HELICOBACTER PYLORI

Global analysis of the human gastric epithelial transcriptome altered by *Helicobacter pylori* eradication in vivo

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Full list of *H pylori* gene microarray is available at http://gut.bmjjournals.com/supplemental

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Objective: The transcriptional profile of gastric epithelial cell lines cocultured with *Helicobacter pylori* and the global gene expression of whole gastric mucosa has been described previously. We aimed to overcome limitations of previous studies by determining the effects of *H pylori* eradication on the transcriptome of purified human gastric epithelium using each patient as their own control.

Design: Laser capture microdissection (LCM) was used to extract mRNA from paraffin-embedded antral epithelium from 10 patients with peptic ulcer disease, before and after *H pylori* eradication. mRNA was reverse transcribed and applied on to Affymetrix cDNA microarray chips customised for formalin-fixed tissue. Differentially expressed genes were identified and a subset validated by real-time polymerase chain reaction (PCR).

Results: A total of 13 817 transcripts decreased and 9680 increased after *H pylori* eradication. Applying cut-off criteria ($p<0.02$, fold-change threshold 2.5) reduced the sample to 98 differentially expressed genes. Genes detected included those previously implicated in *H pylori* pathophysiology such as interleukin 8, chemokine ligand 3, β defensin and somatostatin, as well as novel genes such as GDDR (TFIZ1), chemokine receptors 7 and 8, and gastrokine.

Conclusions: LCM of archival specimens has enabled the identification of gastric epithelial genes whose expression is considerably altered after *H pylori* eradication. This study has confirmed the presence of genes previously implicated in the pathogenesis of *H pylori*, as well as highlighted novel candidates for further investigation.

Helicobacter pylori chronically colonises the stomach of many people worldwide and is associated with the development of peptic ulcer disease and non-cardia gastric cancer in a minority of those infected.[1] After adhering to gastric epithelial cells, *H pylori* subtly influences their function and phenotype via changes in cell signal transduction, thereby influencing multiple gastric cellular and molecular events.[2] Owing to the association of chronic *H pylori* colonisation with gastric carcinogenesis, there has been much interest in defining the bacterial and epithelial cell components of the gastric epithelial cell pathways activated by *H pylori*, particularly as they relate to carcinogenesis and in the generation of the complex inflammatory response to *H pylori*.

The gene-transcription profile of *H pylori* infection has been studied by the culture of gastric epithelial cell lines with *H pylori* by many groups of investigators,[3–11] whereas relatively few studies have examined gene expression in human gastric tissue in vivo.[12 13] The main advantage of cell culture systems is that the response of a pure epithelial cell population is examined without stromal or inflammatory cell influence. However, coculture model systems carry a major disadvantage, as the interplay between stromal, inflammatory and epithelial cells at the tissue level is critical in the generation of the inflammatory response. Furthermore, the use of cancer-derived cell lines in short-term coculture may add further experimental artefacts to the changes in gene expression occurring during chronic *H pylori* infection of the non-neoplastic gastric mucosa.

Several studies have examined the *H pylori*-induced gastric transcriptome in tissue samples. Three studies, two on humans and one on macaques, examined the transcription profile of gastric mucosa after *H pylori* infection, using biopsy specimens that comprised both epithelial and stromal elements.[12–14] However, both published studies on humans examined the transcriptome of patients infected with *H pylori* and that of controls (providing additional complexity to deciphering meaningful differences in gene expression between likely heterogeneous patient groups). Only in the study on macaques were the same hosts examined before and after *H pylori* infection, simplifying the interpretation of differences in gene expression in the presence and absence of *H pylori*.

Laser capture microdissection (LCM) can be used to examine gene expression in a purified cell population extracted from intact tissue samples. Only one study using Balb/c mice infected with the mouse-adapted SS1 strain of *H pylori* used LCM to specifically examine the gastric epithelial response to *H pylori*.[15] We describe the first human study to use LCM in order to obtain a purified population of gastric antral epithelial cells from patients infected with *H pylori*. Furthermore, the transcriptome was analysed using paired biopsy samples from the same patient before and after *H pylori* eradication.

METHODS

Patients and biopsies

Archival formalin-fixed, paraffin-embedded (FFPE) endoscopic gastric antral biopsy specimens that had been collected

Abbreviations: FFPE, formalin fixed, paraffin embedded; LCM, laser capture microdissection; PCR, polymerase chain reaction; reg 3α, regenerating gene family member 3 α; TFF1, trefoil peptide 1

for clinical purposes were obtained from the Department of Medicine, Uijongbu St Mary Hospital, Uijongbu, South Korea, in accordance with the guidelines of the Declaration of Helsinki. As inclusion criteria, we considered well-oriented biopsy specimens that had been taken from the same patient before and 6 weeks after the eradication of *H pylori* using a 7-day course of a triple therapy comprising a proton pump inhibitor, clarithromycin and amoxicillin. None of the patients were taking non-steroidal anti-inflammatory drugs at either time point. Initial *H pylori* infection was documented by histological examination and rapid urease testing, and eradication in all patients was confirmed by negative histology and urea breath tests.[1] Paired biopsy samples from 10 suitable patients were then selected for LCM to extract mRNA for gene array analysis. The ages of these patients ranged from 23 to 69 (mean 58) years, eight were men, and the clinical findings at initial endoscopy were duodenal ulcer,[6] gastric ulcer,[2] and duodenal and gastric ulcers.[2] The presence of the *cagA* gene of *H pylori* was determined by the nested polymerase chain reaction (PCR) method of Koehler *et al*[16] and was found to be positive in 9 of the 10 patients before eradication treatment. Biopsy tissue available for the tenth patient was insufficient for testing.

Histopathological evaluation

Sections of thickness 5 μm were cut from each paraffin block and stained by haematoxylin and eosin. The slides were carefully reviewed by the gastrointestinal pathologist (MBR). Specimens were acceptable for study if chronic actively inflamed gastric mucosa and *H pylori* organisms were identified, populating the surface foveolar epithelium in pre-eradication sections. No active inflammation (neutrophils) or *H pylori* organisms were detected in the eradicated samples. Tissue sections that were poorly oriented, those with extensive intestinal metaplasia that would not yield sufficient normal (non-metaplastic) gastric epithelial glands and those with moderate to severe atrophy were excluded.

Presence of RNA

Owing to the potential for RNA degradation in the routinely collected and processed FFPE tissues, it was important to check that both the paired blocks contained RNA of suitable quality. Sections were scraped from several 10-μm sections cut from each paraffin block and total RNA was extracted and purified using the Paradise Reagent Quality Assessment kit (KIT0313; Arcturus, Mt View, California, USA) and genomic DNA was removed with RNAse-Free DNAse (Qiagen, Valencia, California, USA). The quality of RNA was evaluated by Agilent 2100 Bioanalyser using an RNA 6000 Nano LabChip kit (Agilent Technologies, Wilmington, Delaware, USA).

Laser capture microdissection and RNA extraction

The Paradise FFPE Reagent System protocol (Kit 0311; Arcturus) was followed throughout according to the manufacturer's instructions. Briefly, 7-μm sections were air dried, stained, dehydrated through graded alcohols and subjected to LCM within 1.5 h of deparaffinisation. About 2500 surface and foveolar epithelial cells were microdissected from the tissue sections and captured on LCM HS Capsure caps (Arcturus) using an Autopix Automated Laser Capture Microdissection instrument (Arcturus). Areas of intestinal metaplasia were specifically excluded.

From the microdissected cells, total RNA was extracted, purified and amplified through 1.5 rounds of linear amplification using T7 bacteriophage RNA polymerase-driven in vitro transcription (KIT 0311; Arcturus). After first-strand cDNA synthesis, the quality of RNA was evaluated by real-time PCR, using primers for the 3′ and 5′ ends of β actin as recommended by the manufacturers. RNA was considered acceptable for analysis if the quantity of RNA was >15 ng and the 3′ end:5′ end ratio for β actin was <10. The final amplification and labelling of the dsDNA product was carried out using an Enzo BioArray HighYield RNA Transcript Labeling Kit (Enzo Life Sciences, Farmingdale, New York, USA).

Microarray hybridisation

Labelled cRNA was fragmented and then hybridised on to cDNA microarray chips customised for RNA extracted from FFPE tissues (human U133-X3P expression arrays, Affymetrix, Santa Clara, California, USA) at the Affymetrix Gene Chip Resource at the WM Keck Foundation Biotechnology Resource Laboratory (Yale University, New Haven, Connecticut, USA). Labelled cRNA was fragmented to a size of 35–200 bases by incubation at 94℃ for 35 min in fragmentation buffer (40 mM TRIS-acetate, pH 8.1, 100 mM potassium acetate and 30 mM magnesium acetate). Array hybridisation buffer (100 mM MES, 1 M [Na^+], 20 mM EDTA and 0.01% Tween 20) was used to prehybridise the U133-X3P expression array for 10–15 min at 45℃. The prehybridised solution was removed and replaced with 80 μl of hybridisation mixture containing hybridisation buffer, fragmented cRNA (0.05 μg/μl) and herring sperm DNA (0.1 mg/ml; Promega, Wisconsin, USA). Also included in the hybridisation buffer were acetylated bovine serum albumin (0.5 mg/ml) and four control bacterial and phage cRNA (1.5 pM BioB, 5 pM BioC, 25 pM BioD and 100 pM Cre) samples to serve as internal controls for hybridisation efficiency. The arrays were hybridised for 16 h at 45℃ in a rotisserie oven. After hybridisation, arrays were washed using an Affymetrix fluidics station, stained with streptavidin phycoerythrin (10 μg/ml, Molecular Probes, Carlsbad, California, USA) and scanned on an Affymetrix GeneChip Scanner 3000. Scanned output files were visually inspected for hybridisation artefacts and then analysed by using Affymetrix GeneChip Operating Software. Arrays were scaled to an average intensity of 500 and analysed independently. The quality of the data was evaluated by checking the following quality-control parameters: (a) presence of spiked control cRNAs; (b) low background noise; (c) Q value (pixel-to-pixel variations in signal intensities); and (d) scaling factor that provides a measure of the overall brightness of the array. Scaling is a mathematical technique used by the Affymetrix GeneChip Operating Software to minimise differences in overall signal intensities between two arrays, allowing for more reliable detection of biologically relevant changes. This allows most experiments to become scaled to one target intensity, allowing comparisons between any two experiments.

Bioinformatics and data mining

The expression signals were normalised using the standardisation and normalisation of microarray data (SNOMAD) program.[17] Concordantly absent expression signals were removed from the analysis. A two-tailed Wilcoxon's signed rank sum test was used to perform paired comparisons of the gene expression levels before and after *H pylori* eradication. A 5% false discovery rate correction was used to control for multiple comparisons. The q value of a test measures the minimum false discovery rate that is incurred when calling that test significant. q Values were computed from the unadjusted p values, using the Q-VALUE program.[18] Significantly and differentially expressed genes were grouped into functional categories using the GenMAPP 2 (http://www.GenMAPP.org) and MAPPFinder programs by integrating the annotations of the Gene Ontology Project (ftp://ftp.geneontology.org/go/gene-associations).[19 20]

Confirmation of microarray results by real-time PCR

Several genes of interest that were expressed at a >2.5-fold difference between pre-eradication and post-eradication samples were selected for PCR analysis to verify the result from gene chip analysis. Real-time PCR was carried out on at least three different paired pre-eradication and post-eradication samples per gene. Wherever possible, gene-specific primers for real-time PCR were designed to span an intron (to rule out artefacts from genomic DNA contamination) and to amplify about 100 bp from within 400 bases of the 3′ end of the gene, because the Paradise kit uses oligo dT priming for first-strand synthesis and formalin-fixed RNA is often fragmented to <400 bases. Primers were designed using Primer3 shareware and synthesised by Operon Technologies (Huntsville, Alabama, USA). Real-time PCR was carried out on an MX4000 real-time instrument (Stratagene, Cedar Creek, Texas, USA) using Brilliant SYBR Green Master Mix reagents (Stratagene) according to the manufacturer's instructions, with the exception that the reaction volume was reduced to 25 µl. In parallel with measuring the expression of genes of interest, reactions were carried out using primers for the 3′ end of the human β actin gene, to which all data were then normalised. Amplification conditions yielded efficiencies >90% and linear regression coefficients >0.990. β-Actin was amplified from serial 10× dilutions of cDNA reverse transcribed from Stratagene Reference RNA; values were then used to construct a calibration curve for each PCR run to relate the threshold cycle to the log input amount of template used and to determine relative amounts of gene transcripts. Table 1 lists the sequence of each primer pair and the amplicon size. Thermocycling was carried out for 45 cycles, with denaturation at 95℃ for 30 s, annealing at 55℃ for 1 min, and extension at 72℃ for 1 min. All samples were run in duplicate. A dissociation temperature gradient was included at the end of each run to confirm the absence of high-molecular-weight DNA and primer dimers.

RESULTS

Expression array analysis of gastric antral epithelial genes

After removing the concordantly absent microarray signals, 13 817 transcripts decreased and 9680 increased after *H pylori* eradication. When further applying a fold-change threshold of 2.5, the total number of signals decreased to 871. After correcting for the multiple hypothesis testing effect ($p<0.02$ and $q<0.05$), a final list of 98 genes of interest was obtained. Multiple gene function categories were represented in this list of differentially expressed genes, including immune response, transcriptional regulation, signal transduction, cell-cycle regulation, apoptosis, cell adhesion, growth factors, metabolism, ion channels and structural genes (fig 1). Table 2 gives a list of the 98 genes divided into categories. A complete list of all differentially expressed genes and their expressed signals is available (see addendum).

Confirmation of selected genes by real-time PCR

Real-time quantitative PCR was used to verify changes in gene expression for the 10 genes whose expression according to the microarray analysis changed the most after *H pylori* eradication (fig 2). In every case, there was agreement in terms of increase or decrease between microarray data and the real-time PCR data. As T7 bacteriophage RNA polymerase-driven linear amplification of RNA was used for the chip analysis, it is not surprising that the absolute fold values did not agree between the two methodologies (PCR and microarray). In the cases of gastrokine-1 and β defensin, microarray chip results underestimated the fold change detected by PCR, whereas in the case of regenerating gene family member 3 α (reg 3α), chip results overestimated the fold change found by PCR.

DISCUSSION

This is the first study to use LCM to obtain a purified population of gastric antral epithelial cells for analysis of the *H pylori*-induced transcriptome in humans. It is also unique in that the transcriptome was analysed using paired biopsy samples from the same patient before and after *H pylori* eradication. As expected, most of the genes identified seem to be epithelial in origin, although a few differentially expressed genes (eg, CD137) may be related to a minor population of contaminating intraepithelial lymphocytes, neutrophils or stromal cells included in the microdissected samples. The fact that we targeted the epithelial cell population explains important differences in the *H pylori* gastritis transcriptome identified here, as opposed to other studies where many of the differentially expressed genes may be due to lamina propria immune and stromal cells. For example, in the studies by Mannick *et al*[12] and Wen *et al*[13] many of the up-regulated genes can be attributed to activation of the cellular immune response to *H pylori*.

Many of the genes found to be up-regulated in the *H pylori* gastritis samples in this study have also been shown to be up-regulated in clinical samples, as well as in a variety of in vitro and in vivo models of *H pylori*-induced gastritis, thus adding validity to our approach. Most of the differentially expressed genes can be categorised into well-defined functional categories that are regulated by *H pylori* infection, such as inflammation and apoptosis. As can be expected from any complex biological system possessing circuits and compensatory mechanisms, the gene expression pattern is likely to reflect those genes that are involved in the promotion of a biological or pathological process, as well as those that serve to limit it. Specific examples will be discussed later.

Active *H pylori* infection is characterised by an influx of both acute and chronic inflammatory cells into the gastric lamina propria and epithelium. In this study, interleukin 8 (IL8) expression was the most markedly overexpressed gene in the inflamed mucosa. IL8 is a proinflammatory cytokine, which has a major role in polymorphonuclear chemotaxis (reviewed by Kunkel *et al*[21]); increased gastric epithelial IL8 expression is one of the hallmarks of *H pylori* infection.[22] The expression of the IL8-related chemotactic cytokines GRO-α and GRO-β (chemokine ligands 2 and 3) was also greatly increased in the gastritis samples, in keeping with previous studies that have described increased GRO-α expression in the gastric mucosa during *H pylori* infection.[23] In addition, the expression of IL1 family member 8, a member of the IL1 gene superfamily,[24 25] was also increased in patients with gastritis. The importance of IL1 and other cytokines in the pathogenesis of *H pylori* infection is further emphasised after determining the associations between functional polymorphisms in certain cytokine genes and increased gastric cancer risk after *H pylori* infection.[26] This is the first report to describe expression of the chemokine receptors 7 and 8 in *H pylori*-associated gastritis, although increased expression of other chemokine receptors (5 and 7) has been previously reported.[27 28] β Defensin, an antimicrobial protein expressed by both neutrophils and mucosal epithelial cells, has previously been shown to be expressed in *H pylori*-associated gastritis[29] and to have a direct antibacterial effect. Our data confirm this observation.

Several novel genes which have not been previously implicated in the pathophysiology of *H pylori* gastritis were identified in our study. The GDDR gene, which was the most strongly decreased in gastritis and the most differentially expressed in the entire analysis, is a novel gene shown (in the Chinese literature) to be down regulated in gastric cancer.[30]

Table 1 Reverse transcription-polymerase chain reaction primers for confirmation of microarray results

Gene	Symbol	GenBank ID	Sense and anti-sense primers	Amplicon
β Actin*	ACTB	NM_001101	5′-TCCCCCAACTTGAGATGTATGAAG-3′ 5′-AACTGGTCTCAAGTCAGTGTACAGG-3′	91
Calcyclin	S100A6	NM_014624.3	5′-ACAAGCACACCCTGAGCAAGA-3′ 5′-CCATCAGCCTTGCAATTTCA-3′	99†
Defensin β4	DEFB4	NM_004942.2	5′-GCCTCTTCCAGGTGTTTTTG-3′ 5′-GAGACCACAGGTGCCAATTT-3′	118†
Gastrokine 1	GKN1	NM_019617.2	5′-CAAAGTCGATGACCTGAGCA-3′ 5′-CTTGCCTCTTGCATCTCCTC-3′	93†
GDDR	GDDR	AI821357	5′-TGAGAAACAGGCTCTGGACA-3′ 5′-CAGGAACCAATCCACGTCTT-3′	97†
Interleukin 8	IL8	NM_000584.2	5′-CAGCCAAAACTCCACATGCA-3′ 5′-GCCTTGTATTTAAAAATGCAGTCA-3′	114
Prothymosin α	PTMA	AF348514.1	5′-GGTGATGGTGAGGAAGAGGA-3′ 5′-TCGGTCTTCTGCTTCTTGGT-3′	116†
Regenerating islet-derived 3α	REG3A	NM_002580	5′-TTTGCATGGGAGAGAAATCC-3′ 5′-TTTCCACCTCAGAAATGCTGT-3′	87†
Secretoglobin 2A1	SCTG2A1	NM_002407	5′-ACGCACGACTGAACACAGAC-3′ 5′-TGCAGCCAGAATCTGCATAG-3′	102†
Somatostatin	SST	NM_001048.2	5′-CCAACCAGACGGAGAATGAT-3′ 5′-CCATAGCCGGGTTTGAGTTA-3′	111
Survivin	BIRC5	AA648913	5′-AGGACTGTGACAGCCTCAAC-3′ 5′-GCAGTGTCCCTTTTGCTAGAG-3′	100

IL8, interleukin 8; RT-PCR, reverse transcription-polymerase chain reaction.
All primers were designed to amplify the 3′ end of their respective transcripts.
*Housekeeping gene. †Intron spanning primers.

Sequence analysis indicates that the product of the GDDR gene is identical to a protein recently identified and named TFIZ1, which is secreted by gastric mucosal cells to form a heterodimer with the gastric trefoil peptide 1 (TFF1).[31] TFF1 has the properties of a tumour suppressor protein[32] and TFF1 expression is frequently lost in human gastric cancer through several diverse mechanisms.[30–35] Recent studies indicate that TFF1 may be an adhesin for *H pylori,* as its distribution in gastric glands mirrors bacterial location in the foveolar epithelium and specific binding has been shown.[36] Although the precise function of TFIZ1 is currently not known, the marked down regulation of expression of the

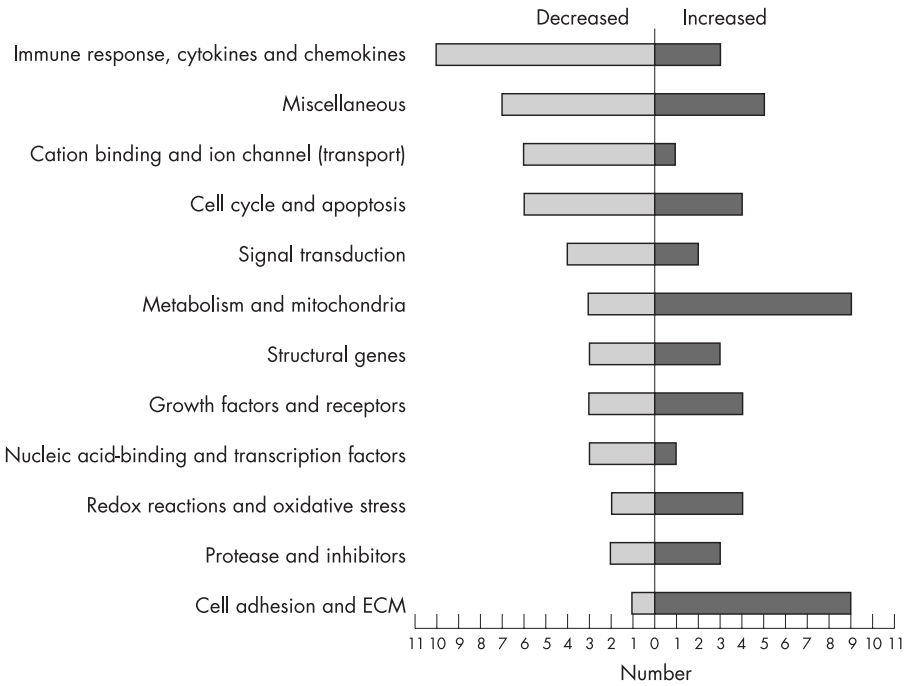

Figure 1 Differentially expressed genes divided into functional categories. Number of genes whose expression was increased in biopsy specimens with *Helicobacter pylori* gastritis compared with post-treatment biopsy specimens (increased) as opposed to genes whose expression decreased in biopsy specimens with *H pylori* gastritis compared with post-treatment biopsy specimens (decreased). ECM, extracellular matrix.

Table 2 Differentially expressed genes after *Helicobacter pylori* eradication

Functional gene category	Accession No	Gene name	Fold change	p Values
Immune response	NM_000584	Interleukin 8	−12.82	0.001
Cytokines and chemokines	NM_014438	Interleukin 1 family, member 8 (eta)	−4.03	0.013
	NM_002090	Chemokine (C-X-C motif) ligand 3 (GRO 3)	−3.86	0.017
	AB009597	Killer cell lectin-like receptor subfamily D, member 1	−3.36	0.005
	NM_004942	Defensin, beta 4	−3.32	0.017
	M57731	Chemokine (C-X-C motif) ligand 2 (GRO 2)	−3.18	0.004
	NM_005201	Chemokine (C-C motif) receptor 8	−3.14	0.005
	M27487	Major histocompatibility complex, class II, DP alpha 1	−3.07	0.009
	NM_002029	Formyl peptide receptor 1	−2.96	0.005
	NM_001838	Chemokine (C-C motif) receptor 7	−2.51	0.02
	M18767	Complement component 1, s subcomponent	2.55	0.007
	NM_005532	Interferon, alpha-inducible protein 27	2.57	0.007
	NM_013352	Squamous cell carcinoma antigen recognised by T cells 2	3.23	0.005
Nucleic acid-binding and transcription factors	NM_018488	T-box 4	−3.92	0.017
	NM_080743	Serine-arginine repressor protein (35 kDa)	−3.71	0.017
	AK024083	Histone deacetylase 6	−2.66	0.013
	X03348	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	4.08	0.007
Cell cycle and apoptosis	NM_000465	BRCA1-associated RING domain 1 (BARD1)	−3.48	0.017
	AI435073	Programmed cell death 6 (apoptosis linked gene 2)	−3.34	0.013
	AA648913	Baculoviral IAP repeat-containing 5 (survivin)	−2.97	0.007
	NM_001561	TNF receptor superfamily, member 9 (CD137)	−2.79	0.007
	AW070323	Serine/threonine kinase 17b (apoptosis-inducing)	−2.77	0.007
	AA971429	CASP8 and FADD-like apoptosis regulator (FLIP)	−2.60	0.017
	NM_002371	Mal, T cell differentiation protein	2.71	0.005
	NM_001759	Cyclin D2	2.89	0.005
	NM_000560	CD53 antigen	2.97	0.013
	AF348514	Prothymosin, alpha (gene sequence 28)	6.59	0.007
Signal transduction	NM_015715	Phospholipase A2, group III	−4.50	0.013
	BC040474	Rho guanine nucleotide exchange factor (GEF) 10	−4.25	0.005
	NM_018485	G protein-coupled receptor 77	−2.82	0.013
	NM_018972	Ganglioside-induced differentiation-associated protein 1	−2.54	0.013
	BC001359	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	2.51	0.001
	AA130247	Protein kinase, cAMP-dependent, catalytic, beta	3.71	0.013
Cell adhesion and ECM	NM_001941	Desmocollin 3	−2.95	0.013
	BC001120	Lectin, galactoside binding, soluble, 3 (galectin 3)	2.50	0.007
	NM_002293	Laminin, gamma 1 (formerly LAMB2)	2.55	0.007
	NM_002305	Lectin, galactoside binding, soluble, 1 (galectin 1)	2.71	0.002
	M98399	CD36 antigen (collagen type I receptor, thrombospondin receptor)	2.95	0.017
	NM_000129	Coagulation factor XIII, A1 polypeptide	3.10	0.013
	AF089868	Melanoma cell adhesion molecule	3.12	0.005
	AV681579	Amyloid beta precursor protein (cytoplasmic tail)-binding protein 2	3.16	0.005
	NM_004684	SPARC-like 1 (mast9, hevin)	3.94	0.013
	NM_000130	Coagulation factor V (proaccelerin, labile factor)	5.50	0.005
Cation binding and ion channel (transport)	BC006404	Suppressor of potassium transport defect 3	−4.47	0.005
	AF336127	Solute carrier family 4, sodium bicarbonate transporter-like, member 11	−3.05	0.005
	AY083533	Mucolipin 2	−3.03	0.017
	AF257080	Potassium channel, subfamily K, member 9	−2.80	0.017
	AA682371	Porin, putative	−2.80	0.017
	NM_006815	Coated vesicle membrane protein	−2.58	0.013
	NM_014624	S100 calcium-binding protein A6 (calcyclin)	2.97	0.001
Metabolism and mitochondria	NM_002649	Phosphoinositide-3-kinase, catalytic, gamma polypeptide	−3.32	0.001
	NM_017827	Seryl-tRNA synthetase 2	−3.01	0.017
	AA702810	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	−2.67	0.017
	AF161387	N-acetylneuraminic acid synthase (sialic acid synthase)	2.51	0.009
	NM_001353	Aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid-binding protein; 3-alpha hydroxysteroid dehydrogenase, type III)	2.66	0.001
	AF126782	Dehydrogenase/reductase (SDR family) member 7	2.68	0.007
	NM_013379	Dipeptidylpeptidase 7	2.87	0.017
	NM_004267	Carbohydrate(N-acetylglucosamine-6-O)sulfotransferase2	3.05	0.005
	NM_002489	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4, 9kDa	3.14	0.009
	AI674647	Signal peptide peptidase-like 2A	3.29	0.005
	J05594	Hydroxyprostaglandin dehydrogenase 15-(NAD)	3.41	0.005
	N21458	Sorbin and SH3 domain containing 1	3.71	0.005
Redox reactions and oxidative stress	L24553	Nitric oxide synthase 2A (inducible, hepatocytes)	−3.58	0.013
	AU144855	Cytochrome P450, family 1, subfamily B, polypeptide1	−3.34	0.013
	NM_001866	Cytochrome c oxidase subunit VIIb	2.50	0.001
	NM_001863	Cytochrome c oxidase subunit Vib polypeptide 1	2.65	0.017
	AF313911	Thioredoxin	3.13	0.002
	NM_000846	Glutathione S-transferase A2	3.68	0.013

Table 2 Continued

Functional gene category	Accession No	Gene name	Fold change	p Values
Growth factors and receptors	NM_002580	Regenerating islet-derived 3 alpha (REG-3)	−5.98	0.013
	M60485	Fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	−2.87	0.013
	BC001422	Placental growth factor, vascular endothelial growth factor-related protein	−2.50	0.013
	NM_019617	Gastrokine 1	2.51	0.007
	NM_002178	Insulin-like growth factor-binding protein 6	3.18	0.005
	NM_001048	Somatostatin	3.34	0.005
	AL575922	Secreted protein, acidic, cysteine-rich (osteonectin)	5.17	0.005
Protease and inhibitors	NM_000185	Serine (or cysteine) proteinase inhibitor, clade D (heparin cofactor), member 1	−2.93	0.013
	NM_001085	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	−2.60	0.009
	NM_003122	Serine protease inhibitor, Kazal type 1	2.71	0.005
	NM_005213	Cystatin A (stefin A)	3.03	0.005
	NM_000014	Alpha-2-macroglobulin	4.14	0.007
Structural genes	NM_016239	Myosin XVA	−2.66	0.017
	U89330	Microtubule-associated protein 2	−2.57	0.013
	AK023821	Microtubule–actin cross-linking factor 1	−2.53	0.009
	NM_001613	Actin, alpha 2, smooth muscle, aorta	2.66	0.005
	AF092128	Integral membrane protein 2B (ITM2B)	2.90	0.005
	AF141347	Tubulin, alpha 3	2.95	0.005
Miscellaneous	X67513	Cholinergic receptor, nicotinic, beta polypeptide 3	−4.59	0.013
	AF063002	Four and a half LIM domains 1	−3.92	0.009
	NM_003469	Secretogranin II (chromogranin C)	−3.50	0.005
	NM_003552	Olfactory receptor, family 1, subfamily D, member 5	−3.45	0.005
	NM_018687	Hepatocellular carcinoma-associated gene TD26	−3.07	0.017
	AL036350	Myeloma overexpressed 2	−2.90	0.005
	NM_031211	LAT1-3TM protein	−2.53	0.013
	BE260771	Keratinocyte-associated protein 2	3.05	0.017
	NM_002407	Secretoglobin, family 2A, member 1	3.32	0.005
	NM_002933	Ribonuclease, RNAse A family, 1 (pancreatic)	4.72	0.007
	AW188940	Beta-2-microglobulin	4.92	0.009
	AI821357	Down-regulated in gastric cancer GDDR	7.06	0.007

CASP8, caspase 8; FADD, Fas (TNFRSF6)-associated via death domain; FLIP, FLICE-inhibitory proteins; TNF, tumour necrosis factor.

gene encoding this TFF1-binding protein in the presence of *H pylori* infection suggests a link between *H pylori* adhesion and abnormal gastric epithelial cell growth.

Our novel findings include the identification of two growth factors, gastrokine and reg 3α, as being differentially regulated during *H pylori* infection. Gastrokine, whose expression was increased after *H pylori* eradication, is a mitogen postulated to play a part in the maintenance of normal gastric epithelial integrity, is highly expressed in the normal gastric foveolar epithelium and down regulated in gastric cancer.[37 38] The expression of reg 3α (also known as pancreatitis-associated protein 1) was decreased after eradication of *H pylori*. Interestingly, other members of the reg family have been shown to be involved in gastric mucosal growth[39] and to be up regulated in gastric cancer.[40–42] More recently, expression of another reg family protein was identified in gastric neuroendocrine cells during *H pylori* gastritis.[43] The expression level of the insulin growth factor-binding protein 6 was increased after *H pylori* eradication. Insulin growth factor-binding protein 6 was shown to be increased in gastric cancer cell lines[44] and in the serum of patients with gastric carcinoma,[45] consistent with a role for insulin growth factor signalling in gastric carcinogenesis.

H pylori gastritis is associated with increased epithelial apoptosis and many studies have shown that within a month of the eradication of *H pylori*, apoptosis returns to normal (reviewed by Shirin *et al*[46]). We detected increased expression of several pro-apoptotic genes during *H pylori* infection, including the tumour necrosis factor receptor superfamily member 9 (CD137), which is expressed by activated B and T cells,[47] and BARD1 (BRCA-mediated associated ring domain 1).[48] Expression of prothymosin α, CD53, caspase 8 and Fas (TNFRSF6)-associated via death domain-like apoptosis regulator (FLICE-inhibitory proteins; all involved in the inhibition of apoptosis[49–51]) were also increased, suggesting that they may serve to limit the apoptotic response to *H pylori*. Although survivin was initially thought to have an important anti-apoptotic function (based on sequence homology with the baculovirus IAP gene), recent evidence suggests that its major function in vivo is in the regulation of chromosomes on the mitotic spindle during cytokinesis.[52] Thus, increased survivin expression during *H pylori* infection may be related to increased gastric epithelial proliferation.

Although there is a substantial body of evidence that RNA extracted from laser-captured cell populations can be isolated, amplified and used for microarray analysis of both animal and plant tissues,[53–55] this is only the second report of gene array analysis from formalin-fixed human tissues. Ma *et al*,[56] who used the Arcturus-Agilent custom-designed array, reported a two-gene expression ratio predictive of clinical outcome in patients with breast cancer treated with tamoxifen. We report the first successful use of the Arcturus-Affymetrix X3P array to explore the human genome. We have found that, with careful selection of tissue blocks and attention to ensuring the quality of extracted RNA, the genomic profile of gastric epithelial cells from FFPE archival tissue can be uncovered. Moreover, real-time PCR analysis confirmed the data generated by the Affymetrix chip for all 10 genes examined. There are, of course, limitations to this technology. For example, changes in protein expression regulated at the post-translational level, such as those recently described for the p27 gene during *H pylori* infection, could not be detected using this system.[57 58]

In summary, using high-throughput gene expression screening of microdissected human *H pylori* samples, we

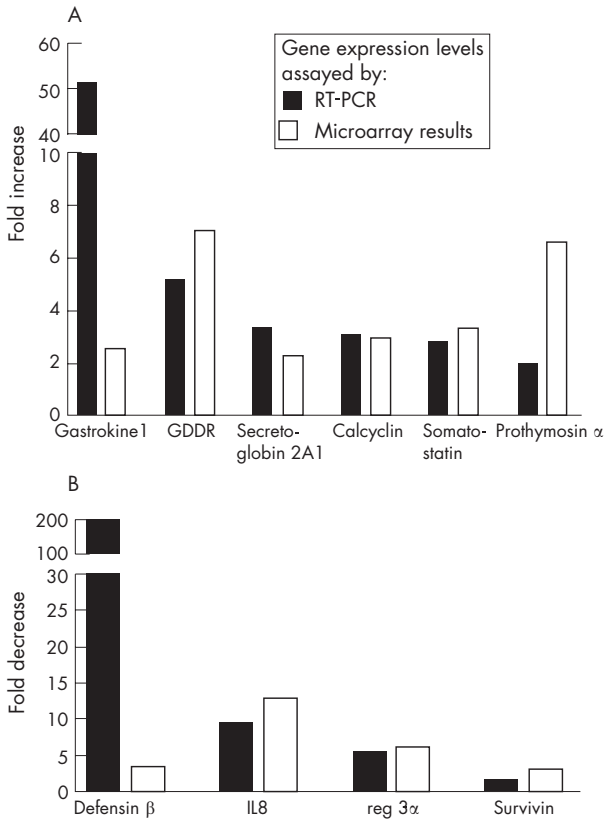

Figure 2 Confirmation of microarray results by real-time polymerase chain reaction (RT-PCR). Microarray results (A) are compared with quantitative gene expression analysis (B) by SYBR green RT-PCR after laser capture microdissection of surface and foveolar epithelium, from formalin-fixed paraffin-embedded tissue samples. Relative fold change in gene expression in microdissected tissues before and after eradication of *Helicobacter pylori* was calculated relative to expression of β actin as a housekeeping gene. Expression of the different transcripts was examined in the same first-strand transcription reaction amplified for array analysis. RT-PCR was carried out on at least three paired pre-eradication and post-eradication samples per gene. A significant correlation was found between both methods (Spearman's r test: r = 0.68, p = 0.029). IL8, interleukin 8; reg 3α, regenerating gene family member 3 α.

have validated the results from other in vitro and in vivo model systems and discovered certain novel candidates which may have key roles in the pathophysiology of gastritis due to *H pylori*. The method used has wide applicability for the analysis of cell-type gene expression from tissues already existing in many clinical pathology archives.

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