World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v16.i11.1385

World J Gastroenterol 2010 March 21; 16(11): 1385-1396
ISSN 1007-9327 (print)

ORIGINAL ARTICLE

Time-series gene expression profiles in AGS cells stimulated with *Helicobacter pylori*

Yuan-Hai You, Yan-Yan Song, Fan-Liang Meng, Li-Hua He, Mao-Jun Zhang, Xiao-Mei Yan, Jian-Zhong Zhang

Yuan-Hai You, Yan-Yan Song, Fan-Liang Meng, Li-Hua He, Mao-Jun Zhang, Xiao-Mei Yan, Jian-Zhong Zhang, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, PO Box 5, Changping District, Beijing 102206, China
Author contributions: You YH and Song YY performed the majority of experiments and wrote the manuscript; Meng FL, He LH, Zhang MJ and Yan XM provided the vital reagents and materials; Zhang JZ designed the study and provided financial support for this work.
Supported by The National Natural Science Foundation of China, No. 39870032; Key Projects in the National Science & Technology Pillar Program in the Eleventh Five-Year Plan Period
Correspondence to: Jian-Zhong Zhang, Professor, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, PO Box 5, Changping District, Beijing 102206, China. zhangjianzhong@icdc.cn
Telephone: +86-10-61739456 Fax: +86-10-61739439
Received: November 22, 2009 Revised: December 14, 2009
Accepted: December 21, 2009
Published online: March 21, 2010

Abstract

AIM: To extend the knowledge of the dynamic interaction between *Helicobacter pylori* (*H. pylori*) and host mucosa.

METHODS: A time-series cDNA microarray was performed in order to detect the temporal gene expression profiles of human gastric epithelial adenocarcinoma cells infected with *H. pylori*. Six time points were selected to observe the changes in the model. A differential expression profile at each time point was obtained by comparing the microarray signal value with that of 0 h. Real-time polymerase chain reaction was subsequently performed to evaluate the data quality.

RESULTS: We found a diversity of gene expression patterns at different time points and identified a group of genes whose expression levels were significantly correlated with several important immune response and tumor related pathways.

CONCLUSION: Early infection may trigger some important pathways and may impact the outcome of the infection.

Key words: *Helicobacter pylori*; Gene expression; Microarray; Time-series

Peer reviewer: Dr. Yutao Yan, Medicine Department, Emory University, 615 Michael ST, Whitehead Building/265, Atlanta, GA 30322, United States

You YH, Song YY, Meng FL, He LH, Zhang MJ, Yan XM, Zhang JZ. Time-series gene expression profiles in AGS cells stimulated with *Helicobacter pylori*. *World J Gastroenterol* 2010; 16(11): 1385-1396 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i11/1385.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i11.1385

INTRODUCTION

Helicobacter pylori (*H. pylori*) have been shown to be the principal cause of acute and chronic gastritis and a major risk factor in gastric cancer development. A chronic inflammatory process induced by the pathogen is thought to be the cause of tumor development. It is well known that *H. pylori* binding to epithelial cells can induce tyrosine phosphorylation of host cell proteins and rearrangement of the cytoskeleton, which may contribute to inflammation and oncogenic transformation[1]. *H. pylori* colonization to the mucosa may also induce a systemic immune response and be susceptible to Ab-dependent complement-mediated phagocytosis and killing. Infected epithelial cells may also induce a mucosal inflammation under a mechanism of autoantibody-mediated destruction[2]. Some host factors like interleukin (IL)-1β, tumor necrosis factor (TNF)-α,

and IL-10 may influence the disease outcome. One investigation on nuclear factor (NF)-κB signaling pathway and iNOS suggests that NF-κB activation may play an important role in protecting mucosol cells from apoptosis through upregulating iNOS[3]. Many previous studies have performed expression profiling to investigate host changes induced by *H. pylori* infection. These studies have provided some useful and significant information and shed some light for exploring the potential mechanism of *H. pylori* infection and host immunity[4-10]. However, none of them is designed based on a time-series scheme, the global and sequential profile of *H. pylori* infection that may be involved in the pathogenetic mechanism by which *H. pylori* infects and contributes to gastric carcinogenesis remains poorly understood. In this study, human gastric epithelial adenocarcinoma cells (AGS) co-cultured with an *H. pylori* 26695 strain at different time points were separated and analyzed by a whole genome Illumina microarray. Computer-assisted bioinformatics analysis was conducted to analyze the differential gene expression pattern.

MATERIALS AND METHODS

H. pylori and AGS cell co-culture

H. pylori strain 26695 was routinely cultured for 24 h on Columbia agar plates (Oxoid) containing 5% goat blood under microaerophilic conditions at 37℃, following a wash in sterile PBS and estimation of the quantity of bacteria by OD600. The human gastric epithelial adenocarcinoma cell line AGS (ATCC CRL 1739) was cultured in RPMI 1640 without antibiotic or antifungal agents, and supplemented with 4 mmol/L L-glutamine and 10% fetal calf serum (Gibco) at 37℃ in a humidified atmosphere of 5% CO_2. A monolayer of AGS cells grown to 80% confluence was co-cultured with *H. pylori* at a multiplicity of infection of 300:1 in culture media for 0.5, 1, 2, 4, and 6 h.

RNA isolation

Co-culture was stopped at each time point and followed by washing three times with PBS. Total RNA was isolated using Trizol extraction (Gibco/BRL). The quality of the RNA was verified by 1% agarose gel containing ethidium bromide.

Microarray expression profiling and data analysis

Illumina Human-6 v2 BeadChips used for this study contains probes for well characterized genes, gene candidates and splice variants for a total number of 48000 features. The "Detection Score > 0.99" was used to determine the expression. It was a statistical measure in the BeadStudio software, which was computed based on the *Z*-value of a gene relative to that of the negative controls. The data were normalized using a cubic spline method, which was generally used as a normalization algorithm in BeadStudio. The differentially expressed genes in different time point were identified using the Illumina custom error model implemented in BeadStudio. DiffScore, the expression difference score, takes into account background noise and sample variability[11]. The formula for the calculation of the DiffScore is: $DiffScore = 10\ \mathrm{sgn}(\mu_{cond} - \mu_{ref})\log_{10}(p)$. The differentially expressed genes with a |Diffscore| > 13 were selected for further analysis. The genes with a fold change > 1.5 were integrated and hierarchically clustered using Mev_4_0 (Multiple Experiment Viewer, TIGR). Gene enrichment in KEGG pathways (Kyoto Encyclopedia of Genes and Genomes) and Gene Ontology (GO) were accomplished with Onto-Tool (Pathway Express, OE2GO)[12,13], and co-expression gene clustering by short time-series expression miner (STEM, Carnegie Mellon University)[14] with a maximum number of model profiles set as 245, and a maximum unit change in model profiles between time points set at 2. Four interesting co-expression profiles were selected for further analyses. To obtain an optimized GO distribution, we also took all differentially expressed genes including those with a fold change < 1.5 as input for STEM analysis, and chose four profiles for GO enrichment using OE2GO. For pathway level analysis, those genes with a fold change > 1.5 were imported into Pathway-Express to obtain the significantly perturbed pathway list and gene mapping. This program was based on an impact analysis that included the classical statistics but also considered other crucial factors such as the magnitude of each gene's expression change, their type and position in the given pathways, their interactions, *etc*. The *IF* of a pathway is calculated as the sum of the following two terms:

$$IF(P_i) = \log(1/p_i) + \frac{\sum_{g \in P_i} |PF(g)|}{|\Delta E| \cdot N_{de}(P_i)}$$

$$PF(g) = \Delta E(g) + \sum_{u \in US_g} \beta_{ug} \cdot \frac{PF(u)}{N_{ds}(u)}$$

Then a simplified network construction was completed based on the genes enriched and mapped to KEGG pathways using STRING (version 8.2)[15], which is a known Predicted Protein-Protein Interactions Database (http://string.embl.de/).

Real-time polymerase chain reaction for confirmation of microarray results

Real-time reverse-transcriptase polymerase chain reaction (Q-RT-PCR) validation of microarray results was carried out for the *GFPT2* gene at the five time points which were significantly altered according to the microarray data. RNA samples of different time points were prepared as previously described in RNA isolation. Briefly, 2 g total RNA of each sample was used for cDNA synthesis. Real time PCR was performed on the Rotor-Gene RG-3000 Real-Time Thermal Cycler with the SYBR Premix Ex Taq™ (TakaRa) and *GAPDH* was used as an internal control. The relative quantification of mRNA expression at each time point was calculated and compared with that of the untreated AGS cells as control. The primers of selected gene for RT-PCR were: (1) *GFPT2* forward primer (5'-GACAAGCAGATGCCCGTCAT-3') and reverse primer (5'-AACTTGGAACTTTCAGTATCGTCCTT-3'); and (2) *GAPDH* forward primer

(5'-AGAAGGCTGGGGCTCATTTG-3') reverse primer (5'-AGGGGCCATCCACAGTCTTC-3').

RESULTS

Definition of differentially expressed genes

Microarray hybridization results showed that about 3577 genes in total ($P < 0.05$, DiffScore > 13, named dataset1 in this study) expressed differentially compared with 0 h group. This dataset was generated by taking an integration and alignment for the gene list of different time points using Microsoft Excel software, and the repeated genes were thus excluded. Rows were gene names and columns were differential expression values in different time points. Those genes without fold changes in some time points were set as a value equal to 0. The gene numbers at each time point for the 808 genes ($P < 0.05$, a fold change > 1.5, named dataset2 in this study) are listed in Table 1 and were selected for further emphatically analysis.

Table 1 Number of different genes expressed at different time points compared with those of control AGS cells

Time point (h)	Up-regulation (*n*)	Down-regulation (*n*)	Total
0.5	109	209	318
1	140	242	382
2	151	203	354
4	126	291	417
6	198	156	354

$P < 0.05$, fold-change > 1.5, dataset2.

Microarray data analysis

Taking dataset2 as input, hierarchical cluster analysis showed some differentially expressed genes down-regulated at 4 h and up-regulated at 6 h (Figure 1A and B). Eighty of the most differentially expressed genes were extracted by sorting their fold change and were hierarchically clustered as shown in Figure 1C. Immunity and tumor-related genes were labeled with triangles and circles, respectively. Ten significant profiles were obtained by STEM and four interesting profiles were shown with genes in detail (Figure 2 and Table 2). However, GO analysis did not provide significant terms. Taking dataset1 as input, the GO analysis results for the four profiles clustered are listed in Table 3 and Figure 3. Table 4 shows the GO distribution change of each time point by up-regulation and down-regulation, respectively. Analysis of KEGG pathways revealed many enrichment-related pathways including cell adhesion molecules, MAPK signaling, p53 signaling, and TGF-β signaling pathways, complement and coagulation cascades, and epithelial cell signaling in *H. pylori* infection. The top four significantly perturbed pathways are listed in Table 5. Related networks extracted from significant pathways are shown in Figure 4.

Real-time PCR confirmation of microarray results

Relative expression levels of each time point were consistent with that of the microarray profile except at 0.5 h, for which a little higher fold-change was obtained in microarray data.

DISCUSSION

Some previous studies have reported that *H. pylori* type I strains that harbor the cag pathogenicity island (PAI) and cagA are associated with increased bacterial virulence and a more severe inflammatory response in gastric epithelial cells. These virulence factors have also been considered to be associated with induction of interleukin through an NF-κB-dependent pathway in host mucosa[16]. In addition, host protein phosphorylation, cytoskeletal rearrangement, and differential activation of MAP kinases have been described in host cells after infection of type I strains[1]. Although CagA and Cag PAI are considered to be factors highly involved in the development of gastritis and carcinoma, more complex as yet undiscovered mechanisms may exist between *H. pylori* and host cells. We aimed to take a global view of gene expression profiles of host response to infection in a time-series interaction model, which may help understand the pathogenesis of *H. pylori* related diseases.

Considering that only genes with fold changes > 1.5 were included in the analysis, the number of differential genes was only 808. This may lead to an ignorance for many important genes. Therefore, we initiated co-expression clustering analysis using STEM for both the 3577 differentially expressed genes (dataset1) and 808 genes (dataset2) with fold-change > 1.5. For the 808 genes, four significant clusters showed four different co-expression profiles (Figure 2). One hundred and twenty-six genes down-regulated at 4 h were clustered into profile 123, but no significant GO terms were enriched for these genes. In profile 3, some genes related to tumors were consistently down-regulated. For instance, *cdkn1c* had consistently decreased expression of theses genes, which may be involved in promotion of tumor formation. Profile 144 was mainly involved in factors regulating cell bioactivity and morphology such as *rflb*, *gdf15*, *sqstm1* and *adm2*. DNA-damage-inducible transcript and *csf2* also had increased gene expression at 4 and 6 h, suggesting that some potential mechanisms for cell differentiation and damage may be triggered beginning at 4 h. Hierarchically clustered results also showed two gene clusters with down-regulation at 4 h and up-regulation at 6 h. Analysis of all differentially expressed genes showed four interesting profiles whose GO distributions included nucleic acid binding, regulation of transcription, oxido-reductase activity *etc*. For the GO distribution of dataset1, profile 71 and profile 83 showed a similar co-expression profile as well as GO terms including nucleus, nucleic acid binding *etc*. (Table 3, Figure 3B and D). However, profile 83 showed an obvious and continuous up- regulated gene cluster. Profile 111 and 108 mainly focused on cell surface and showed a down-regulated

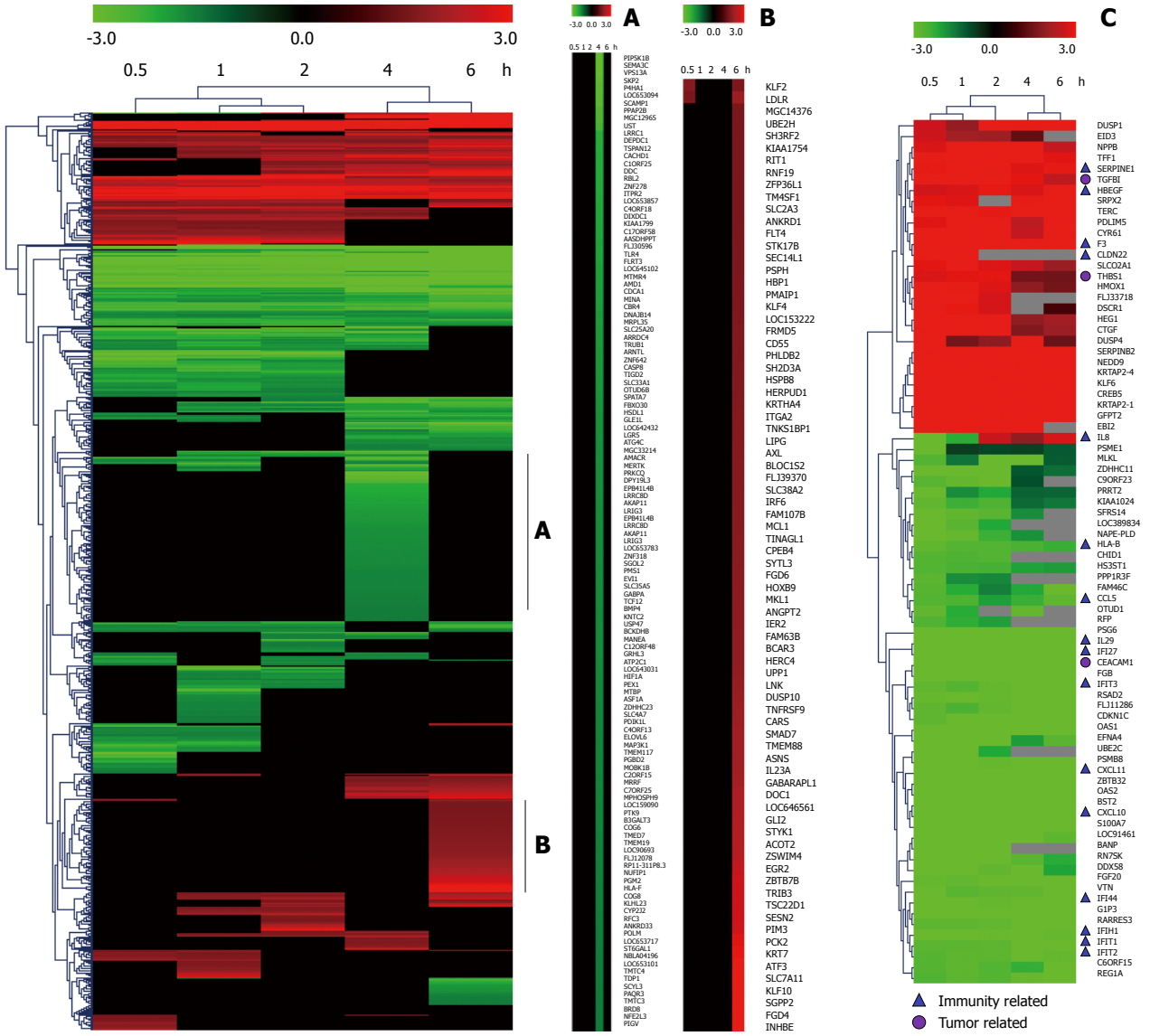

Figure 1 Hierarchical cluster analysis of time-series gene expression alteration after infection of *Helicobacter pylori* at 5 time points. Genes that significantly changed during infection were included in hierarchical clustering analysis using average linkage and Euclidean dissimilarity methods. Significant clusters A and B show the details of genes including name of the gene down-regulated at 4 h and up-regulated at 6 h. Eighty of the most differentially expressed genes were clustered in C. Immunity and tumor related genes are labeled.

gene cluster (Figure 3A and C). All profiles illustrated an obvious expressional change at 4 h. Statistically significant changes in gene ontology at each time point showed that apoptosis appeared from 1 h in up-regulated genes. At the same time, in down-regulated genes, chemokine activity became the most significant term (Table 4). This seemed consistent with results of the pathway analysis, which showed that the P53 signaling pathway became the most significantly perturbed pathway at 1 h in up-regulated genes. In down-regulated genes, the cytokine-cytokine receptor interaction pathway became more significant. Genes involving immune response and other responses to viruses were at the top of the GO list of down-regulated genes. This suggested an inhibition of immune response by *H. pylori* during early infection. Tumor-related pathways like P53 and MAPK may play an important role in determining the development of special phenotype and disease outcomes according to the results of pathway analysis. For the top 80 differentially expressed genes, 43 (54%) were related to immunity (29, 36%) and tumor development (14, 18%). Many immune factor-related down-regulated genes showed a consistently increasing expression levels. The cell adhesion molecules (CAM) pathway was the most significantly perturbed pathway at several time-points. The increased expression of CAM induced by *H. pylori* may contribute to cell adhesion, invasion and cell proliferation in gastric epithelial cells[17].

From the reconstructed simplified pathway, we can inspect some important nodes with several interaction edges like *stat1*, *stat2*, *fos*, *csf2*, *pdgfb* and *ccl5* genes. These genes may be the trigger and linker of the pathway net during early infection, which however requires further studies. From Figure 4 and the expression value of each

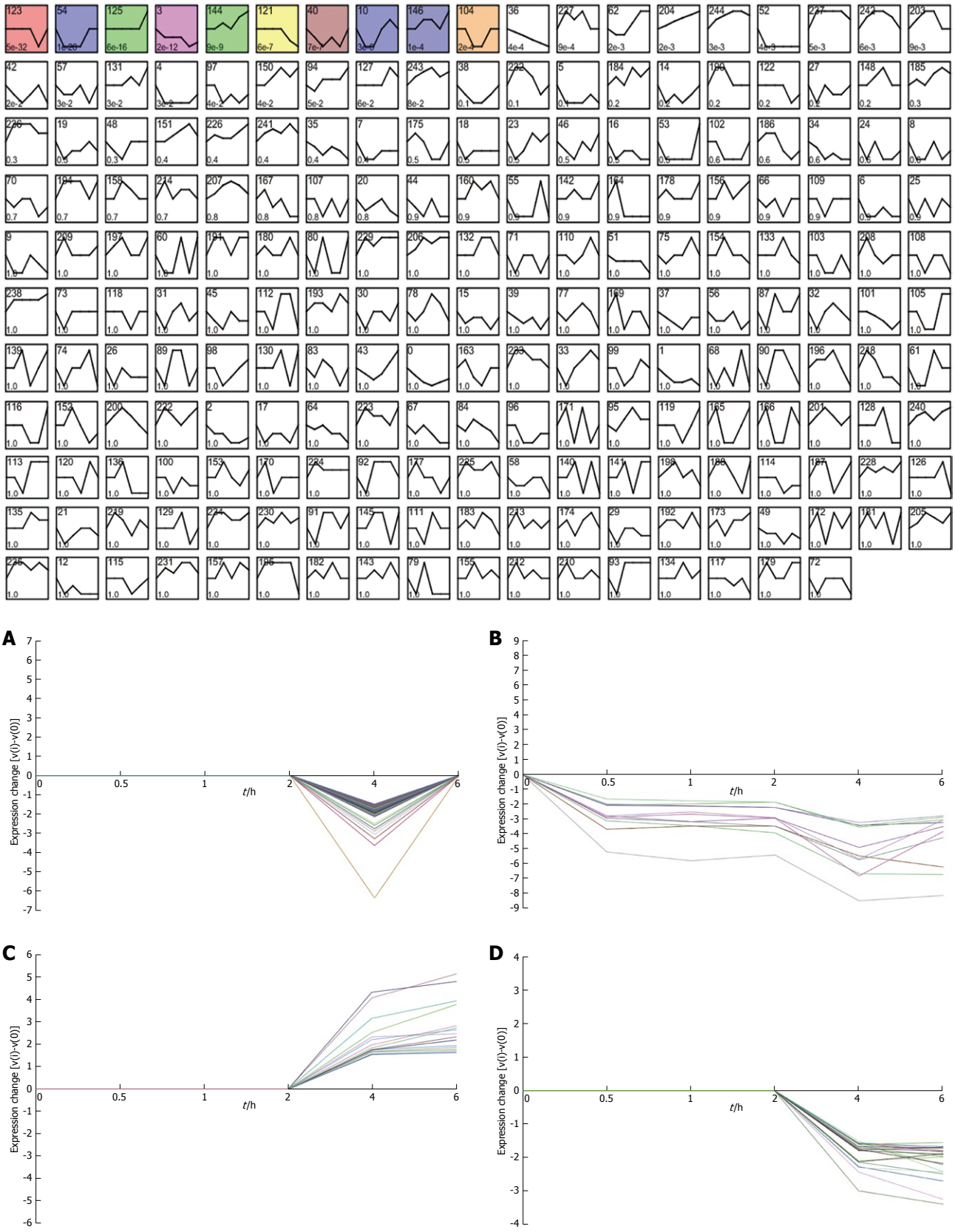

Figure 2 Short time-series expression miner (STEM) clustering of the differentially expressed genes. All profiles are ordered based on the *P* value significance of the number of genes assigned *vs* expected. A: Profile 123 (0, 0, 0, 0, -1, 0): 126.0 genes assigned, 37.8 genes expected, *P*-value = 5.4E-32 (significant); B: Profile 3 (0, -2, -2, -2, -4, -3): 11.0 genes assigned, 0.4 genes expected, *P*-value = 1.9E-12 (significant); C: Profile 144 (0, 0, 1, 0, 2, 3): 16.0 genes assigned, 2.5 genes expected, *P*-value = 8.5E-9 (significant); D: Profile 121 (0, 0, 0, 0, -2, -3): 21.0 genes assigned, 5.7 genes expected, *P*-value = 6.3E-7 (significant).

gene, we could learn that most immunity-related genes were down-regulated while many tumor-related genes were up-regulated. *Il-24* is an important oncogene and could inhibit specifically the tumor growth. The protein

Table 2 Description of selected clustered genes from short time-series expression miner (STEM) using dataset2 as input

Cluster ID	Symbol									
Profile 123	C4ORF18	USP47	CYP2J2	LGR5	FLRT3	LOC643031	TMEM117	CACHD1	C12ORF48	MTMR4
	RBL2	ZDHHC23	TTC13	NUFIP1	FLJ30596	AASDHPPT	C2ORF15	PGBD2	LRRC8D	EVI1
	SKP2	ZNF318	VPS13A	AMACR	ST6GAL1	AMD1	ELOVL6	PGM2	SLC35A5	CBR4
	EPB41L4B	C1ORF25	C1GALT1	ATG4C	MERTK	FANCL	LRIG3	RHPN1	PIP5K1B	SEMA3C
	P4HA1	LOC653094	SCAMP1	PPAP2B	MGC12965	UST	LRRC1	DEPDC1	DDC	ZNF278
	ITPR2	LOC653857	DIXDC1	KIAA1799	C17ORF58	TLR4	LOC645102	CDCA1	MINA	DNAJB14
	MRPL35	SLC25A20	ARRDC4	TRUB1	ARNTL	ZNF642	CASP8	TIGD2	SLC33A1	OTUD6B
	SPATA7	FBXO30	HSDL1	GLE1L	LOC642432	MGC33214	PRKCQ	DPY19L3	AKAP11	LOC653783
	SGOL2	PMS1	GABPA	TCF12	BMP4	KNTC2	BCKDHB	MANEA	GRHL3	ATP2C1
	HIF1A	PEX1	MTBP	ASF1A	SLC4A7	PDIK1L	C4ORF13	MAP3K1	MOBK1B	MRRF
	C7ORF25	MPHOSPH9	LOC159090	PTK9	B3GALT3	COG6	TMED7	TMEM19	LOC90693	FLJ12078
	RP11-311P8.3	ZNF181	COG8	KLHL23	RFC3	NBLA04196	LOC653101	TMTC4	TDP1	SCYL3
	PAQR3	TMTC3	BRD8	NFE2L3	PIGV	TSPAN12				
Profile 3	PSG6	FGB	CEACAM1	CDKN1C	IFIT3	RSAD2	PSG7	FLJ11286	BTN3A2	STAT1
	FLJ20035									
Profile 144	EHD2	RELB	COL16A1	GDF15	GNA15	LETM2	STX11	FOSL1	LOC647512	SQSTM1
	C12ORF59	ADM2	DDIT3	CHAC1	CSF2	DDIT4				
Profile 121	ZC3HAV1	PSG9	LYZ	FGG	PSG2	PAGE4	REG4	GAD1	PPM1H	TMEM70
	LRP8	PAQR8	SH3BGRL	MYLIP	ROR1	C5ORF14	SUSD4	MGC3265	CADPS2	IDUA
	EPSTI1									

Table 3 Statistically significant changed gene ontology of the four selected profiles

Profile	GO name	*n*	Corrected *P* value	Function code
111	Apical part of cell	2	0.00842	CC
71	Nucleic acid binding	12	2.7E-4	MF
	Zinc ion binding	23	0.00308	MF
	Regulation of transcription	22	0.01027	BP
	Myeloid cell differentiation	2	0.01577	BP
	Nucleus	39	2.9E-4	CC
	Intracellular	23	3.5E-4	CC
108	Small GTPase binding	2	0.01173	MF
	Oxido-reductase activity	6	0.02544	MF
	GPI anchor biosynthetic process	2	0.02591	BP
	Female pregnancy	3	0.02622	BP
	Golgi membrane	5	0.03987	CC
	Cell surface	3	0.03987	CC
83	DNA binding	6	0.00577	MF
	Metal binding	6	0.03346	MF
	Nucleus	10	0.01029	CC

Corrected *P* value < 0.05, derived from dataset1.

encoded by this gene can induce apoptosis selectively in various cancer cells. Overexpression of this gene has been shown to lead to elevated expression of several GADD family genes, which correlates with the induction of apoptosis[18-20]. In this study, we examined *il-24* levels which gradually increased more than two-fold from 2 to 6 h. At 6 h, there was a ten-fold change, indicating that after perturbation of P53 and MAPK, *il-24* may participate in maintaining the immune defense against invading pathogens. We also examined an increased level of *gadd45* which can stimulate DNA excision-repair *in vitro* and inhibits entry of cells into S phase. This gene is a member of a group of genes whose transcript levels are increased following stressful growth arrest and treatment with DNA-damaging agents. In the network, both *c-Fos* and *c-Jun*, two genes considered to mediate inflammation and carcinogenesis, have been found to be up-regulated, which is consistent with the results of this study[21].

We also analyzed expression profiles of some other important infection-related genes that were reported previously and may play an important role in *H. pylori*-induced diseases, although these genes were not clustered into a special profile in this study using the current analytical tools. MMP is a mucosal matrix metalloproteinase. Previous studies have demonstrated elevated MMP-9 levels in *H. pylori*-infected gastric mucosa, and eradication of *H. pylori* can significantly decrease MMP9 expression levels consistently[22,23]. MMP1 has been the subject of studies of inflammatory gene profiles in gastric mucosa[2,24]. MMP7 has been reported to be up-regulated in gastric cancer tissues[25,26]. However, few studies have reported on MMP24. In this study, the profile of MMP24 showed a consistent and increased level from 1 to 6 h, which suggested a similar function with MMP9 during *H. pylori* infection. Some other genes with similar expression profiles are *il-27ra*, *il-32*, *il-23a*, *il-11*, *il-8* and *ccl20*. This gene cluster showed down-regulation or no change at the first two or three time points and up-regulation in the last two or three time points. *Il-29*, *ccl5*, *cxcl10* and *cxcl11* showed a consistent down-regulation at all time points with high fold-change. Expression of these genes suggested that the immune defense system may be suppressed during the first 1 or 2 h of *H. pylori* infection and some tumor-related genes and pathways were activated. After this short interaction and competition for about 2 h, the immune defense system may have regained the advantage with increasing expression levels of inflammatory and tumor suppressor factors. CagA translocation might occur 30 min after infection and may be at its maximum level in a time range of about 4-5 h[27,28]. In this study, the differentially expressed genes significantly increased at the time point of 4 h. This also

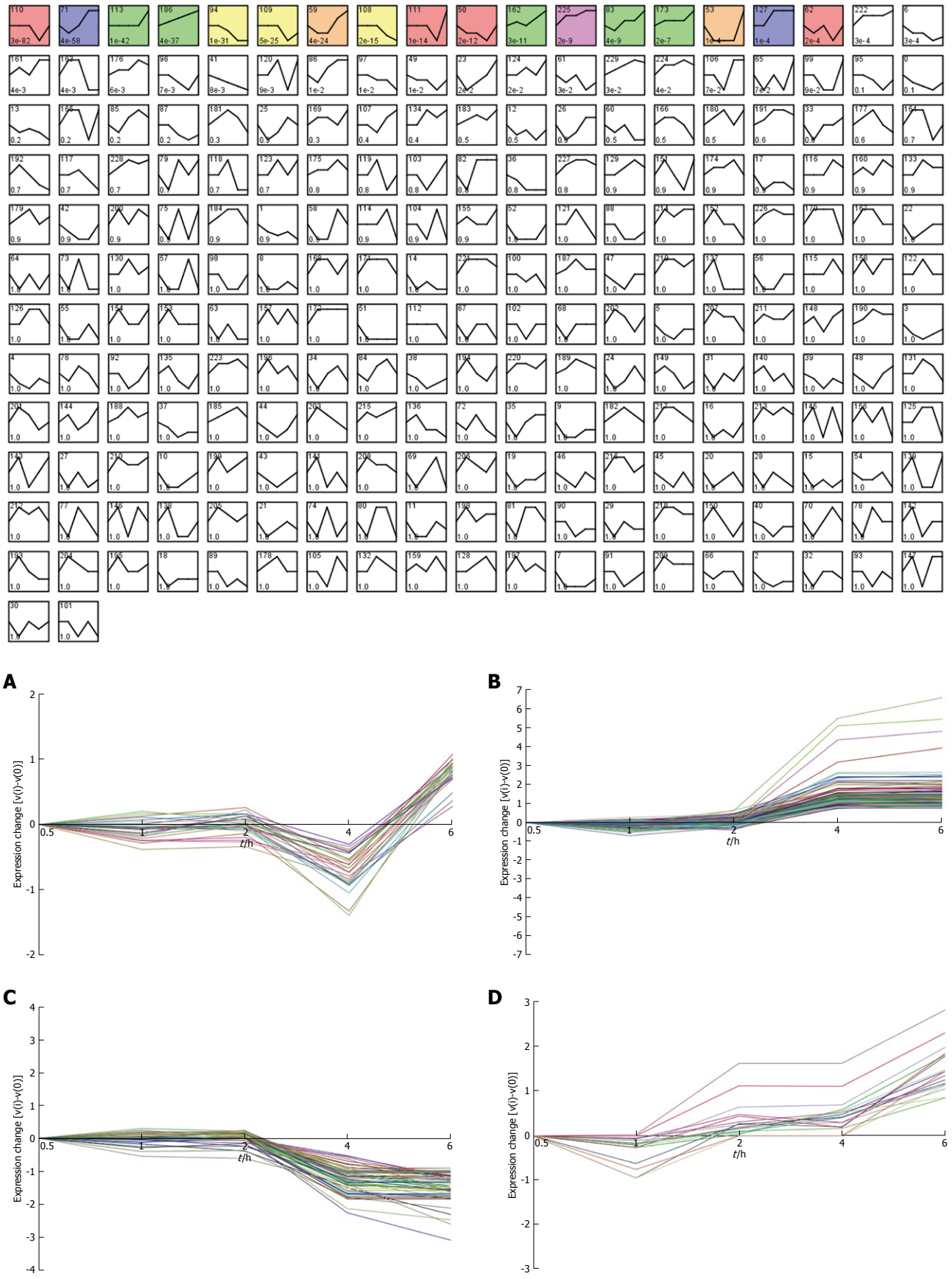

Figure 3 STEM clustering of all the 3577 differentially expressed genes labeled by accession number. All profiles were ordered based on the *P* value significance of the number of genes assigned *vs* expected. A: Profile 111 (0, 0, 0, -1, 1): 28.0 genes assigned, 4.2 genes expected, *P*-value = 1.2E-14 (significant); B: Profile 71 (0, -1, 0, 2, 2): 123.0 genes assigned, 19.0 genes expected, *P*-value = 4.4E-58 (significant); C: Profile 108 (0, 0, 0, -2, -3): 57.0 genes assigned, 16.2 genes expected, *P*-value = 1.5E-15 (significant); D: Profile 83 (0, -1, 1, 1, 3): 17.0 genes assigned, 2.7 genes expected, *P*-value = 4.3E-9 (significant).

Table 4 Statistically significant changed gene ontology at each time point

Time point (h)	Up-regulation					Down-regulation				
	GO ID	GO name	Genes	*P* value	Code	GO ID	GO name	Genes	*P* value	Code
0.5	GO:0008201	Heparin binding	5	7.1E-4	MF	GO:0006955	Immune response	20	0.00000	BP
	GO:0008134	Transcription factor binding	4	0.01585	MF	GO:0009615	Response to virus	10	0.00000	BP
	GO:0003700	Transcription activity	10	0.02835	MF	GO:0008150	Biological process	15	0.00896	BP
	GO:0008083	Growth factor activity	4	0.03882	MF	GO:0007267	Cell-cell signaling	10	0.00966	BP
	GO:0005576	Extracellular region	15	0.02875	CC	GO:0006935	Chemotaxis	6	0.01581	BP
	GO:0005634	Nucleus	28	0.03452	CC	GO:0006954	Inflammatory response	8	0.01581	BP
						GO:0008285	Negative regulation of cell proliferation	7	0.03430	BP
						GO:0007275	Multicellular organismal development	16	0.03576	BP
						GO:0008009	Chemokine activity	7	0.00000	MF
						GO:0046870	Cadmium ion binding	3	0.00194	MF
						GO:0016779	Nucleotidyl transferase activity	5	0.02486	MF
						GO:0005576	Extracellular region	37	0.00000	CC
						GO:0005615	Extracellular space	14	2.0E-4	CC
						GO:0005634	Nucleus	56	7.0E-4	CC
1	GO:0008201	Heparin binding	5	0.00265	MF	GO:0008009	Chemokine activity	6	3.5E-4	MF
	GO:0003700	Transcription factor activity	13	0.00886	MF	GO:0046870	Cadmium ion binding	3	0.00264	MF
	GO:0005515	Protein binding	38	0.01716	MF	GO:0003677	DNA binding	26	0.00264	MF
	GO:0045766	Positive regulation of angiogenesis	3	0.01125	BP	GO:0046872	Metal ion binding	36	0.01144	MF
	GO:0001558	Regulation of cell growth	6	0.01502	BP	GO:0008270	Zinc ion binding	34	0.02041	MF
	GO:0006915	Apoptosis	8	0.02591	BP	GO:0003674	Molecular function	15	0.02257	MF
	GO:0008285	Negative regulation of cell proliferation	6	0.02591	BP	GO:0003676	Nucleic acid binding	13	0.02257	MF
	GO:0005634	Nucleus	36	0.00597	CC	GO:0016779	Nucleotidyl transferase activity	4	0.02571	MF
	GO:0005575	Cellular component	10	0.02160	CC	GO:0005515	Protein binding	61	0.03204	MF
						GO:0003704	Specific RNA polymerase Ⅱ transcription factor activity	3	0.04080	MF
						GO:0009615	Response to virus	10	0.00000	BP
						GO:0006955	Immune response	18	0.00000	BP
						GO:0006355	Regulation of transcription DNA-dependent	39	4.0E-5	BP
						GO:0006350	Transcription	31	4.5E-4	BP
						GO:0008150	Biological process	18	0.00348	BP
						GO:0007267	Cell-cell signaling	11	0.00480	BP
						GO:0006954	Inflammatory response	8	0.03385	BP
						GO:0045087	Innate immune response	5	0.04274	BP
						GO:0005634	Nucleus	71	0.00000	CC
						GO:0005576	Extracellular region	37	1.1E-4	CC
						GO:0005615	Extracellular space	13	0.00474	CC
						GO:0005622	Intracellular	31	0.01344	CC
						GO:0005575	Cellular component	15	0.03381	CC
2	GO:0003700	Transcription factor activity	18	1.4E-4	MF	GO:0009615	Response to virus	10	0.00000	BP
	GO:0008201	Heparin binding	5	0.00193	MF	GO:0006955	Immune response	16	0.00000	BP
	GO:0043565	Sequence-specific DNA binding	10	0.01819	MF	GO:0008150	Biological process	18	6.6E-4	BP
	GO:0008083	Growth factor activity	5	0.01885	MF	GO:0007267	Cell-cell signaling	10	0.01111	BP
	GO:0005178	Integrin binding	3	0.02722	MF	GO:0006954	Inflammatory response	8	0.01911	BP
	GO:0008134	Transcription factor binding	4	0.02722	MF	GO:0045087	Innate immune response	5	0.02866	BP
	GO:0008009	Chemokine activity	3	0.02849	MF	GO:0007565	Female pregnancy	5	0.03928	BP
	GO:0046872	Metal ion binding	23	0.04806	MF	GO:0005576	Extracellular region	36	1.0E-5	CC
	GO:0045944	Positive regulation of transcription from RNA polymerase Ⅱ promoter	7	0.00234	BP	GO:0005615	Extracellular space	13	0.00113	CC
	GO:0006955	Immune response	10	0.00470	BP	GO:0005634	Nucleus	51	0.00899	CC
	GO:0008285	Negative regulation of cell proliferation	7	0.00681	BP	GO:0046870	Cadmium ion binding	3	0.01145	MF
	GO:0000122	Negative regulation of transcription from RNA polymerase Ⅱ promoter	6	0.00681	BP	GO:0016831	Carboxy-lyase activity	3	0.02198	MF
	GO:0006915	Apoptosis	9	0.00713	BP	GO:0030674	Protein binding bridging	4	0.04373	MF
	GO:0006954	Inflammatory response	7	0.00769	BP					
	GO:0001558	Regulation of cell growth	5	0.00914	BP					
	GO:0009611	Response to wounding	3	0.01457	BP					
	GO:0005615	Extracellular space	12	8E-5	CC					
	GO:0005634	Nucleus	42	2.4E-4	CC					
	GO:0005576	Extracellular region	22	4E-4	CC					
	GO:0030173	Integral to Golgi membrane	3	0.02101	CC					

4	GO:0008083	Growth factor activity	8	1.0E-5	MF	GO:0009615	Response to virus	12	0.00000	BP
	GO:0005125	Cytokine activity	6	3.7E-4	MF	GO:0007565	Female pregnancy	9	1.6E-4	BP
	GO:0046983	Protein dimerization activity	6	0.00123	MF	GO:0006955	Immune response	17	5.0E-4	BP
	GO:0005100	Rho GTPase activator activity	3	0.00268	MF	GO:0001525	Angiogenesis	7	0.02671	BP
	GO:0008201	Heparin binding	4	0.00826	MF	GO:0007267	Cell-cell signaling	10	0.02671	BP
	GO:0003700	Transcription factor activity	13	0.00826	MF	GO:0008150	Biological process	19	0.02928	BP
	GO:0008047	Enzyme activator activity	3	0.01045	MF	GO:0016477	Cell migration	5	0.03984	BP
	GO:0005178	Integrin binding	3	0.01447	MF	GO:0005576	Extracellular region	45	0.00000	CC
	GO:0016563	Transcription activator activity	4	0.02237	MF	GO:0005577	Fibrinogen complex	3	6.0E-4	CC
	GO:0005515	Protein binding	33	0.03960	MF	GO:0005615	Extracellular space	14	0.00843	CC
	GO:0043565	Sequence-specific DNA binding	8	0.03960	MF	GO:0031093	Platelet α granule lumen	4	0.01203	CC
	GO:0006955	Immune response	11	2.4E-4	BP	GO:0016020	Membrane	61	0.03962	CC
	GO:0006915	Apoptosis	9	0.00440	BP	GO:0005794	Golgi apparatus	15	0.03962	CC
	GO:0030183	B cell differentiation	3	0.01798	BP					
	GO:0045944	Positive regulation of transcription from RNA polymerase Ⅱ promoter	5	0.03323	BP					
	GO:0000079	Regulation of cyclin-dependent protein kinase activity	3	0.03704	BP					
	GO:0007050	Cell cycle arrest	4	0.03704	BP					
	GO:0008284	Positive regulation of cell proliferation	5	0.03704	BP					
	GO:0007267	Cell-cell signaling	6	0.03704	BP					
	GO:0001558	Regulation of cell growth	5	0.04074	BP					
6	GO:0005515	Protein binding	65	0.00000	MF	GO:0046870	Cadmium ion binding	3	0.00135	MF
	GO:0003700	Transcription factor activity	24	1.0E-5	MF	GO:0003674	Molecular function	13	0.00852	MF
	GO:0008083	Growth factor activity	8	3.5E-4	MF	GO:0008009	Chemokine activity	4	0.00852	MF
	GO:0003714	Transcription co-repressor activity	7	3.5E-4	MF	GO:0003950	NAD+ADP-ribosyl transferase activity	3	0.01169	MF
	GO:0005125	Cytokine activity	8	3.5E-4	MF	GO:0030674	Protein binding bridging	3	0.04041	MF
	GO:0005100	Rho GTPase activator activity	4	3.5E-4	MF	GO:0009615	Response to virus	12	0.00000	BP
	GO:0003700	Transcription factor activity	7	6.2E-4	MF	GO:0006955	Immune response	16	0.00000	BP
	GO:0046983	Protein dimerization activity	7	6.9E-4	MF	GO:0007565	Female pregnancy	9	0.00000	BP
	GO:0008270	Zinc ion binding	32	0.00504	MF	GO:0008150	Biological process	14	0.00612	BP
	GO:0046872	Metal ion binding	32	0.00827	MF	GO:0007267	Cell-cell signaling	8	0.00676	BP
	GO:0005085	Guanyl-nucleotide exchange factor activity	5	0.02023	MF	GO:0006952	Defense response	5	0.00728	BP
	GO:0043565	Sequence-specific DNA binding	11	0.03272	MF	GO:0030168	Platelet activation	3	0.01864	BP
	GO:0008201	Heparin binding	4	0.03502	MF	GO:0051258	Protein polymerization	3	0.03966	BP
	GO:0005178	Integrin binding	3	0.04652	MF	GO:0005576	Extracellular region	44	0.00000	CC
	GO:0006915	Apoptosis	13	0.00173	BP	GO:0005615	Extracellular space	13	6.0E-5	CC
	GO:0006950	Response to stress	7	0.00173	BP	GO:0005577	Fibrinogen complex	3	6.0E-5	CC
	GO:0007050	Cell cycle arrest	6	0.00788	BP	GO:0031093	Platelet α granule lumen	4	8.1E-4	CC
	GO:0045944	Positive regulation of transcription from RNA polymerase Ⅱ promoter	7	0.01021	BP					
	GO:0045740	Positive regulation of DNA replication	3	0.01720	BP					
	GO:0008360	Regulation of cell shape	4	0.02121	BP					
	GO:0008285	Negative regulation of cell proliferation	8	0.02486	BP					
	GO:0000122	Negative regulation of transcription from RNA polymerase Ⅱ promoter	7	0.02486	BP					
	GO:0009611	Response to wounding	3	0.02698	BP					
	GO:0030183	B cell differentiation	3	0.02698	BP					
	GO:0007229	Integrin-mediated signaling pathway	5	0.02698	BP					
	GO:0006954	Inflammatory response	7	0.02698	BP					
	GO:0007179	Transforming growth factor β receptor signaling pathway	4	0.02698	BP					
	GO:0043066	Negative regulation of apoptosis	4	0.02698	BP					
	GO:0006935	Chemotaxis	5	0.04499	BP					
	GO:0007010	Cytoskeleton organization and biogenesis	5	0.04841	BP					
	GO:0006955	Immune response	10	0.04843	BP					
	GO:0005576	Extra cellular region	31	9.0E-5	CC					
	GO:0005615	Extra cellular space	14	6.6E-4	CC					
	GO:0005622	Intracellular	29	0.00843	CC					
	GO:0005737	Cytoplasm	40	0.03660	CC					

ONTO-TOOLS/OE2GO was used to identify the differentially expressed GO terms based on the hypergeometric distribution and corrected *P* value (< 0.05). The GO identified number (GOID), GO term name (GO name), the number of genes changed within each functional gene category, *P* values are listed. GO terms with at least 3 genes changed and corrected *P* values < 0.05 are listed in Table 4.

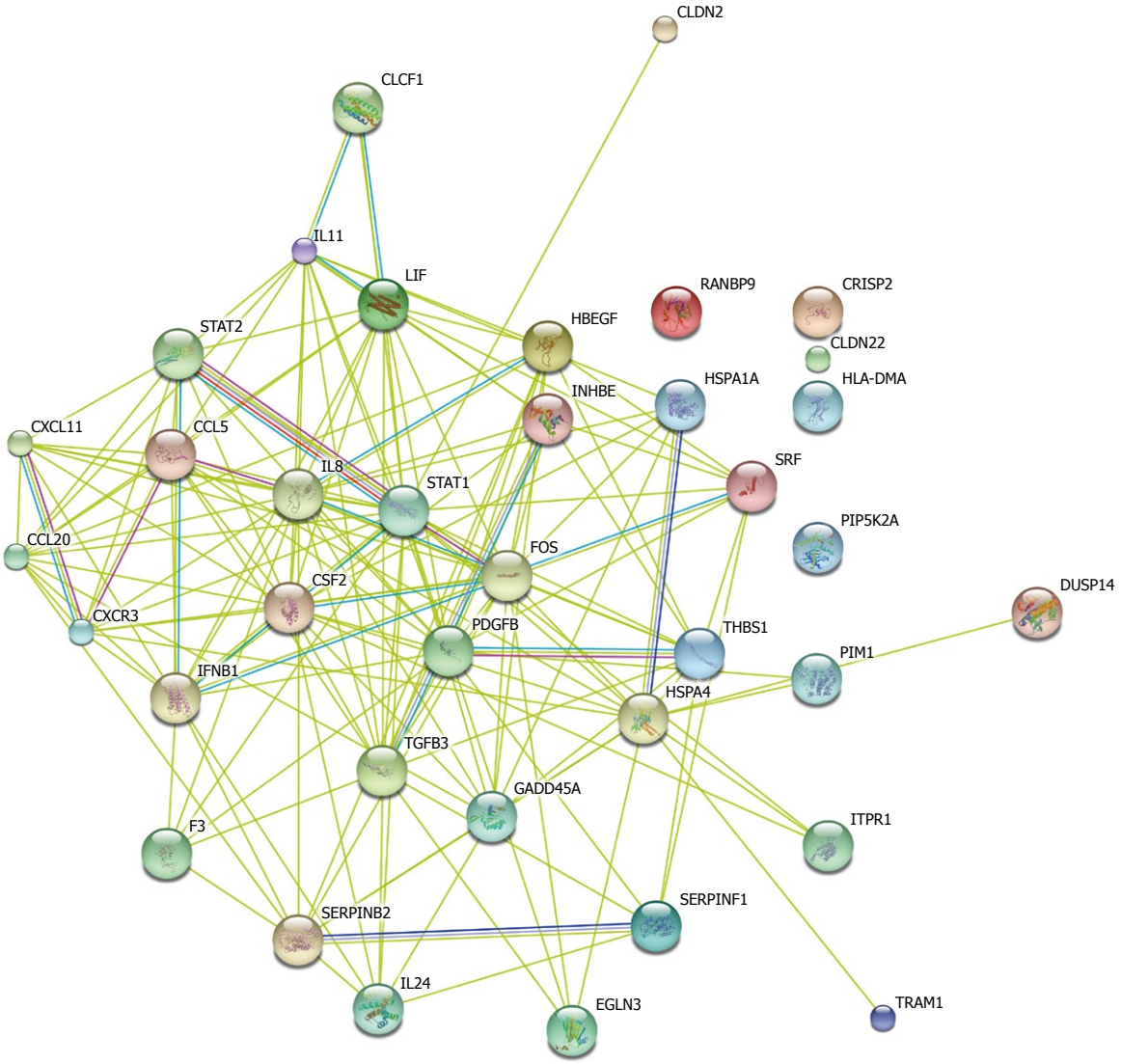

Figure 4 A simplified gene network extracted from significant pathways using STRING database.

Table 5 Top four significantly perturbed pathways at each time point

Time point	0.5 h	1 h	2 h	4 h	6 h
Gene mapping					
Up-regulation	CAM	P53	MAPK	CAM	CAM
	MAPK	TGF	ECHP	CY-CY	CY-CY
	P53	MAPK	RCC	MAPK	JAK-STA
	TGF	CCC	P53	JAK-STA	MAPK
Down-regulation	APP	APP	APP	Phos	APP
	Toll	CY-CY	CY-CY	APP	CY-CY
	CY-CY	Toll	Toll	Toll	Toll
	NKMC	Mela	Mela	Mela	Mela

suggested that it might be an important turning point between infection and host response. Although a model system of the AGS cell line infected with *H. pylori* was used to explore the host response[5,29], it should be noted that this is an isolated cell culture system, and cannot account for the varied effects of conditions in a human stomach. Therefore, the speculation generated from this study represents a valuable, but a simplified view of the situation. More researches are required to confirm these findings. In addition, we also compared our results with the genes with significant change after *H. pylori* infection in another report[30]. Several genes in that report are consistent with our results in dataset1 like *socs2*, *stat6*, *ccl4*, *cxcl2*, *hla-dma*, *hsph1*, *plat*, *ifitm1*, *alox5*, *tlr4*, *faim3*, *cd47*, *ifngr1* and *il8*.

Only part of these genes showed a high fold change > 1.5 in differential expressions, including *il8*, *faim3*, *tlr4*, *alox5*, *hla-dma*, *cxcl2* and *ccl4*.

In summary, the results from this sequential expression microarray have extended previous studies that were limited to the comparison of normal and diseased tissues. We took a global view on the genes and pathway net related to *H. pylori* infection, several co-expressional profiles and important new genes like *mmp24* and *il-24* involved in immune response and tumorigenesis during *H. pylori* infection were also identified. Our study also suggested that the outcome of *H. pylori* infection is probably involved in a complex mechanism, and is associated with a number of immune factors. Formation of tumors may be a result

of an imbalance between bacterial attack and immune defense of host. We speculate that this competition may occur at 1-2 h after infection, and 4 h may be a first time point at which the balance is upset.

COMMENTS

Background

It has been indicated that *Helicobacter pylori* (*H. pylori*) infection may highly contribute to gastritis and carcinogenesis in the past two decades since it was recovered from human gastric mucosa in 1983, and many studies have focused on identification of both bacterial factors and host determinants that may contribute to the pathogenic mechanism.

Research frontiers

Gene expression microarray has been widely used in identifying genes associated with *H. pylori* infection and gastric tumor. However, the time-series gene expression profile of *H. pylori* infection remains unexplored. In this study, the authors extended the knowledge of the dynamic interaction between *H. pylori* and host mucosa using a high density human gene microarray and flexible bioinformatics analysis.

Innovations and breakthroughs

Several important genes that have not been reported previously and a pathway net related to *H. pylori* infection were discovered by the sequential microarrays. Based on the co-expressional profile analysis during infection, a new speculation for the pathogenic mechanism has been set up.

Applications

This study has provided a systemic view of expression profile of time-series *H. pylori* infected AGS cells. The new identified genes and pathway net as well as the hypothesis could help researchers in this field further understand the potential mechanism associated with *H. pylori* infection and carcinogenesis, and provide important information for prevention and control of *H. pylori* related diseases.

Peer review

The scientific and innovative contents as well as readability in this manuscript reflect the advanced levels of the clinical and basic researches in gastroenterology both at home and abroad.

REFERENCES

1 **Segal ED**, Lange C, Covacci A, Tompkins LS, Falkow S. Induction of host signal transduction pathways by Helicobacter pylori. *Proc Natl Acad Sci USA* 1997; **94**: 7595-7599

2 **Wen S**, Felley CP, Bouzourene H, Reimers M, Michetti P, Pan-Hammarström Q. Inflammatory gene profiles in gastric mucosa during Helicobacter pylori infection in humans. *J Immunol* 2004; **172**: 2595-2606

3 **Kim JM**, Kim JS, Jung HC, Oh YK, Chung HY, Lee CH, Song IS. Helicobacter pylori infection activates NF-kappaB signaling pathway to induce iNOS and protect human gastric epithelial cells from apoptosis. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G1171-G1180

4 **Conlin VS**, Curtis SB, Zhao Y, Moore ED, Smith VC, Meloche RM, Finlay BB, Buchan AM. Helicobacter pylori infection targets adherens junction regulatory proteins and results in increased rates of migration in human gastric epithelial cells. *Infect Immun* 2004; **72**: 5181-5192

5 **Bach S**, Makristathis A, Rotter M, Hirschl AM. Gene expression profiling in AGS cells stimulated with Helicobacter pylori isogenic strains (cagA positive or cagA negative). *Infect Immun* 2002; **70**: 988-992

6 **DeRisi J**, Penland L, Brown PO, Bittner ML, Meltzer PS, Ray M, Chen Y, Su YA, Trent JM. Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nat Genet* 1996; **14**: 457-460

7 **Marchet A**, Mocellin S, Belluco C, Ambrosi A, DeMarchi F, Mammano E, Digito M, Leon A, D'Arrigo A, Lise M, Nitti D. Gene expression profile of primary gastric cancer: towards the prediction of lymph node status. *Ann Surg Oncol* 2007; **14**: 1058-1064

8 **Wu CM**, Lee YS, Wang TH, Lee LY, Kong WH, Chen ES, Wei ML, Liang Y, Hwang TL. Identification of differential gene expression between intestinal and diffuse gastric cancer using cDNA microarray. *Oncol Rep* 2006; **15**: 57-64

9 **Lim JW**, Kim H, Kim KH. Cell adhesion-related gene expression by Helicobacter pylori in gastric epithelial AGS cells. *Int J Biochem Cell Biol* 2003; **35**: 1284-1296

10 **Chang YT**, Wu MS, Chang YJ, Chen CC, Lin YS, Hsieh T, Yang PC, Lin JT. Distinct gene expression profiles in gastric epithelial cells induced by different clinical isolates of Helicobacter pylori--implication of bacteria and host interaction in gastric carcinogenesis. *Hepatogastroenterology* 2006; **53**: 484-490

11 **Chudin E**, Kruglyak S, Baker SC, Oeser S, Barker D, McDaniel TK. A model of technical variation of microarray signals. *J Comput Biol* 2006; **13**: 996-1003

12 **Draghici S**, Khatri P, Bhavsar P, Shah A, Krawetz SA, Tainsky MA. Onto-Tools, the toolkit of the modern biologist: Onto-Express, Onto-Compare, Onto-Design and Onto-Translate. *Nucleic Acids Res* 2003; **31**: 3775-3781

13 **Draghici S**, Khatri P, Tarca AL, Amin K, Done A, Voichita C, Georgescu C, Romero R. A systems biology approach for pathway level analysis. *Genome Res* 2007; **17**: 1537-1545

14 **Ernst J**, Bar-Joseph Z. STEM: a tool for the analysis of short time series gene expression data. *BMC Bioinformatics* 2006; **7**: 191

15 **Jensen LJ**, Kuhn M, Stark M, Chaffron S, Creevey C, Muller J, Doerks T, Julien P, Roth A, Simonovic M, Bork P, von Mering C. STRING 8--a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res* 2009; **37**: D412-D416

16 **Censini S**, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA* 1996; **93**: 14648-14653

17 **Chen X**, Leung SY, Yuen ST, Chu KM, Ji J, Li R, Chan AS, Law S, Troyanskaya OG, Wong J, So S, Botstein D, Brown PO. Variation in gene expression patterns in human gastric cancers. *Mol Biol Cell* 2003; **14**: 3208-3215

18 **Papathanasiou MA**, Kerr NC, Robbins JH, McBride OW, Alamo I Jr, Barrett SF, Hickson ID, Fornace AJ Jr. Induction by ionizing radiation of the gadd45 gene in cultured human cells: lack of mediation by protein kinase C. *Mol Cell Biol* 1991; **11**: 1009-1016

19 **Jackman J**, Alamo I Jr, Fornace AJ Jr. Genotoxic stress confers preferential and coordinate messenger RNA stability on the five gadd genes. *Cancer Res* 1994; **54**: 5656-5662

20 **Carrier F**, Smith ML, Bae I, Kilpatrick KE, Lansing TJ, Chen CY, Engelstein M, Friend SH, Henner WD, Gilmer TM. Characterization of human Gadd45, a p53-regulated protein. *J Biol Chem* 1994; **269**: 32672-32677

21 **Hofman VJ**, Moreilhon C, Brest PD, Lassalle S, Le Brigand K, Sicard D, Raymond J, Lamarque D, Hébuterne XA, Mari B, Barbry PJ, Hofman PM. Gene expression profiling in human gastric mucosa infected with Helicobacter pylori. *Mod Pathol* 2007; **20**: 974-989

22 **Lee LY**, Wu CM, Wang CC, Yu JS, Liang Y, Huang KH, Lo CH, Hwang TL. Expression of matrix metalloproteinases MMP-2 and MMP-9 in gastric cancer and their relation to claudin-4 expression. *Histol Histopathol* 2008; **23**: 515-521

23 **Kubben FJ**, Sier CF, Schram MT, Witte AM, Veenendaal RA, van Duijn W, Verheijen JH, Hanemaaijer R, Lamers CB, Verspaget HW. Eradication of Helicobacter pylori infection favourably affects altered gastric mucosal MMP-9 levels. *Helicobacter* 2007; **12**: 498-504

24 **Pillinger MH**, Marjanovic N, Kim SY, Lee YC, Scher JU, Roper J, Abeles AM, Izmirly PI, Axelrod M, Pillinger MY,

Tolani S, Dinsell V, Abramson SB, Blaser MJ. Helicobacter pylori stimulates gastric epithelial cell MMP-1 secretion via CagA-dependent and -independent ERK activation. *J Biol Chem* 2007; **282**: 18722-18731

25 **Ogden SR**, Wroblewski LE, Weydig C, Romero-Gallo J, O'Brien DP, Israel DA, Krishna US, Fingleton B, Reynolds AB, Wessler S, Peek RM Jr. p120 and Kaiso regulate Helicobacter pylori-induced expression of matrix metalloproteinase-7. *Mol Biol Cell* 2008; **19**: 4110-4121

26 **Wei J**, O'Brien D, Vilgelm A, Piazuelo MB, Correa P, Washington MK, El-Rifai W, Peek RM, Zaika A. Interaction of Helicobacter pylori with gastric epithelial cells is mediated by the p53 protein family. *Gastroenterology* 2008; **134**: 1412-1423

27 **Asahi M**, Azuma T, Ito S, Ito Y, Suto H, Nagai Y, Tsubokawa M, Tohyama Y, Maeda S, Omata M, Suzuki T, Sasakawa C. Helicobacter pylori CagA protein can be tyrosine phosphorylated in gastric epithelial cells. *J Exp Med* 2000; **191**: 593-602

28 **Odenbreit S**, Gebert B, Püls J, Fischer W, Haas R. Interaction of Helicobacter pylori with professional phagocytes: role of the cag pathogenicity island and translocation, phosphorylation and processing of CagA. *Cell Microbiol* 2001; **3**: 21-31

29 **Cho SO**, Lim JW, Kim KH, Kim H. Involvement of Ras and AP-1 in Helicobacter pylori-Induced Expression of COX-2 and iNOS in Gastric Epithelial AGS Cells. *Dig Dis Sci* 2009; Epub ahead of print

30 **Kim KK**, Kim HB. Protein interaction network related to Helicobacter pylori infection response. *World J Gastroenterol* 2009; **15**: 4518-4528

S- Editor Wang YR **L- Editor** Ma JY **E- Editor** Lin YP