, DE, USA).

Hybridization, scanning, and feature extraction

The cRNA was hybridized in equal amounts to six arrays using a Gene Expression Hybridization Kit (#5188-5242, Agilent Technologies). Hybridization was performed at 60°C for 17 h using whole rat genome arrays (#G4131F, Agilent Technologies). The arrays were washed using a gene expression wash buffer kit (#5188-5327, Agilent Technologies) before stabilization and dehydration were performed (#5185-5979, Agilent Technologies). The arrays were scanned on a microarray (#G2565BA, Agilent Technologies) and the subsequent data compiled with Agilent feature extraction software. All steps from RNA amplification to the final scanner output were conducted by a private contractor (Shanghai Biochip Co., Ltd, Shanghai, China).

mRNA microarray data analysis

Array normalizations and error detection were carried out using Silicon Genetics' GeneSpring GX Version 10.0 (Agilent Technologies) via the enhanced Agilent feature extraction import preprocessor. First, values of poor quality intensities and low dependability were removed using a "filter on flags" feature, where standardized software algorithms determined which spots were "present", "marginal", or "absent". Filters were set to retain only the present and marginal values for further analysis. Data were normalized using algorithms supplied with the feature extraction software. After data normalization, a final quality-control filter was applied, where genes expressing excessive biological variability were discarded. Data were further analyzed using GeneSpring to reveal genes significantly differing in expression between treatment groups.

miRNA microarray experimental setup and initial data analysis

miRNA isolation, target labeling, and hybridization

miRNA isolation was carried out using pooled total RNA isolated by a mirVana™ RNA Isolation Kit (p/n AM1560, Applied Biosystem, Foster City, CA, USA) and labeled using Agilent's miRNA Complete Labeling and Hybridization Kit (p/n 5190-0456, Agilent Technologies). Labeled RNA was hybridized in equal amounts to arrays using a Gene Expression Hybridization Kit (#5188-5242, Agilent Technologies). Hybridization was performed at 55°C and 20 rpm for 20 h using six rat miRNA microarray arrays

(#G4131F, Agilent Technologies). The arrays were washed using a gene expression wash buffer kit (#5188-5327, Agilent Technologies), stabilized, and dehydrated (#5185-5979, Agilent Technologies). The arrays were scanned on a Microarray (#G2565BA, Agilent Technologies) and the subsequent data was compiled with Agilent feature extraction software. All steps from RNA amplification to the final scanner output were conducted by a private contractor (Shanghai Biochip Co., Ltd, Shanghai, China).

miRNA data analysis

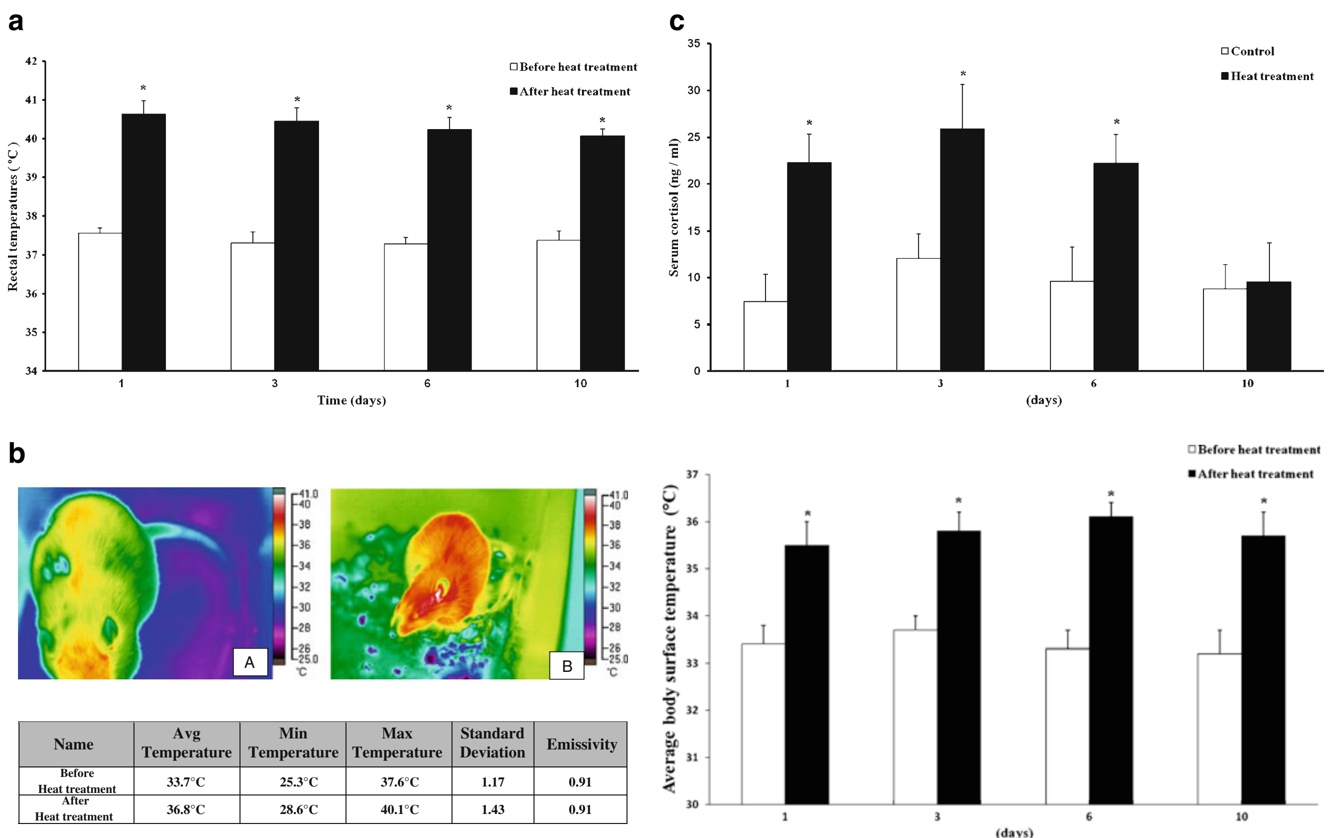
Signal intensities of each spot were normalized to background signal and analyzed using the G4450AA feature extraction software 9.5 and scan control software 7.0 (Agilent Technologies). Data were further analyzed using GeneSpring to reveal miRNA significantly differing in expression between treatment groups.

Integrated analysis of miRNA and mRNA expression profiles

miRNA and mRNA expression profiles were overlapped to generate a miRNA–mRNA interaction database. Integrated analysis was then performed, comparing the predicted mRNA targets of each altered miRNA to experimentally determined (http://www.microrna.org, and mirBase was available at http://microrna.sanger.ac.uk/targets/v2/etc).

Statistical analysis

All results are presented as the mean ± SE. Independent sample *T* tests were performed to identify significant differences between treatment groups using SPSS 12.0 software. A *p* value of less than 0.05 was considered statistically significant. Microarray analyses were conducted using GeneSpring GX_10.0.



Name	Avg Temperature	Min Temperature	Max Temperature	Standard Deviation	Emissivity
Before Heat treatment	33.7°C	25.3°C	37.6°C	1.17	0.91
After Heat treatment	36.8°C	28.6°C	40.1°C	1.43	0.91

Fig. 1 **a** Rat rectal temperature before and after heat treatment. Rat rectal temperature was significantly elevated following 2 h of heat exposure at 40°C at all time points. *$P<0.05$. **b** Body surface temperature of rats before (**a**) and after (**b**) heat treatment. Rat body surface temperature was significantly elevated after 2 h of heat exposure at 40°C at all time points, *$P<0.05$. **c** Serum cortisol concentrations from heat-stressed rats were significantly higher than the control group on the first, third, and sixth day after heat treatment. The greatest increase in cortisol concentration was on the third day of heat treatment before returning to levels equivalent to control by day 10. *$P<0.05$ heat-treated group vs. control

Results

Assessment of heat treatment

Rat rectal and body surface temperatures as well as serum cortisol concentration were all found to be significantly elevated after heat treatment at all four time points (all $P<0.05$), with the exception of serum cortisol concentration at day 10 displaying equivalent levels prior to and following heat treatment (Fig. 1).

Histological changes in the small intestine following heat stress

Sections of the duodenum, jejunum, and ileum stained with hematoxylin and eosin, were observed under microscopy. Bleeding in the intestinal villi and desquamation at the tips of the intestinal villi were observed in the small intestine after heat treatment. Desquamation of mucosal epithelial cells caused the lamina propria to become exposed, exhibiting the most severe injury in the jejunum following 3 days of heat treatment (Fig. 2). Subsequently, tissue from the jejunum on the third day of heat treatment was used for all mRNA and miRNA microarray analyses.

mRNA expression profiling and bioinformatics analysis

Gene expression profiling of rat jejunum following 3 days of heat treatment was performed using six rat genome microarrays. Greater than 41,000 rat genes and transcripts were investigated and the heat-treated and control groups were compared. Genes (270) were found to be significantly up-regulated and 122 genes to be significantly down-regulated by ≥2.0-fold ($P\leq0.01$) in the heat-treatment group compared with control (Fig. 3a). To further characterize the types of genes altered in response to heat stress, the 392 differentially expressed genes were classified into gene ontology (GO) slim terms. GO slim assigns high level terms from each of

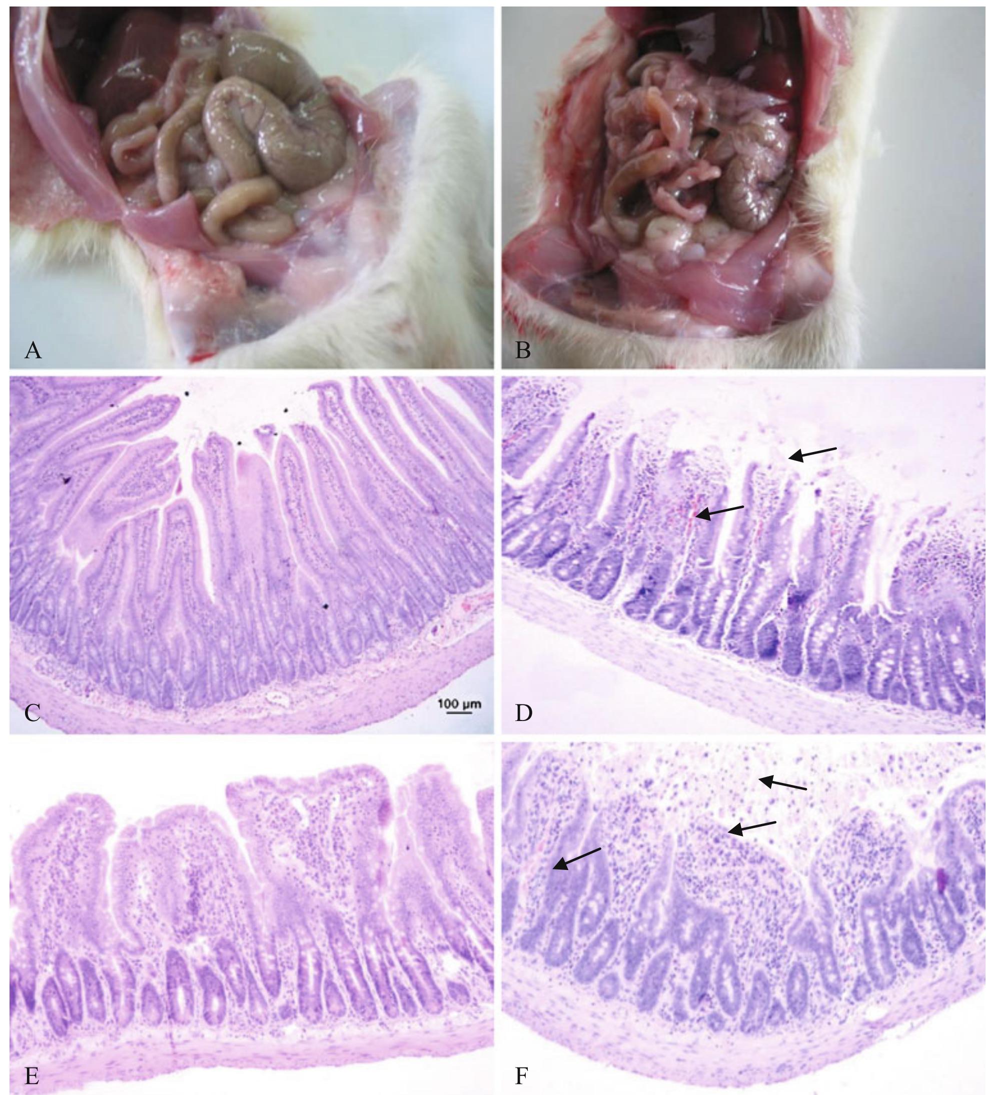


Fig. 2 Photomicrographs of hematoxylin and eosin-stained sections of control (**a**) and heat-treated (**b**) rat jejunum on the third day (×200 magnification). Severe damage to the rat intestinal villi is apparent, with desquamation at the tips of the intestinal villi and exposure of the lamina propria. Abnormal microstructures were indicated with *arrowheads*. *Scale bar* represents 100 μm

the three major gene ontologies: molecular function, biological processes, and cellular components.

Molecular function analysis (Table 1) found that the 392 differentially expressed genes were predominantly related to GO: 0003700 transcription factor activity, GO: 0020037 heme binding, GO: 0004497 monooxygenase activity, GO: 0005524 ATP binding, GO: 0003707 steroid hormone receptor activity, GO: 0005515 protein binding, GO: 0008083 growth factor activity, GO: 0009055 electron carrier activity, and GO: 0016491 oxidoreductase activity.

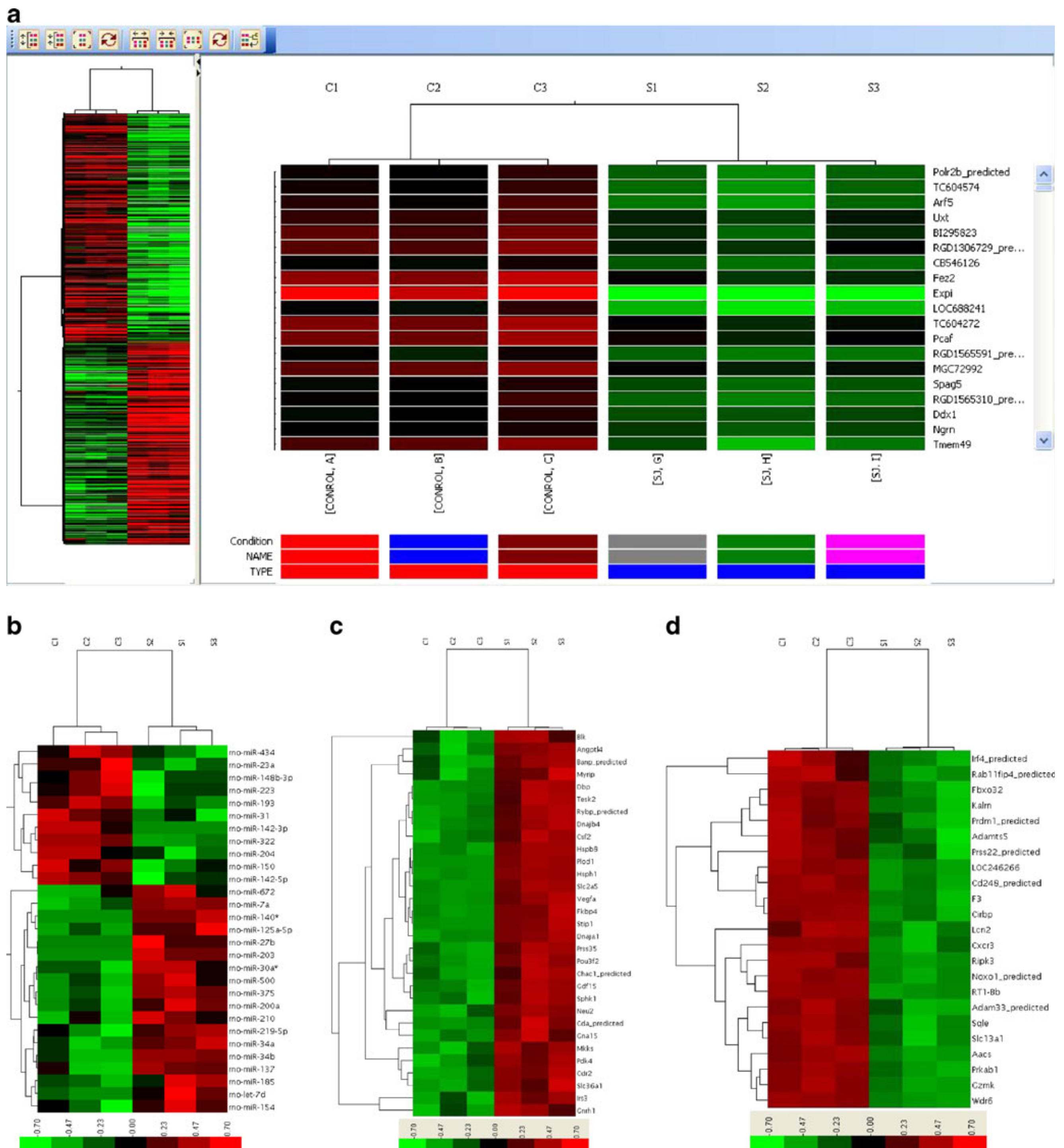


Fig. 3 Hierarchical cluster analysis of the differentially expressed mRNAs (**a**), miRNAs (**b**), and their predicted up- and down-regulated mRNA targets (**c** and **d**, respectively). *Red bars* represent high expression levels relative to the reference sample, *blue bars* represent low expression levels, and *black bars* indicate expression similar to the normalized median gene expression

Table 1 Molecular function analysis of the differentially expressed mRNAs

GO term	Count	*p* value	*q* value
GO:0003700 transcription factor activity	17	7.11E-14	1.42E-12
GO:0020037 heme binding	13	1.71E-13	2.99E-12
GO:0043565 sequence-specific DNA binding	13	9.02E-13	1.22E-11
GO:0008270 zinc ion binding	30	9.57E-13	1.22E-11
GO:0004497 monooxygenase activity	10	4.50E-12	4.84E-11
GO:0005524 ATP binding	24	8.62E-12	8.07E-11
GO:0003707 steroid hormone receptor activity	6	7.53E-10	5.02E-09
GO:0005515 protein binding	36	4.60E-09	2.80E-08
GO:0008083 growth factor activity	7	6.30E-09	3.68E-08
GO:0009055 electron carrier activity	11	7.41E-09	4.15E-08
GO:0016491 oxidoreductase activity	17	1.79E-08	9.29E-08
GO:0005353 fructose transporter activity	3	4.19E-08	2.09E-07
GO:0050660 FAD binding	5	1.42E-07	6.88E-07
GO:0004252 serine-type endopeptidase activity	8	5.58E-07	2.37E-06
GO:0008233 peptidase activity	12	1.85E-06	6.64E-06
GO:0004499 dimethylaniline monooxygenase (N-oxide-forming) activity	2	2.27E-06	7.76E-06
GO:0000334 3-hydroxyanthranilate 3,4-dioxygenase activity	2	4.81E-06	1.53E-05
GO:0004506 squalene monooxygenase activity	2	4.81E-06	1.53E-05
GO:0046983 protein dimerization activity	4	5.94E-06	1.81E-05
GO:0000166 nucleotide binding	22	6.86E-06	2.00E-05
GO:0016493 C-C chemokine receptor activity	2	9.90E-06	2.83E-05
GO:0051082 unfolded protein binding	4	1.17E-05	3.28E-05
GO:0005506 iron ion binding	6	1.32E-05	3.63E-05
GO:0046872 metal ion binding	25	1.55E-05	4.10E-05
GO:0042056 chemoattractant activity	1	4.79E-05	1.15E-04
GO:0031072 heat shock protein binding	4	6.46E-05	1.50E-04
GO:0050661 NADP binding	2	6.52E-05	1.50E-04
GO:0008009 chemokine activity	2	9.76E-05	2.17E-04
GO:0004143 diacylglycerol kinase activity	2	1.21E-04	2.61E-04
GO:0004713 protein-tyrosine kinase activity	5	1.76E-04	3.68E-04
GO:0016702 oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	3	1.90E-04	3.92E-04
GO:0051219 phosphoprotein binding	2	2.61E-04	5.22E-04

Analysis of biological processes (Table 2) revealed the 392 differentially expressed genes to be predominantly associated with GO: 0055114 oxidation reduction, GO: 0006355 regulation of transcription, DNA-dependent, GO: 0009408 response to heat, GO: 0006955 immune response, GO: 0002504 antigen processing and presentation of peptide or polysaccharide antigen via MHC class II, and GO: 0006457 protein folding.

Cellular component analysis (Table 3) identified the 392 differentially expressed genes to be primarily related to GO: 0005634 nucleus, GO: 0016021 integral to the membrane, GO: 0005576 extracellular region, GO: 0016020 membrane, GO: 0005737 cytoplasm, GO: 0005792 microsome, and GO: 0042613 MHC class II protein complex.

To define the biological pathways potentially associated with heat stress in the rat jejunum, microarray data were analyzed using a Molecule Annotation System (http://bioinfo.capitalbio.com/mas/). KEGG pathway analysis revealed that the genes significantly altered in response to heat stress to be associated with cytokine–cytokine receptor interaction, antigen processing and presentation, retinol metabolism, complement and coagulation cascades, the PPAR signaling pathway, fatty acid metabolism, the renin–angiotensin system, asthma, drug metabolism, cytochrome P450, 3-chloroacrylic acid degradation, the MAPK signaling pathway, tryptophan metabolism, circadian rhythm, the adipocytokine signaling pathway, cell adhesion molecules (CAMs), VEGF signaling, the Fc epsilon RI signaling pathway, and the p53 signaling pathway (Table 4).

Table 2 Biological processes analysis of the differentially expressed mRNAs

GO term	Count	*p* value	*q* value
GO:0055114 oxidation reduction	19	2.91E-16	1.02E-14
GO:0006355 regulation of transcription, DNA-dependent	22	3.44E-13	5.35E-12
GO:0009408 response to heat	7	2.71E-12	3.17E-11
GO:0006955 immune response	11	4.73E-10	3.31E-09
GO:0002504 antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	3	1.26E-09	8.01E-09
GO:0006457 protein folding	7	2.39E-07	1.12E-06
GO:0006468 protein amino acid phosphorylation	14	2.90E-07	1.31E-06
GO:0042493 response to drug	6	6.29E-07	2.59E-06
GO:0006508 proteolysis	14	7.17E-07	2.79E-06
GO:0045471 response to ethanol	4	2.16E-06	7.57E-06
GO:0030968 unfolded protein response	2	3.75E-06	1.25E-05
GO:0042789 mRNA transcription from RNA polymerase II promoter	1	4.81E-06	1.53E-05
GO:0007205 protein kinase C activation	3	6.61E-06	1.97E-05
GO:0045071 negative regulation of viral genome replication	1	1.44E-05	3.88E-05
GO:0019835 cytolysis	3	1.80E-05	4.66E-05
GO:0006814 sodium ion transport	5	2.25E-05	5.72E-05
GO:0034612 response to tumor necrosis factor	1	2.88E-05	7.13E-05
GO:0048252 lauric acid metabolism	2	2.88E-05	7.13E-05
GO:0033574 response to testosterone stimulus	2	4.79E-05	1.15E-04
GO:0043576 regulation of respiratory gaseous exchange	2	7.17E-05	1.62E-04
GO:0006983 ER overload response	1	1.00E-04	2.19E-04
GO:0019369 arachidonic acid metabolism	2	1.34E-04	2.83E-04
GO:0006915 apoptosis	7	2.52E-04	5.12E-04
GO:0006935 chemotaxis	3	3.11E-04	6.14E-04
GO:0043065 positive regulation of apoptosis	4	5.02E-04	9.63E-04

Table 3 Cellular component analysis of the differentially expressed mRNAs

GO term	Count	*p* value	*q* value
GO:0005634 nucleus	39	5.61E-19	4.40E-17
GO:0016021 integral to membrane	49	6.29E-19	4.40E-17
GO:0005576 extracellular region	26	1.26E-16	5.86E-15
GO:0016020 membrane	55	1.16E-15	3.24E-14
GO:0005737 cytoplasm	44	3.62E-14	8.44E-13
GO:0005792 microsome	10	8.65E-12	8.07E-11
GO:0042613 MHC class II protein complex	4	1.82E-11	1.59E-10
GO:0005615 extracellular space	11	3.38E-11	2.78E-10
GO:0009897 external side of plasma membrane	6	3.28E-10	2.55E-09
GO:0005783 endoplasmic reticulum	14	3.92E-10	2.89E-09
GO:0005789 endoplasmic reticulum membrane	10	1.79E-08	9.29E-08
GO:0031225 anchored to membrane	5	3.95E-07	1.73E-06
GO:0005886 plasma membrane	17	6.84E-07	2.74E-06
GO:0005625 soluble fraction	6	9.18E-07	3.47E-06
GO:0016324 apical plasma membrane	5	9.73E-07	3.58E-06
GO:0042470 melanosome	3	3.44E-04	6.68E-04

miRNA expression profiling

A microarray platform optimized for the analysis of 350 rat miRNAs was used to analyze and compare the pattern of miRNA expression between heat-treated and control rat jejunum. miRNAs (29) were found to be significantly different between the two groups ($P \leq 0.05$, Fig. 3b) comprising of 18 up-regulated (rno-miR-34b, -137, -154, -672, -219-5p, -375, -7a, -34a, -30a*, -500, -185, -203, -200a, -140*, -let-7d, -125a-5p, -27b, -210) and 11 down-regulated miRNAs (rno-miR-322, -142-5p, -434, -204, -142-3p, -193, -31, -150, -148b-3p, -223, -23a).

Integrated analysis of miRNA and mRNA expression profiles

Integrated analysis comparing the predicted mRNA targets of each altered miRNA to the experimentally determined revealed that the altered miRNAs and their predicted mRNA targets had reciprocal levels of expression (Fig. 3c and d; Table 4 in the Supplementary Material)

Discussion

Heat stress caused significant damage to rat small intestine

The small intestine plays a critical role both in providing a barrier to pathogens as well as an important absorptive function (Turner 2009). Many stressors such as radiation, LPS, pharmacological drugs, endotoxins, and extreme heat can cause damage to the intestinal epithelium. Focusing on heat stress, when mammals are exposed to environmental temperatures greater than their thermoneutral temperature, internal body heat is dissipated by increased peripheral blood flow which, in turn, dramatically reduces blood flow to the GI tract (Shen et al. 2009). The ischemia and hypoxia experienced by the small intestine can result in necrosis and shedding of epithelial cells of the intestinal villi. Intestinal epithelial cell damage disrupts the protective intestinal barrier and reduces its absorptive capability (Yu et al. 2010a, b). The present study provided clear evidence of this, with heat stress causing significant damage to the rat small intestine; found to be most severe in the jejunum after 3 days of heat treatment, in accordance with our previous findings (Liu et al. 2009; Yu et al. 2010a, b). Moreover, elevated body temperature and metabolic rate are known to increase the production of reactive oxygen species, which react with lipids, proteins, and nucleic acids, culminating in cellular injury (Iacobas et al. 2008; Nick 2010).

Integrating miRNA and mRNA expression profiles in the small intestine in response to heat stress

MicroRNAs (miRNAs) are known to regulate the expression of target genes both at the level of mRNA translation and mRNA stability. This ability lets miRNAs activity to be involved in the control of a wide range of biological functions and processes, such as development, differentiation, metabolism, growth, proliferation, and apoptosis

Table 4 KEGG pathway analysis of the differentially expressed mRNAs

Pathway	Count	*p* value	*q* value	Gene
Cytokine–cytokine receptor interaction	9	2.18E-07	4.96E-06	Ccl4; Ccl5; Ccr5; Csf2; Cxcr3; Il18rap; Il22ra2; Fas; Vegfa
Antigen processing and presentation	6	1.79E-06	1.81E-05	Hspa1a; Hspa1l; Hspa8; Hsp90aa1; Hsp90ab1; RT1-Bb
Retinol metabolism	5	3.76E-06	2.85E-05	Adh1; Cyp1a2; Cyp4a8; Cyp4a3; Dgat2
Complement and coagulation cascades	5	4.71E-06	3.30E-05	Cd55; F3; Fgb; Serpine1; Serpinf2
PPAR signaling pathway	5	5.83E-06	3.74E-05	Angptl4; Cyp4a8; Cyp4a3; Ppara; Scd1
Fatty acid metabolism	4	1.43E-05	7.86E-05	Adh1; Aldh1a7; Cyp4a8; Cyp4a3
Renin–angiotensin system	3	3.50E-05	1.68E-04	Cma1; Cpa3; Enpep
Asthma	3	7.51E-05	2.81E-04	Fcer1a; Ms4a2; RT1-Bb
Drug metabolism—cytochrome P450	4	1.00E-04	3.52E-04	Adh1; Cyp1a2; Fmo2; Fmo5
3-Chloroacrylic acid degradation	2	3.50E-04	0.001128	Adh1; Aldh1a7
MAPK signaling pathway	6	3.59E-04	0.001128	Ddit3; Hspa1a; Hspa1l; Hspa8; Hspb1; Fas
Tryptophan metabolism	3	5.68E-04	0.001615	Aldh1a7; Cyp1a2; Haao
Circadian rhythm	2	8.18E-04	0.002067	Nr1d1; Per2
Adipocytokine signaling pathway	3	0.001659	0.003838	Irs3; Ppara; Prkab1
Cell adhesion molecules (CAMs)	4	0.002113	0.004228	Cntn1; Madcam1; RT1-Bb; Spn
VEGF signaling pathway	3	0.002137	0.004228	Hspb1; Sphk1; Vegfa
Fc epsilon RI signaling pathway	3	0.002314	0.00448	Csf2; Fcer1a; Ms4a2
p53 signaling pathway	3	0.002405	0.00456	Cdk6; Serpine1; Fas

(Arvey et al. 2010; Lionetti et al. 2009). Recently, studies in mammals have shown that global miRNA expression profiles are altered in response to a number of cellular and organ stresses, including oxidative stress, DNA damage, UV irradiation, arsenic exposure, drug treatment, nutrient deprivation, ischemia, and hypoxia (Dave and Khalili 2010; Pothof et al. 2009; Rinaldi et al. 2010; Simone et al. 2009; Wilmink et al. 2010; Zhang et al. 2010). miRNAs were considered as sentinels of the cellular stress response as: (1) they are post-transcriptional gene regulators, potentially functioning as “quick responders” to cellular stress; (2) as miRNAs regulate numerous targets, they have the capacity to potently and efficiently coordinate a stress response involving numerous genes; (3) owing to their small size and high stability, miRNAs may be less susceptible to certain types of stress (Babar et al. 2008). In the present study, 29 miRNAs were significantly differentially expressed (comprising 18 up-regulated and 11 down-regulated miRNAs) in the rat small intestine after heat treatment. Studies have recently shown that several miRNAs are closely associated with cellular stress; for example, miR-34a and -34b are effectors of p53 which is implicated in DNA damage and repair, cell-cycle arrest, and apoptosis (Antonini et al. 2010; Hermeking 2010). In the present study, rno-miR-34a and -34b were both significantly up-regulated in rat small intestine after heat treatment, suggesting that miR-34 may also be involved in heat stress-induced apoptosis of the intestinal epithelium. Rno-miR-137 is reported to be implicated in the response to ischemia (Dharap et al. 2009), as its expression is increased following traumatic brain injury in the rat (Lei et al. 2009). In the present study, rno-miR-137was significantly up-regulated in rat small intestine after heat treatment, suggesting it may be also implicated in heat stress-induced ischemia and injury in rat small intestine. In the case of let-7 miRNA, one recent publication reported the miRNA to be involved in the stress response, where it was significantly down-regulated in both normal lung epithelial cells and lung cancer cell lines in response to radiation (Barshack et al. 2010). The present study also found rno-let-7d to be significantly reduced in rat small intestine after heat treatment, supporting let-7d as an important miRNA in the stress response. To further investigate the role of miRNAs in stress-induced damage to the small intestine, we integrated miRNA with mRNA expression profiles and identified positively and negatively correlated miRNA/gene pairs. A total of 29 miRNAs and 392 mRNAs were found to be differentially expressed in response to heat stress, with the altered miRNAs and their 54 predicted mRNA targets displaying reciprocal levels of expression. The negative correlation between miRNAs and predicted target mRNA expression identified in this study supports the hypothesis that miRNAs can significantly modulate gene expression.

In summary, the present study performed microarray-based expression profiling of both miRNA and mRNA of tissue isolated from the rat small intestine following heat stress. A total of 29 miRNAs and 392 mRNAs were found to be significantly differentially expressed in the small intestine of rats exposed to heat stress. Furthermore, a negative correlation between the expression of miRNAs and their predicted gene targets was identified. Although relatively little information is currently available concerning the biological function of miRNAs identified to date, we strongly suggest that miRNAs play an important role in modulating gene expression involved in the pathophysiological response to heat stress in the rat small intestine.

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