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Editorial board member of *World Journal of Gastroenterology*, Toru Mizuguchi, MD, PhD, Associate Professor, Surgeon, Department of Surgery, Surgical Oncology and Science, Sapporo Medical University Hospital, Sapporo 060-8543, Hokkaido, Japan

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Basic Study

Glycosylation-related gene expression in HT29-MTX-E12 cells upon infection by *Helicobacter pylori*

Michael T Cairns, Ananya Gupta, Julie A Naughton, Marian Kane, Marguerite Clyne, Lokesh Joshi

Michael T Cairns, Marian Kane, Lokesh Joshi, Glycoscience Group, National Centre for Biomedical Engineering Science, National University of Ireland Galway, H91 CF50 Galway, Ireland

Ananya Gupta, School of Natural Sciences, National University of Ireland Galway, H91 CF50 Galway, Ireland

Julie A Naughton, Marguerite Clyne, Conway Institute of Biomolecular and Biomedical Research, School of Medicine and Medical Sciences, University College Dublin, Dublin 4, Ireland

Author contributions: Cairns MT, Gupta A and Naughton JA performed the majority of the experiments; Cairns MT analysed the data; Clyne M and Joshi L designed and coordinated the research; Cairns MT, Kane M and Clyne M wrote the paper.

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Correspondence to: Michael T Cairns, Senior Research Fellow, Glycoscience Group, National Centre for Biomedical Engineering Science, National University of Ireland Galway, University Rd, H91 CF50 Galway, Ireland. michael.cairns@nuigalway.ie
Telephone: +353-91492094

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Abstract**AIM**

To identify glycosylation-related genes in the HT29 derivative cell line, HT29-MTX-E12, showing differential expression on infection with *Helicobacter pylori* (*H. pylori*).

METHODS

Polarised HT29-MTX-E12 cells were infected for 24 h with *H. pylori* strain 26695. After infection RNA was isolated from both infected and non-infected host cells. Sufficient infections were carried out to provide triplicate samples for microarray analysis and for qRT-PCR analysis. RNA was isolated and hybridised to Affymetrix arrays. Analysis of microarray data identified genes significantly differentially expressed upon infection. Genes were grouped into gene ontology functional categories. Selected genes associated with host glycan structure (glycosyltransferases, hydrolases, lectins, mucins) were validated by real-time qRT-PCR analysis.

RESULTS

Infection of host cells was confirmed by the isolation of live bacteria after 24 h incubation and by PCR amplification of bacteria-specific genes from the host cell RNA. *H. pylori* do not survive incubation under the adopted culture conditions unless they associate with the adherent mucus layer of the host cell. Microarray analysis identified a total of 276 genes that were significantly differentially expressed ($P < 0.05$) upon *H. pylori* infection and where the fold change in expression was greater than 2. Six of these genes are involved in glycosylation-related processes. Real-time qRT-PCR demonstrated significant downregulation (1.8-fold, $P < 0.05$) of the mucin MUC20. REG4 was heavily expressed and significantly downregulated (3.1-fold, $P < 0.05$) upon infection. Gene ontology analysis was consistent with previous studies on *H. pylori* infection.

CONCLUSION

Gene expression data suggest that infection with *H. pylori* causes a decrease in glycan synthesis, resulting in shorter and simpler glycan structures.

Key words: Glycosylation; Adherent mucus; Gastric; HT29-MTX-E12; *H. pylori* strain 26695; Transcriptomics

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Core tip: Few studies on *Helicobacter pylori* (*H. pylori*) infection focus on glycosylation-related genes in the host cells yet key cell-cell interactions are likely mediated through surface glycoconjugates. We use HT29-MTX-E12 cells, a promising and novel model of the stomach epithelium, to investigate the transcriptomic effects of *H. pylori* infection. HT29-MTX-E12 cells produce a thick adherent mucus layer and show a level of pluripotency that gastric cells naturally present and which some other model cell lines do not. Furthermore both *H. pylori* strain 26695 (lacks BabA adhesin) and HT29-MTX-E12 host cells (TLR2-negative) have some features atypical of more common models of *H. pylori* infection.

Cairns MT, Gupta A, Naughton JA, Kane M, Clyne M, Joshi L. Glycosylation-related gene expression in HT29-MTX-E12 cells upon infection by *Helicobacter pylori*. *World J Gastroenterol* 2017; 23(37): 6817-6832 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i37/6817.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i37.6817>

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a major cause of peptic ulcer disease and chronic gastritis, and is a causative agent for both gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma^[1-3]. Gastric cancer is the second most common cause of

cancer-related deaths and is the fifth greatest killer worldwide after tuberculosis. Eradication of *H. pylori* infection with antibiotics may cause as much as a 40 % decrease in the cases of gastric cancer^[4]. Despite its occurrence in approximately two-thirds of the world's population, infection with *H. pylori* is asymptomatic in the majority of cases. How *H. pylori* manages to establish chronic infection in the hostile environment of the human stomach and to evade a vigorous immune response is not known.

An acute response to *H. pylori* infection is characteristically marked by the induction of interleukin (IL)-8. This is evident in both human biopsies and cell line studies. An acute response is followed by a chronic response which is marked in humans by the production of IgA autoantibodies to IL-8^[5]. A rhesus macaque model of *H. pylori* infection showed a dramatic change in gastric epithelial morphology over the first few hours of infection but a restoration of normal morphology within 24 h despite the continued presence of the pathogen^[6]. These morphological changes were associated with modified O-glycan structures. It has been shown previously that transcript levels of *B3GNT5* were upregulated upon *H. pylori* infection of gastric cell lines MKN45 and AGS and that overexpression of this gene, associated with glycosylation of glycolipids, led to increased sialyl Lewis x presentation and adhesion of *H. pylori*^[7]. Furthermore, host cells in asymptomatic infected human patients appear to modify O-glycans in order to counter the effects of the invading *H. pylori*^[8].

H. pylori resides in the stomach where there is a thick adherent layer of mucus. While most invading pathogens are excluded from epithelial surfaces by the mucous layer, *H. pylori* survives in the mucous layer and can penetrate it to attach to the underlying cells. The main mucin components of mucus in the stomach are MUC5AC, MUC6, MUC1 and MUC16^[9-11] with *H. pylori* displaying a distinct tropism for MUC5AC^[12,13]. Although *H. pylori* may reside in the mucous layer it is through adhesion to the underlying epithelial cells that the bacteria exert their harmful/inflammatory effects. *H. pylori* binds to cells through adhesins, such as BabA that binds Lewis b and H-type 1 antigens on epithelial cell glycoproteins or glycolipids. They can also use the SabA adhesin to bind to sialyl Lewis x and sialyl Lewis a antigens, though this is expected to play a greater part in chronic infection. More recently LabA has been identified which binds to LacdiNAc structures present on MUC5AC^[14]. Only a small fraction of *H. pylori* associated with infected cells is found internally^[15], instead the bacteria inject the effector protein CagA and subvert host cell signalling^[16,17].

Transcriptomic studies, through the use of high density DNA microarrays, have been applied to various aspects of *H. pylori* infection including analysis of human biopsies^[18-23], analysis of human tissue after eradication treatments^[24,25], the analysis of animal models^[6,26,27] and the analysis of various cell

lines^[7,28-30]. High variability in the data sets has been attributed to different model systems, different host cells and the diverse nature of the different *H. pylori* strains used.

Human cell lines, isolated from the gastrointestinal tract (GIT), have been commonly used to study *H. pylori* infection. The responses of different gastric cell lines to *H. pylori* infection have been detailed in a recent paper^[31]. The HT29 colon cell line and its derivatives have been used frequently as a model of the gastrointestinal tract because the cells are multipotent and can be directed along different paths of selection and differentiation. Some HT29 derivatives have an absorptive (enterocyte-like) phenotype while others have a mucus-secreting (goblet-like) phenotype. This makes HT29 cells particularly suited to the study of glycan structures in the gastrointestinal tract and mucus-associated effects on bacterial infection. The HT29-MTX-E12 (E12) cell line is a HT29 derivative selected on the basis of tight junction formation and adherent mucus production, which has many of the characteristics of a gastric cell^[32-36]. In particular, differentiated E12 cells express MUC5AC, MUC1, MUC 6 and only trace amounts of MUC2^[35], a composition which more closely resembles the stomach mucin makeup than the intestine.

The aim of this study was to determine the transcriptomic response of the mucus-secreting E12 cells to *H. pylori* infection with special focus on the glycomic response. Because known *H. pylori* adhesins bind glycosylated structures on the cell surface and in the mucous layer and glycosylation is known to play a major role in general host-pathogen and cell-cell interactions, it is of interest to know how glycosylated structures may change in response to infection.

MATERIALS AND METHODS

E12 culture

E12 cells, a kind gift from Per Artursson of the University of Uppsala, were maintained in flasks in complete Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (Life Technologies), 2 mmol/L L-glutamine (Life Technologies), 1% non-essential amino acids and antibiotics (penicillin (50 U/mL) and streptomycin (50 µg/mL)). Cells (2×10^5) were seeded on transwell filters (0.4 µm pore polycarbonate membrane insert) in 6-well plates. Cells were incubated at 37 °C in a humidified 5 % CO₂ atmosphere and media was added every second day. Transepithelial electrical resistance (TEER) measurements were taken at 20 d. Antibiotics were removed from the cells at least 24 h before infection assays.

H. pylori culture

Bacteria (*H. pylori* strain 26695) were plated on Columbia blood agar (Oxoid) and grown for 48 h in a microaerophilic environment using gas packs (Oxoid,

BR0038). Bacteria were harvested from plates into Brucella broth, the OD₆₀₀ adjusted to 0.1 and grown in T25 tissue culture flasks at 37 °C under microaerophilic conditions with shaking at 70-80 rpm until the OD₆₀₀ reached 0.6. Gram staining of bacterial cultures was used to check coccoid levels were less than 5%. Washed bacteria were resuspended to an OD₆₀₀ of 1.8 with antibiotic-free RPMI.

Infection protocol

On day 20 E12 cells in transwells were washed with HBSS and 2 mL antibiotic-free complete DMEM media was added to the base of each well and 1 mL of RPMI to the apical surface of each well. *H. pylori* do not survive in DMEM^[37] but will in RPMI. On day 21 200 µL bacteria (6×10^8) were added to each well at a multiplicity of infection (MOI) of 300. Plates were incubated at 37 °C for 24 h. Cells were harvested for RNA isolation. Replicate transwells were washed gently with sterile PBS and harvested using trypsin EDTA as described previously^[35,38]. Serial dilutions of the trypsinised cells were plated in triplicate on Columbia blood agar plates and incubated under microaerophilic conditions. Colonies were counted after 4-5 d incubation to determine the number of bacteria (CFU/mL) that were associated with the cells. Though some infections resulted in low IL8 transcript levels this did not appear to correlate with low CFU values.

RNA isolation and microarray hybridisation

RNA for microarray hybridisations and qPCR analysis was isolated by TRIzol extraction followed by standard RNA purification protocols (Qiagen). RNA (100 ng) was labelled with biotin using the Affymetrix 3' IVT Express Protocol. All hybridisations were carried out using the hybridisation and wash solutions as recommended in the Affymetrix 3' IVT Express Protocol and were carried out by DNA Vision, Charleroi, BE.

Microarray analysis

Spot intensities provided (DNA Vision) were normalised and analysed in GeneSpring (Ver 12.3). Data were log transformed and quantile normalised. Subsequently all normalised data was baseline transformed to the median of all samples. Intensity values for all entities (54675) were initially filtered to exclude values which were less than 50 in all three samples of both conditions (uninfected, infected). Significantly differentially expressed genes were identified based on log values using an unpaired *t*-test (variance assumed equal)^[39], with a FDR cut-off value of 0.05. Multiple test correction was carried using the Benjamini and Hochberg correction. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus^[40] and are accessible through GEO Series accession number GSE74492 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74492>). Where microarray data is

graphed, the raw signals for uninfected samples were the actual spot intensities and for the infected samples the signals were corrected using the fold change determined from normalised data for each gene. Standard deviations are shown for the three samples in each condition using raw spot intensities (uninfected) and raw spot intensities corrected for fold change (infected). Where the microarray data is shown as a log fold change, standard deviations were generated using propagation of errors rules.

For Gene Ontology analysis, DAVID (Database for Annotation, Visualization and Integrated Discovery, v6.7) was used^[41] (<http://www.david.ncifcrf.gov/>). The number of significant genes (6034) was reduced further using more stringent parameters (FDR < 0.01, fold change > 1.2). Where directional analysis was carried out, only the top 500 upregulated genes (FDR < 0.005, fold change > 1.2) were included in the GO analysis and this was compared with whatever number of downregulated genes (714) had *P*-values below the *P*-value of the 500th upregulated gene (FDR = 0.0046) in order to maintain a balanced comparison. GO analysis also excluded GO terms where the number of genes in the GO term was more than 10% of all the genes on the array; this therefore removed broader gene ontology terms which were not generally informative. These restrictions were not imposed for GO analysis of the glycosylation-related genes because of the lower number of genes involved.

qRT-PCR

qRT-PCR validation was carried out on RNAs from samples collected independently of the microarray samples. The RNA samples for qRT-PCR validation were also from several different infections; this adds some of the variability seen in the data and is an issue that is discussed further in the manuscript. For real time qRT-PCR RNA (1 µg) was reverse transcribed using the VILO cDNA Synthesis Kit (Invitrogen) according to the manufacturer's instructions. PCR was carried out using diluted template (2 µL) and GoTaq[®] qPCR Master Mix (18 µL) on a Mx3000P qPCR System (Stratagene). Cycling conditions for all primer pairs was 95 °C for 5 min followed by 40 cycles of 95 °C 10 s and 60 °C 10 s. Dissociation curves were determined using a temperature gradient from 95 °C to 56 °C. Primer sequences were either retrieved from primer repositories (PrimerBank, <https://pga.mgh.harvard.edu/primerbank/> and qPrimerDepot, <https://primerdepot.nci.nih.gov/>), designed using Primer3 (http://primer3plus.com/web_3.0.0/primer3web_input.htm) or taken from relevant papers (Supplementary Table 1 and references therein). Several primer pairs were tested for their potential as housekeeping genes and geNorm analysis^[42] was used to select an optimal set of three genes for subsequent normalisation (*GAPDH*, *RPLP0*, *UAP1*).

All fold change values were calculated within geNorm which also generated standard deviations based on the rules for the propagation of errors. Standard two-tailed *t*-tests were used to determine where fold changes were significant. As an approximate measure of gene expression level, cycle threshold (Ct) values determined by qRT-PCR for each gene (uninfected cells) were compared to a Ct_{max}, in this case *IL32* the lowest expressed of our selected genes

RESULTS

This study investigated the effects of *H. pylori* (strain 26695) colonisation on the transcriptome of E12 cells, a specialised derivative of the HT29 cell line. The cell line forms a functional layer of polarised cells and produces an adherent mucous layer when grown on transwells for 21 d.

Microarray analysis

E12 cells were grown on transwells for 21 d and infected with *H. pylori* strain 26695 at a MOI of 300. Cells were harvested for RNA isolation after incubation at 37 °C for 24 h. *H. pylori* maintains viability during infection despite its normal requirement for a microaerophilic environment, possibly as a consequence of close association with the mucous layer surrounding the E12 cells. Microarray analysis of all samples showed separate clustering of infected E12 (*n* = 3) and uninfected E12 samples (*n* = 3) (Supplementary Figure 1). The infected samples, originating from triplicate wells in a single infection experiment, clustered very tightly. Although greater variability was seen in the uninfected E12 samples, this is probably because these samples originate from two separate infection experiments.

Confirmation of a global modulation of E12 genes in response to *H. pylori* infection

Statistical analysis of the microarray data suggested significant differential expression of 6034 genes (FDR < 0.05) upon infection. Of these, 276 unique genes (< 5 %) showed fold changes greater than two (see Table 1 for the top 15 upregulated and downregulated genes; Supplementary Table 2 for all 276 genes). GO analysis of a reduced list (2639 genes) of regulated genes (FDR < 0.01, fold change > 1.2) is presented in Figure 1A. This analysis was sensitive to the selected fold change cut-off therefore we considered it appropriate to choose quite a low value (1.2-fold). To provide some additional perspective, GO analysis was repeated separately on the most significantly upregulated and downregulated genes (data not shown). This analysis gave an idea of the direction in which each significant process (as shown in Figure 1A) was regulated. GO analysis of the top 500 upregulated genes (FDR < 0.005, fold change > 1.2) revealed

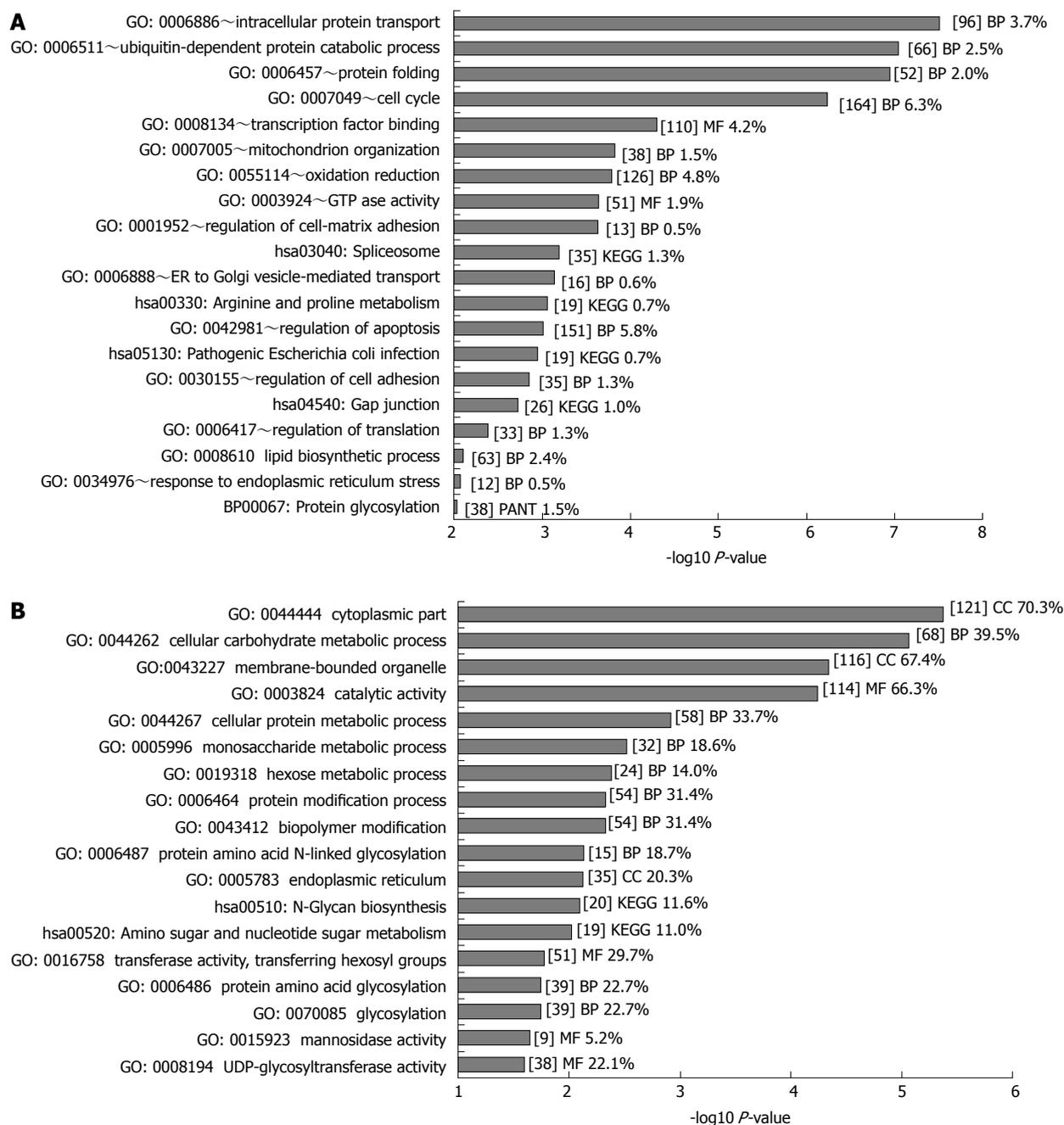


Figure 1 GO analysis of significant differentially expressed genes. A: GO analysis of 2639 genes deemed significantly differentially expressed ($P < 0.01$, fold change > 1.2) was carried out in DAVID. Only GO terms with a P -value below 0.01 are shown ($-\log_{10} P$ -value > 2). GO terms were excluded where the number of genes belonging to that GO term exceeded 10% of all genes. B: GO analysis of 374 significantly differentially expressed glycosylation-related genes ($P < 0.001$) was carried out in DAVID. Only GO terms with a P -value below 0.1 are shown ($-\log_{10} P$ -value > 1). Some Panther (PANT) gene ontology and KEGG pathway terms were included from the DAVID analysis. The number of genes included in each term is given within square brackets [] together with the percentage of significant genes relative to the total number of genes of that term on the array. BP: Biochemical process; MF: Molecular function; CC: Cellular component.

significant processes related to protein ubiquitination, protein folding, cell redox homeostasis, oxidative phosphorylation and the mitochondrial membrane. GO analysis of the top downregulated genes (FDR = 0.005, fold change > 1.2 , 714 genes) revealed significant processes associated with ECM-receptor interaction, focal and cell adhesion, phosphorylation and modulation of the cytoskeleton. Some GO processes included genes with approximately equal numbers

of up and downregulated genes: these additional GO terms included Golgi vesicle mediated transport, dolichol-linked oligosaccharide biosynthetic processes, phosphorylation-related signalling, apoptosis, cell junction function, molecular adaptor activity, cell cycle, transcription, antigen presentation and apical junction complex. These terms are consistent with the reported effects of *H. pylori* on cells and therefore support our supposition that the observed effects on glycosylation,

Table 1 Differentially expressed genes associated with *Helicobacter pylori* infection of E12 cells

Gene symbol	P-value	Fold change	Gene name
Top 15 upregulated			
¹ <i>CLIP1</i>	0.0025	5.42	CAP-GLY domain containing linker protein 1
<i>C10orf118</i>	0.0031	5.21	chromosome 10 open reading frame 118
<i>TOP1</i>	0.0352	5.09	topoisomerase (DNA) I
<i>LARS</i>	0.0054	5.02	leucyl-tRNA synthetase
<i>CDC27</i>	0.0073	4.95	cell division cycle 27 homolog (<i>S. cerevisiae</i>)
<i>STIP1</i>	0.0042	3.92	stress-induced-phosphoprotein 1
<i>MALAT1</i>	0.0077	3.82	metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)
<i>IL32</i>	0.0021	3.54	interleukin 32
<i>SLC39A6</i>	0.0204	3.33	solute carrier family 39 (zinc), member 6
<i>AHNAK2</i>	0.0070	3.25	AHNAK nucleoprotein 2
<i>HSP90AB1</i>	0.0014	3.24	heat shock protein 90 kDa alpha (cytosolic), B1
<i>ACTR2</i>	0.0035	3.20	ARP2 actin-related protein 2 homolog (yeast)
<i>EIF4G1</i>	0.0349	3.16	eukaryotic translation initiation factor 4 gamma, 1
<i>ACBD3</i>	0.0253	3.14	acyl-CoA binding domain containing 3
<i>CCND1</i>	0.0222	3.09	cyclin D1
Top 15 downregulated			
<i>PDK4</i>	0.0011	3.25	pyruvate dehydrogenase kinase, isozyme 4
<i>BCAT1</i>	0.0042	3.24	branched chain amino-acid transaminase 1, cytosolic
<i>PELI1</i>	0.0059	3.24	Pellino homolog 1 (<i>Drosophila</i>)
<i>CYP3A5</i>	0.0015	3.12	cytochrome P450, family 3, subfamily A, polypeptide 5
<i>HPGD</i>	0.0032	3.10	hydroxyprostaglandin dehydrogenase 15-(NAD)
<i>LRRC31</i>	0.0030	3.05	leucine rich repeat containing 31
<i>KIAA1984</i>	0.0082	2.97	KIAA1984
<i>HNRNPD</i>	0.0054	2.86	Heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)
<i>IL28A</i>	0.0026	2.85	interleukin 28A (interferon, lambda 2)
<i>DDX60</i>	0.0026	2.84	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60
<i>PHF14</i>	0.0021	2.82	PHD finger protein 14
<i>LOC100288092</i>	0.0025	2.82	Hypothetical protein LOC100288092
<i>ZNF207</i>	0.0027	2.82	zinc finger protein 207
<i>NCOA2</i>	0.0025	2.81	nuclear receptor coactivator 2
<i>AHSA2</i>	0.0064	2.72	AHA1, activator of heat shock 90 kDa protein ATPase homolog 2 (yeast)

¹qRT-PCR analysis showed *CLIP1* was downregulated.

the focus of this study, relate to infection by *H. pylori*.

Modulation of glycosylation-related genes in response to *H. pylori* infection

In order to study changes in the glycome of E12 cells upon *H. pylori* infection we examined those gene probes on the Affymetrix U133 Plus 2 Array that were associated with a glycosylation role, selected with reference to the Consortium for Functional Glycomics (CFGv3) array. We identified 170 significantly differentially expressed genes upon *H. pylori* infection that were glycosylation related (see Table 2 for the top 20 upregulated and downregulated genes; Supplementary Table 3 for all 170 genes). Only 6 (< 4 %) of these genes showed fold changes greater than 2-fold. The most upregulated gene from the microarray analysis was *LGALS7/7B*, (lectin, galactoside-binding, soluble, 7/7B, 2.3-fold) and the most downregulated gene was *REG4* (regenerating islet-derived family, member 4, 2.5-fold) followed by *OGT* (O-linked N-acetylglucosamine (GlcNAc) transferase, 2.2-fold) and *AMIGO2* (adhesion molecule with Ig-like domain 2, 2.2-fold). These small changes in glycosylation-related genes are not uncommon in significant processes that involve glycosylation^[7,43].

GO analysis of differentially regulated glycosylation-related genes was referenced against this Affymetrix array-derived glycome to determine which GO terms, relevant to glycosylation, were significant in the *H. pylori* infected E12 cells (Figure 1B). The most relevant terms were nucleotide sugar metabolism, N-glycan biosynthesis, cell death and galactose metabolic process, all of which generally related to upregulated genes, and hexose metabolic process, mannosidase activity and UDP-glycosyltransferase activity, which related to downregulated genes (Figure 1B).

Changes in terminal sugars of E12 glycoconjugates upon *H. pylori* infection

Microarray signal strength is not a robust measure of gene expression because probes bind with different affinities to the array. However, fold change measurements are robust because the same probe is used to compare two conditions. In this and the following sections the fold change differences between infected and uninfected gene levels are therefore quantitative. However, this data alone omits useful information about the expression level of one gene relative to another, always bearing in mind that two probes could have very different binding efficiencies for

Table 2 Glycosylation-related differentially expressed genes associated with *Helicobacter pylori* infection of E12 cells

Gene symbol	Fold change	P value	Gene name
Top 20 upregulated			
<i>LGALS7/7B</i>	2.31	0.0115	Lectin, galactoside-binding, soluble, 7 /7B
<i>UGGT1</i>	1.98	0.0059	UDP-glucose glycoprotein glucosyltransferase 1
<i>ST3GAL5</i>	1.90	0.0035	ST3 beta-galactoside alpha-2,3-sialyltransferase 5
<i>PARM1</i>	1.87	0.0026	Prostate androgen-regulated mucin-like protein 1
<i>NAGK</i>	1.79	0.0016	N-acetylglucosamine kinase
<i>VSIG1</i>	1.71	0.0240	V-set and immunoglobulin domain containing 1
<i>EDEM2</i>	1.69	0.0032	ER degradation enhancer, mannosidase alpha-like 2
<i>MPDU1</i>	1.69	0.0029	Mannose-P-dolichol utilization defect 1
<i>DPM2</i>	1.61	0.0015	Dolichyl-phosphate mannosyltransferase polypeptide 2, reg. s/u
<i>LMAN2</i>	1.60	0.0021	Lectin, mannose-binding 2
<i>GMPPB</i>	1.59	0.0019	GDP-mannose pyrophosphorylase B
<i>ALG3</i>	1.59	0.0214	Asn-linked glycosylation 3, alpha-1,3- mannosyltransferase hom.
<i>GALK2</i>	1.57	0.0027	Galactokinase 2
<i>DDOST</i>	1.53	0.0094	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase
<i>MCAM</i>	1.53	0.0111	Melanoma cell adhesion molecule
<i>B3GALT6</i>	1.53	0.0097	UDP-Gal:betaGal beta 1,3-galactosyltransferase polypeptide 6
<i>SLC35B1</i>	1.51	0.0025	Solute carrier family 35, member B1
<i>ST6GALNAC4</i>	1.50	0.0024	ST6 N-acetylglucosaminide alpha-2,6-sialyltransferase 4
<i>GMPPA</i>	1.49	0.0031	GDP-mannose pyrophosphorylase A
<i>ALDOC</i>	1.48	0.0020	Aldolase C, fructose-bisphosphate
Top 20 downregulated			
<i>REG4</i>	2.46	0.0018	Regenerating islet-derived family, member 4
<i>OGT</i>	2.25	0.0037	O-linked N-acetylglucosamine (GlcNAc) transferase (OGT)
<i>AMIGO2</i>	2.22	0.0039	Adhesion molecule with Ig-like domain 2
<i>GALNT12</i>	2.09	0.0033	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylglucosaminyltransferase 12
<i>EXT1</i>	1.99	0.0256	Exostosin 1
<i>LY75</i>	1.86	0.0075	Lymphocyte antigen 75 (CLEC13B)
<i>PIGZ</i>	1.82	0.0072	Phosphatidylinositol glycan anchor biosynthesis, class Z
<i>UGP2</i>	1.79	0.0320	UDP-glucose pyrophosphorylase 2
<i>MANEA</i>	1.79	0.0225	Mannosidase, endo-alpha
<i>ALG13</i>	1.78	0.0138	Asparagine-linked glycosylation 13 homolog (<i>S. cerevisiae</i>)
<i>GBAP1</i>	1.75	0.0035	Glucosidase, beta, acid pseudogene 1
<i>SLC35A3</i>	1.73	0.0071	Solute carrier family 35 (UDP-N-acetylglucosamine transporter), member A3
<i>ST3GAL4</i>	1.71	0.0032	ST3 beta-galactoside alpha-2,3-sialyltransferase 4
<i>B4GALNT3</i>	1.71	0.0129	Beta-1,4-N-acetyl-galactosaminyl transferase 3
<i>HMMR</i>	1.70	0.0039	Hyaluronan-mediated motility receptor (RHAMM)
<i>B4GALT6</i>	1.69	0.0134	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 6
<i>MAN2A1</i>	1.60	0.0034	Mannosidase, alpha, class 2A, member 1
<i>LGALS2</i>	1.58	0.0127	Lectin, galactoside-binding, soluble, 2
<i>MAN1A1</i>	1.53	0.0021	Mannosidase, alpha, class 1A, member 1
<i>MUC13</i>	1.53	0.0038	Mucin 13, cell surface associated
<i>CD164</i>	1.52	0.0015	CD164 molecule, sialomucin
<i>B4GALT5</i>	1.51	0.0071	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 5

the array. In Figure 2, where three selected groups of glycosylation related genes are presented, a gene has only been included if microarray analysis showed that it was either significantly differentially expressed or that it was represented on the array by more than one probe. In addition a signal strength cut-off was set to 20 to exclude weak genes which were less likely to be relevant (though technically significant in the original analysis). For sialylation (Figure 2A) *ST6GALNAC1*, *NEU1*, *SLC35A1*, *ST3GAL5* and *NANP* were significant but because they were each represented by only one probe their absolute expression levels relative to the other genes shown may be less representative. Where a gene was not significant but represented by multiple probes (e.g., *ST3GAL2*), the data was considered sufficiently reliable to warrant presenting expression levels relative to the other genes.

Terminal glycosylation of cell surface glycoconjugates directly determines the interaction of bacteria with the host cell. Therefore, sialylation and fucosylation were expected to be key processes affected by *H. pylori* infection. Terminal sialylation of glycoconjugates is a consequence of sialyltransferase expression and therefore can be predicted based on the transcript expression levels of the sialyltransferase enzymes from the microarray data. *ST3GAL4*, which can sialylate most glycoconjugate types and is believed to be involved in sialyl Lewis x and sialyl T antigen formation, was significantly downregulated (1.7-fold) whereas *ST3GAL5*, which is involved in ganglioside GM3 synthesis in glycolipids, was significantly upregulated (1.9-fold) on *H. pylori* infection (Figure 2A). *ST6GALNAC1* (down, 1.3-fold) and *ST6GALNAC4* (up, 1.8-fold) were also significantly differentially

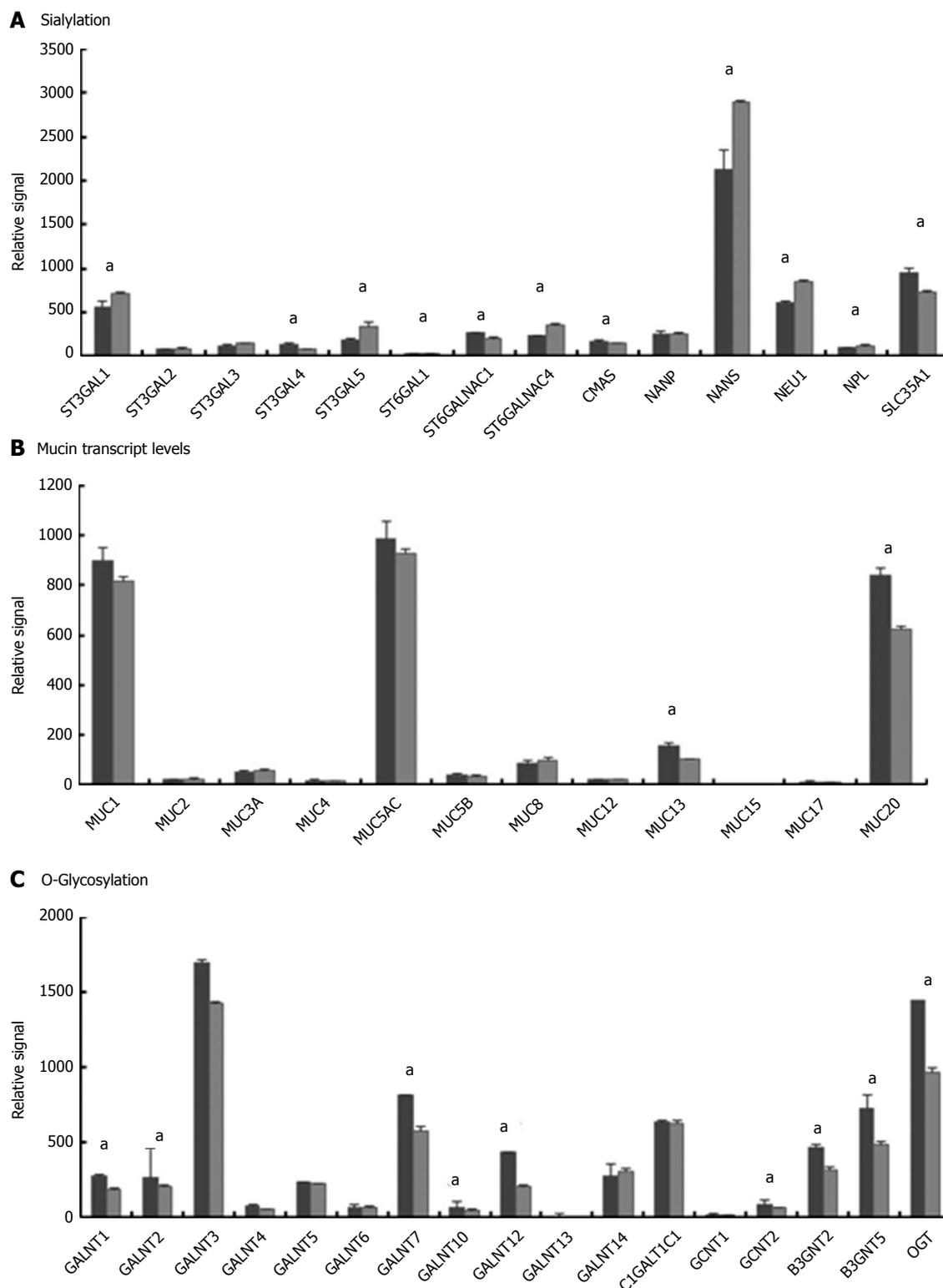


Figure 2 Transcript levels determined by microarray analysis of selected glycosylation related processes. A: Sialylation-related genes; B: Mucin genes; C: O-glycosylation genes. Raw signals reported for each gene were the strongest signals where multiple probes were available (except for MUC2 where the negligible signal from the single probe, indicating very weak expression, was confirmed by qRT-PCR). All signals for uninfected samples were raw values whereas all infected samples were extracted using fold change values of normalised data as detailed in Materials and Methods. Uninfected (dark grey) or Infected (light grey). Error bars show standard deviations ($n = 3$). ($^{\#}FDR < 0.05$).

expressed upon *H. pylori* infection. *ST6GALNAC4* is involved in the biosynthesis of ganglioside (GD1A from GM1B) and the transfer of NeuAc to GalNAc residues of O-glycans to form the stunted sialyl Tn antigen.

Based on signal intensities, *ST3GALS* 2-5 were all moderately expressed (raw control intensity < 500), *ST3GAL1* was highly expressed (> 500) and *ST3GAL6* was negligibly expressed (< 10). *ST6GALS* were lowly

Table 3 qRT-PCR significance of selected genes

Gene	Fold Change	Regulation	Significance
<i>GNE</i>	1.84	Down	1
<i>MUC20</i>	1.77	Down	1
<i>REG4</i>	3.06	Down	1
<i>MAN2A2</i>	1.76	Down	1
<i>MAN2B2</i>	1.91	Down	NS $P < 0.1$
<i>SLC35A2</i>	1.53	Down	NS $P < 0.1$
<i>RHOA</i>	1.35	Down	NS $P < 0.1$
¹ <i>TFF1</i>	10.37	Down	NS $P < 0.2$
<i>MAN2A1</i>	1.42	Down	NS $P < 0.2$
<i>ST3GAL5</i>	2.00	Down	NS $P < 0.2$
<i>MUC13</i>	3.66	Down	NS
<i>PDK4</i>	1.65	Down	NS
<i>GALNT10</i>	2.74	Down	NS
<i>GALNT7</i>	1.68	Down	NS
<i>MGAT2</i>	2.09	Down	NS
<i>MUC1</i>	1.66	Down	NS
<i>NEU1</i>	1.54	Up	NS
<i>CLIP1</i>	2.37	Down	NS
<i>LGALS2</i>	1.27	Up	NS
<i>OGT</i>	1.43	Down	NS
<i>AMIGO2</i>	1.14	-	NS
<i>ALG8</i>	1.15	-	NS
<i>GALK2</i>	1.14	-	NS
<i>SLC35A3</i>	1.23	Down	NS
<i>FPGT</i>	1.07	-	NS
<i>CGN</i>	1.26	Down	NS
<i>TJP3</i>	1.01	-	NS
<i>OCLN</i>	1.66	Down	NS
<i>EPHA2</i>	1.07	-	NS
<i>IL32</i>	1.00	-	NS
<i>IL8</i>	1.53	Up	NS

¹*TFF1* is not shown in Figure 3, and four further genes (*MGAT1*, *LGALS7*, *OSTC* and *LGALS7*) are only shown in Supplementary Figure 2.

(< 50) (*ST6GAL1*) to negligibly expressed (*ST6GAL2*) and all *ST8SIAs* (1-5) were also negligibly expressed. This is in line with the predominance of NeuAca2-3Gal linkages over NeuAca2-6Gal in HT29 cells^[44] and the predominance of O-glycans over N-glycans. The overall effect to be expected is therefore one of increased sialyl Tn antigen, decreased sialyl Lewis x/a determinants on glycoproteins with potential effects on glycolipids. This may result in shorter glycan chains and may affect packing density because some GALNTs require recognition of neighbouring GalNAc residues, now increasingly masked by sialylation, to initiate new glycan chains.

Although a number of fucosyltransferases (*FUT1*, *FUT3*, *FUT4*, *FUT5*, *FUT6* and *FUT11*) were moderately expressed, only *FUT1* was upregulated significantly (1.2-fold) upon *H. pylori* infection (data not shown). This would suggest no major role for fucosylation in the E12 response to *H. pylori* infection. *FUCA1* is a lysosomal fucose hydrolase. Its strong expression and upregulation on infection (1.2-fold) might suggest net removal of surface expressed fucose residues.

Changes in O-glycosylation upon *H. pylori* infection

As stated above, E12 cells produce a copious adherent

mucous layer of which the main components are O-glycans. The data presented here show expression of *MUC5AC*, *MUC1*, *MUC20* and *MUC13*. Only *MUC13* and *MUC20* were significantly downregulated (Figure 2B). *MUC5AC* was represented by two high signal probes on the array but neither showed any change upon *H. pylori* infection. (It is noted that *MUC5AC* appears as a significantly upregulated gene (1.2-fold) in Supplementary Table 3, however, this probe produced only a weak signal).

Mucin O-glycosylation initiates with the transfer of N-acetylgalactosamine (GalNAc) to a Ser/Thr residue on the protein catalysed by the polypeptide N-acetylgalactosamine transferases (GALNTs). Of 14 GALNTs represented on the array all 5 that were significant were downregulated upon *H. pylori* infection - *GALNT1* (1.5-fold), *GALNT2* (1.3-fold), *GALNT7* (1.4-fold), *GALNT10* (1.4-fold) and *GALNT12* (2.1-fold) (Figure 2C). *GALNT12* is believed to be involved in the initial glycosylation of *MUC5AC* and *MUC1* but not of some other mucins^[45]. It has different activities towards already glycosylated *MUC5AC* and *MUC1* peptides suggesting that its activity may determine packing density of multiple O-linked glycans and that specifically glycan density may be reduced on *H. pylori* infection where *GALNT12* appears downregulated. Sialylation of the polypeptide-linked GalNAc by *ST6GALNACs* may also change the recognition of initiation sites by specific polypeptide GalNAc transferases. Changes in O-glycan initiation have been reported previously in serum proteins of patients with gastric carcinoma^[46]. Differential expression of GalNAc transferases in gastric cancer cell lines and gastric carcinoma may be associated with occupancy of potential O-glycosylation sites in mucins^[47,48]. GalNAc transferase expression has been reported to be variable among gastric cell lines with GALNTs 1, 2 and 3 showing ubiquitous expression while other GalNAc transferases show more restricted expression^[49].

No genes coding enzymes that have the potential to produce core 1 to core 8 O-linked glycan structures were significantly regulated upon *H. pylori* infection. *C1GALT1* and *C1GALT1C1* (*COSMC*) were expressed at moderate and high levels respectively based on signal intensities suggesting the likely presence of core 1 structures on E12 O-glycans. Extension of the core structures by B4GALTs and B3GNTs seemed likely with moderate expression of several B4GALTs and B3GNTs. The galactose transferases and N-acetylglucosamine transferases that were significantly downregulated were those more often associated with N-glycan (*B4GALT5*, 1.5-fold; *B4GALT1*, 1.2-fold) or glycosphingolipid synthesis (*B4GALT5*, *B4GALT1* and the strongly expressed *B3GNT5*, 1.5-fold). We detected downregulation of *B3GNT5* and *B3GALT5* whereas a gastric biopsy study showed upregulation of the same genes^[50]. The downregulated *B3GNT2* (1.4-fold) is commonly involved in polyactosamine

synthesis so may be involved in N-glycans, O-glycans and/or glycosphingolipids. The other common form of non-mucin O-glycosylation in E12 cell glycoconjugates initiated by O-GlcNAc protein glycosyltransferase was significantly downregulated on *H. pylori* infection (*OGT*, 2.3-fold) according to microarray data (Supplementary Table 3).

Changes in N-glycosylation upon *H. pylori* infection

N-glycosylation can be split into the lipid-linked oligosaccharide (LLO) precursor synthesis process generating Glc₃Man₉GlcNAc₂ and the processes of trimming and extension. There was a general upregulation of the genes involved in the LLO step except for *ALG13* which instead showed downregulation. *ALG13* showed the largest fold change (1.8-fold, Table 2) of all the genes involved in LLO precursor synthesis. There was a general downregulation of the genes involved in trimming and extension. All mannosidases, most moderately expressed, were downregulated (Supplementary Table 3) and one, *MAN2A2*, was consequently confirmed by qPCR (below).

The significance of changes in N-glycosylation in an O-glycan-dominated environment is unclear. Also, whether the altered N-glycans are on mucins, other transmembrane glycoproteins, or both, cannot be determined from the available data. It has been suggested previously that LLO-precursor synthesis could be a constitutive process where precursors are available in excess and control is elsewhere^[51]. *ALG13* may, however, play a key role because it showed the largest fold change of all the genes in the precursor synthesis pathway, it was downregulated unlike other genes in the LLO-precursor synthesis pathway and interestingly it adds on the first GlcNAc of the LLO-precursor. Downregulated mannosidases (*MAN2A1*, 1.9-fold; *MAN1A1*, 1.5-fold; *MAN2B2*, 1.3-fold) are suggestive of an overall reduction of trimming and an increased level of the Man₉GlcNAc₂ glycan. Overall the effects suggest shorter and less complex N-glycan chains following *H. pylori* infection.

Changes in availability of activated sugars upon *H. pylori* infection

Glycan synthesis could be limited by changes in the abundance and availability of activated sugars. The UDP-galactose (*SLC35A2*, 1.3-fold; *SLC35B1*, 1.5-fold) and GDP-fucose (*SLC35C2*, 1.3-fold) transporters were significantly upregulated while the CMP-sialic acid (*SLC35A1*, 1.3-fold) and UDP-GlcNAc (*SLC35A3*, 2.1-fold) transporters were downregulated upon infection (Supplementary Table 3). The UDP-Gal, UDP-GlcNAc and CMP-sialic acid transporters were all strongly expressed while the GDP-fucose transporter was moderately expressed. N-Acetylneuraminic acid synthase (*NANS*) was very strongly expressed and significantly upregulated on infection (Figure 2A). This suggests the availability of Neu5Ac, however, the

downregulation of both *SLC35A1* and *CMAS* expression (though marginal) (Figure 2A) would possibly make a subsequent rise in activated Neu5Ac (CMP-Neu5Ac) levels seem less likely. Increased levels of sialidase (*NEU1*) may lead to cell shedding of sialylated epitopes with a compensatory switch on of Neu5Ac biosynthesis (Figure 2A). GO analysis had indicated the significance of hexose and, more specifically, galactose metabolism. All significant galactose metabolism enzymes were upregulated as were all significant mannose metabolism enzymes. All significant fucose metabolism enzymes were downregulated as were all sialic acid metabolic enzymes.

Modulation of lectin levels upon *H. pylori* infection

The two LMAN lectins *LMAN1* (1.5-fold down) and *LMAN2* (1.6-fold up) were expressed strongly and differentially (Supplementary Table 3). Both lectins are expressed in the endoplasmic reticulum and Golgi body and are believed to be involved in N-glycan sorting and recycling. Of the Gal binding lectins, *LGALS1* (1.4-fold) and *LGALS7* (2.3-fold) were both upregulated significantly, and *LGALS2* (1.6-fold) and *LGALS8* (1.4-fold) were both downregulated significantly, all being expressed at moderate levels (Supplementary Table 3). *LGALS7* is a myoepithelial cell marker, associated positively with cancer progression and metastasis, while *LGALS8* is involved in integrin-like cell interactions and is negatively associated with malignancy. The opposite regulation of *LGALS7* and *LGALS8*, therefore, is consistent with an altered cell phenotype. *LGALS3* and *LGALS4* were expressed very strongly (raw signal >5000) but not differentially upon infection according to microarray data. Where oligosaccharide chains are not terminated with sialic acid, the major exposed sugar is galactose. It might therefore be relevant that two Gal binding lectins (LGALs) were expressed very strongly (*LGALS3* and *LGALS4*) and four others were significantly regulated upon infection. Most other lectins (CLECs, collectins, selectins, siglecs) were either weakly or negligibly, but not differentially, expressed.

qRT-PCR validation of prediction changes in gene expression level

qRT-PCR validation was carried out on RNA from samples collected independently of the microarray samples and from several different infection experiments in an effort to identify variability between infections. qRT-PCR analysis was carried out on a selected set of genes. Initially two non-glycogenes (*PDK4*, *CLIP1*) were selected based on the maximum fold changes observed on the microarray. *PDK4* expression was confirmed as downregulated though with a smaller fold change. It was shown subsequently that *CLIP1* was not upregulated though initial microarray analysis had suggested upregulation. Only one (1558924_s_at) of six *CLIP1* probes on the array

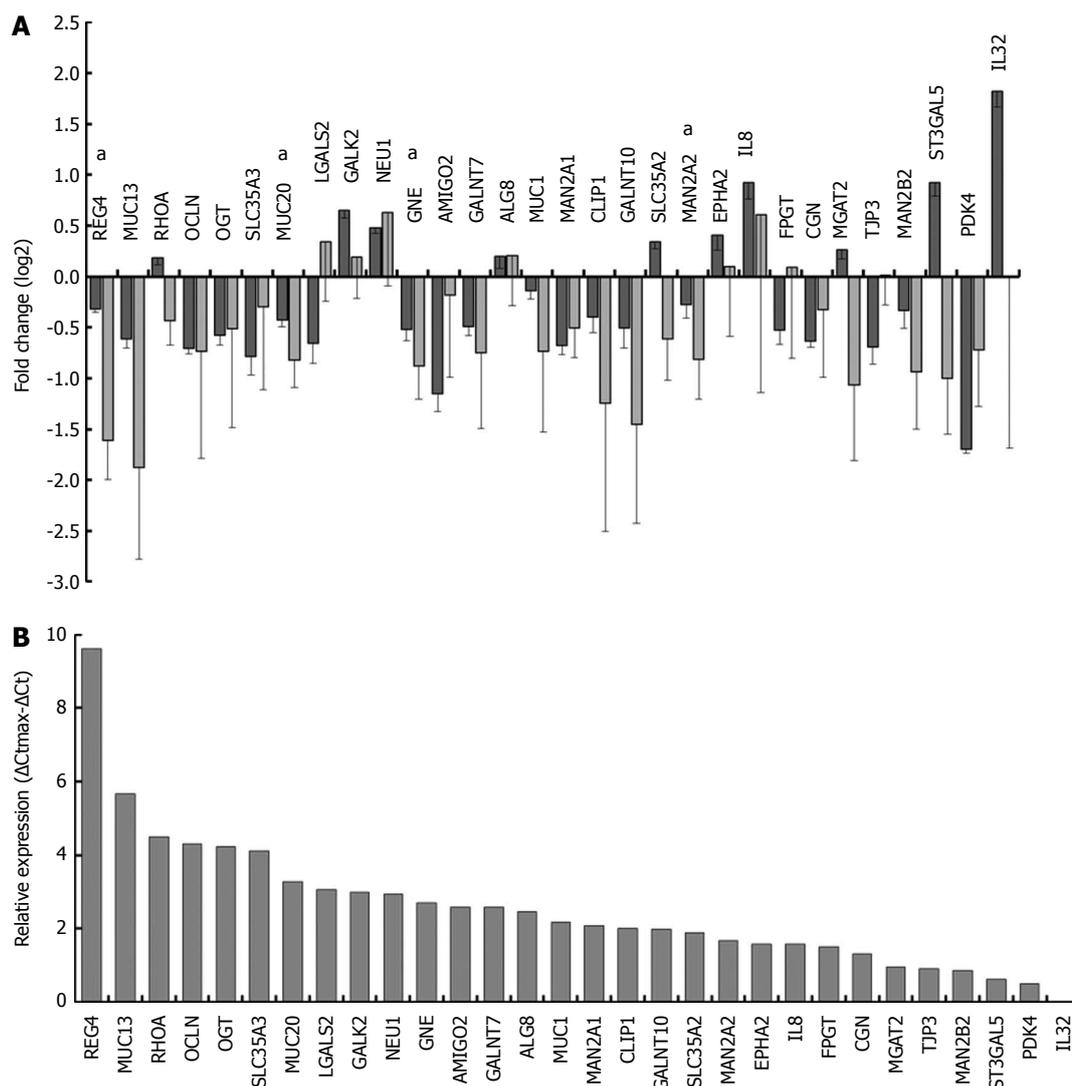


Figure 3 qRT-PCR validation of selected genes. Differential expression of selected genes upon *Helicobacter pylori* (*H. pylori*) infection of E12 cells was compared between microarray (dark grey) and qRT-PCR (light grey) analyses. qRT-PCR validation was carried out on RNAs from samples collected independently of the microarray samples. A: Microarray data ($n = 3$) was normalised across samples with GeneSpring. qRT-PCR data ($n = 3$) were normalised using geNorm and a panel of three housekeeping genes. Error bars show standard deviations determined using propagation of error rules; B: Relative expression of each gene in uninfected E12 cells was estimated by qRT-PCR using ΔC_t determinations. ΔC_{tmax} was the ΔC_t for the lowest expressing gene (IL32) after correcting Ct values across samples with the housekeeping gene GAPDH. Further details are given in Materials and Methods. All genes were significantly differentially expressed in microarray analysis and genes marked with asterisks (a) were confirmed significant by qRT-PCR.

suggested upregulation: a probe producing a much higher intensity signal (201975_at) instead suggested 1.3-fold significant downregulation and it is this probe that correlated with the qRT-PCR analysis (Figure 3). In general *H. pylori*-infected samples showed qRT-PCR-corroborated upregulation of *IL8* and downregulation of *REG4* (Figure 3A), though the upregulation of *IL8* (and *IL32*) was highly variable across samples (see Supplementary Figure 2) resulting in an absence of significance. The stomach mucin *MUC1* showed no significant change in expression by either microarray analysis or qRT-PCR. This was true also for *MUC13*, a gene found at low levels in normal gastric mucosa but often expressed in metaplasia^[52,53]. These two genes did however show consistent (not significant) downregulation in both microarray analysis and qRT-

PCR. A third mucin gene, *MUC20*, was shown to be significantly downregulated (1.4-fold). *MUC5AC* showed no significant expression change based on microarray data (Figure 2B) or in a preliminary screen by qRT-PCR (data not shown). qRT-PCR did not corroborate regulation of a number of genes where the expected fold changes by microarray analysis were less than 2 (*LGALS2*, *ST3GAL5*, *MGAT2*, *FPGT* and *SLC35A2*).

Four genes (*REG4*, *MUC20*, *GNE* and *MAN2A2*) showed significant differential regulation by qRT-PCR ($P < 0.05$), and others approached significance ($P < 0.1$ - *MAN2B2*, *SLC35A2*, *RHOA*; $P < 0.2$ - *TFF1*, *ST3GAL5* and *PDK4*) (Table 3). The absence of significance in some cases was partly due to the small fold changes detected both by microarray and qRT-PCR analyses,

but it was also due to variability in the samples used for qRT-PCR. In several cases one of the three uninfected samples behaved differently from the other two. This was very striking for the interleukins *IL8* and *IL32*, and for *TFF1* which showed high downregulation (10.4-fold) on infection, but was not significant because one uninfected sample (3 in Supplementary Figure 2) showed much lower levels than the other two uninfected samples (1 and 2, Supplementary Figure 2). Hierarchical clustering (Pearson correlation with average linkage clustering) clearly shows the inconsistency across the qRT-PCR samples (Supplementary Figure 2) and how inconsistent genes cluster with other inconsistent genes suggesting a biological rather than a technical reason for this variability. This anomalous behaviour in the uninfected samples had also been seen in the microarray analysis. A small cluster of seven genes including all of the MUC genes assayed and *GALNT10*, suggests some common regulatory feature. *REG4*, *TFF1* and *ST3GAL5* also formed a tight cluster (Supplementary Figure 2).

DISCUSSION

A recent study of gastric cell lines has shown a wide range of responses to *H. pylori*^[31]. Commonly used cell lines differ in their ability to polarise, to maintain a stomach-like epithelial morphology and differ in their pattern recognition receptors. *H. pylori* is, for the most part, a non-invasive bacterium that colonises the mucous layer surrounding the stomach and does not survive without this mucous layer. We have chosen for our study the HT29 derivative E12 cells because they produce an adherent mucous layer and the cells support *H. pylori* infection while the parent HT29 cells do not support *H. pylori* infection^[36]. The main mucin components of the stomach mucous layer *in vivo* are the secreted gel-forming mucin, MUC5AC, and the cell surface mucin, MUC1. Other mucins include MUC4, MUC12, MUC13 and MUC6^[54]. E12 cells have been shown by immunofluorescent methods to express high levels of MUC5AC and MUC1, and to a lesser extent MUC2^[35]. The transcriptomic data presented here show expression of *MUC5AC*, *MUC13*, *MUC1* and *MUC20* with little or no expression of either *MUC2* or *MUC6*. E12 cells have therefore a mucin profile approximating that of the stomach and provide a good model for *H. pylori* infection studies.

The cellular response to pathogenic organisms is orchestrated by Toll-like receptors (TLRs). Strong evidence suggests that *H. pylori* infection is TLR2-mediated^[55-57] yet there is some evidence that TLR4 is involved^[58,59]. E12 cells however produce the hallmark response of IL-8 production upon *H. pylori* infection despite being TLR2 negative. The cellular response will also be determined by the receptor/adhesin characteristics of the host-pathogen interaction. Some *H. pylori* strains can bind to cells through the

BabA adhesin which binds to the fucosylated Lewis b antigens on epithelial cell surface glycoconjugates^[60,61]. Strain 26695, used throughout this study, possess an active *cag* pathogenicity island (PAI) associated with virulence^[62] yet it has an inactive BabA adhesin^[36]. Furthermore, fucosylated structures are relatively scarce on mucins secreted by E12 cells^[36]. Therefore in this infection system the BabA adhesin interaction with Lewis antigens is not a factor in the IL-8 response to *H. pylori* and other *H. pylori* adhesin-host cell receptor combinations^[63] are likely to play a role in mediating these responses.

Expression patterns of *H. pylori*-infected and uninfected E12 cells indicated variability especially in the uninfected E12 cells. The reasons for this are unclear. It might suggest that some E12 cells did not fully differentiate to form a fully polarised cell layer. However, TEER measurements were consistently high (about 600 Ω /cm²) across all uninfected samples and could not differentiate E12 subpopulations. E12 cells, like the parent HT29 cells, are not a clonal population^[64,65]. It has been shown that E12 cells show the occasional MUC2-expressing cell and that these cells can be expanded under the appropriate conditions^[34]. It may therefore be that random samples contain differing proportions of cell types and so produce different expression profiles. It is likely that clonal cell lines would give more consistent results, however, we would argue that *in vivo* cells lining the stomach are not clonal, and therefore that non-clonal E12 cells may better reflect variation that is present *in vivo*.

Infection of the E12 cells was confirmed in this work by the presence of *cagA* RNA in exposed cells (data not shown). All significant expression changes on *H. pylori* infection were small with only 4%-5% being greater than 2-fold. This has been noted previously in similar experiments with *H. pylori* infection. The altered expression of glycosyltransferases and other glycosylation-related genes identified in this study is different in some specifics to that seen in related studies. This could be because E12 cells are derived from the colon where similar studies of *H. pylori* infection have used gastric cell lines^[7,28,29]. But variability in gene expression response is also seen across gastric cell lines possibly as a result of differences in mucin presentation. We would therefore argue that E12 cells, with their gastric-like mucin composition, constitute a valid model of *H. pylori* infection. Although the expression of specific genes did not correspond with that seen in similar studies, gene ontology terms were in general agreement suggesting that, in the broader functional picture, there are strong similarities across the different *H. pylori* infection studies. Variability in gene response could equally be due to the choice of *H. pylori* strain.

One consistently downregulated gene was *REG4*, a 17 kD secreted C-type lectin, expressed in selected

enteroendocrine cells throughout the GIT and some goblet cells^[66]. The functional role of REG4 is still unclear though it appears to be coexpressed with various enteroendocrine hormones. Although it is reportedly not expressed in normal stomach tissue, it is expressed, and has potential as a biomarker, in gastric carcinoma^[67]. qPCR confirmed microarray data that expression of the *REG4* transcript was very high. Why this should be in E12 cells is unclear. It could be a manifestation of the colonic origins of HT29/E12 cells, or of the selection process for generating the E12 derivative, or it may be related to the high mucin production of this cell line. Another member of the REG family, REG3 γ , has been shown to be intimately involved in the mucous layer protecting the underlying epithelial cells from bacterial colonisation^[68] but there is no evidence yet that REG4 has any anti-bacterial activity.

The overall shortening of O-glycans and the decreased complexity of both N- and O-glycans that this study suggests follow *H. pylori* infection may be driven by the infecting bacteria in their efforts to ease access through the mucus to the underlying enterocytes. It could equally be a host cell response to reduce potential bacterial adhesion sites or a way to facilitate shedding of the mucous layer and the attached bacteria. The results presented from the E12 model will require confirmation by detailed oligosaccharide analysis. Alternative approaches, such as lectin histochemistry or direct biophysical analysis of released glycans from glycoproteins and glycolipids, may not be able to detect the responses seen at the transcriptomic level because of overlapping specificities of lectins. However, it is of interest that the results presented are consistent with the Rhesus Macaque *in vivo* model of *H. pylori* infection^[6] and with the analysis of adherent mucins isolated from asymptomatic *H. pylori* infected patients^[8].

In conclusion, E12 cells are a promising model of the stomach epithelium; the cells form tight junctions, they polarise, they produce a thick adherent layer of mainly MUC5AC and have a mucin composition similar to that of the stomach. The glycosyl transferase gene expression profiles suggested core 1 and core 2 extended structures on O-glycans, with little or no core 3-8 structures, which is consistent with mass spectroscopy studies of isolated E12 glycoproteins. This study has shown that *H. pylori* infection increased expression of some sialyltransferases which may lead to shortened O-glycan chains. Furthermore, differential changes to various ppGalNAc transferase transcripts (mainly downregulation) suggested effects on O-glycan packing density and O-glycan site occupancy. The enzyme profiles would also suggest a change in N-glycan structures upon infection to more high-mannose and less complex oligosaccharides. Such

changes may not affect the overall glycocalyx since it is so O-glycan dominated but could affect specific N-glycoproteins. Many of the transferase enzyme transcripts that are regulated upon *H. pylori* infection have a noticeable preference for glycolipids which is consistent with the presence of Lewis b ligands on glycolipids rather than glycoproteins in E12 cells^[36]. While the overall changes to glycosyltransferase transcripts upon infection were small, small changes in enzyme activities that determine physicochemical properties of the mucus and the range of exposed epitopes could lead to profound effects on the phenotype of these infected cells.

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COMMENTS

Background

Key pathogen-host cell interactions are likely mediated through the glycan components of surface glycoproteins and glycolipids. *Helicobacter pylori* (*H. pylori*) is protected in the harsh environment of the stomach by the mucus layer of the gastric epithelium. Here we have used a HT29-derivative cell line that secretes a thick adherent mucus layer. This study explores changes in host cell gene transcription, specifically the genes that code mucins, glycosyltransferases, glycosyl hydrolases and lectins that may be associated with *H. pylori* infection.

Research frontiers

H. pylori infections are currently effectively cleared by antibiotics, however, the occurrence of antibiotic resistant strains is increasing and it is inevitable that new treatments will need to be developed. Understanding the key interactions between host and pathogen will drive the development of new drugs possibly analogues of surface sugars such as sialic acid and fucose.

Innovations and breakthroughs

Different strains of *H. pylori* display different levels of virulence. Some strains of *H. pylori* bind via BabA adhesins to Lewis antigens on the host cells and the host response is mediated through the TLR2 receptor. Here they use a common virulent strain (26695) that does not present one of the typical adhesins (BabA) commonly involved in *H. pylori* adhesion.

Applications

Changes in glycan structure on host cells in response to *H. pylori* infection will be changes initiated by the host to fight off infection and changes in the host induced by the infecting bacteria. By understanding which enzymes are involved in these host and pathogen modified glycan structure changes, it may be possible to design specific inhibitors of these enzymes.

Terminology

Transepithelial electrical resistance is a measure of the integrity of a cell layer and indicates that tight junctions have formed between cells.

Peer-review

This is a well written manuscript that attempts to identify genes significantly differentially expressed upon *H. pylori* infected in the HT29 derivative cell line by using microarray analysis and qRT-PCR analysis.

REFERENCES

- 1 **Blaser MJ**, Atherton JC. Helicobacter pylori persistence: biology and disease. *J Clin Invest* 2004; **113**: 321-333 [PMID: 14755326 DOI: 10.1172/jci200420925]
- 2 **Peek RM Jr**, Crabtree JE. Helicobacter infection and gastric neoplasia. *J Pathol* 2006; **208**: 233-248 [PMID: 16362989 DOI: 10.1002/path.1868]
- 3 **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 [PMID: 9207137]
- 4 **Ma JL**, Zhang L, Brown LM, Li JY, Shen L, Pan KF, Liu WD, Hu Y, Han ZX, Crystal-Mansour S, Pee D, Blot WJ, Fraumeni JF Jr, You WC, Gail MH. Fifteen-year effects of Helicobacter pylori, garlic, and vitamin treatments on gastric cancer incidence and mortality. *J Natl Cancer Inst* 2012; **104**: 488-492 [PMID: 22271764 DOI: 10.1093/jnci/djs003]
- 5 **Crabtree JE**, Peichl P, Wyatt JL, Stachl U, Lindley IJ. Gastric interleukin-8 and IgA IL-8 autoantibodies in Helicobacter pylori infection. *Scand J Immunol* 1993; **37**: 65-70 [PMID: 8418474 DOI: 10.1111/j.1365-3083.1993.tb01666.x]
- 6 **Cooke CL**, An HJ, Kim J, Canfield DR, Torres J, Lebrilla CB, Solnick JV. Modification of gastric mucin oligosaccharide expression in rhesus macaques after infection with Helicobacter pylori. *Gastroenterology* 2009; **137**: 1061-1071, 1071.e1-1071.e8 [PMID: 19375420 DOI: 10.1053/j.gastro.2009.04.014]
- 7 **Marcos NT**, Magalhães A, Ferreira B, Oliveira MJ, Carvalho AS, Mendes N, Gilmartin T, Head SR, Figueiredo C, David L, Santos-Silva F, Reis CA. Helicobacter pylori induces beta3GnT5 in human gastric cell lines, modulating expression of the SabA ligand sialyl-Lewis x. *J Clin Invest* 2008; **118**: 2325-2336 [PMID: 18483624 DOI: 10.1172/jci134324]
- 8 **Curt MJC**, Lecointe K, Mihalache A, Rossez Y, Gosset P, Leonard R, Robbe-Masselot C. Alteration or adaptation, the two roads for human gastric mucin glycosylation infected by Helicobacter pylori. *Glycobiology* 2015; **25**: 617-631 [DOI: 10.1093/glycob/cwv004]
- 9 **vanKlinken BJW**, Dekker J, Buller HA, DeBolos C, Einerhand AWC. Biosynthesis of mucins (MUC2-6) along the longitudinal axis of the human gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol* 1997; **273**: G296-G302
- 10 **Wang Y**, Cheon DJ, Lu Z, Cunningham SL, Chen CM, Luo RZ, Xing D, Orsulic S, Bast RC Jr, Behringer RR. MUC16 expression during embryogenesis, in adult tissues, and ovarian cancer in the mouse. *Differentiation* 2008; **76**: 1081-1092 [PMID: 18637025 DOI: 10.1111/j.1432-0436.2008.00295.x]
- 11 **McGuckin MA**, Lindén SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. *Nat Rev Microbiol* 2011; **9**: 265-278 [PMID: 21407243 DOI: 10.1038/nrmicro2538]
- 12 **Van de Bovenkamp JH**, Mahdavi J, Korteland-Van Male AM, Büller HA, Einerhand AW, Roerén T, Dekker J. The MUC5AC glycoprotein is the primary receptor for Helicobacter pylori in the human stomach. *Helicobacter* 2003; **8**: 521-532 [PMID: 14535999 DOI: 10.1046/j.1523-5378.2003.00173.x]
- 13 **Van den Brink GR**, Tytgat KM, Van der Hulst RW, Van der Loos CM, Einerhand AW, Büller HA, Dekker J. H pylori colocalises with MUC5AC in the human stomach. *Gut* 2000; **46**: 601-607 [PMID: 10764701 DOI: 10.1136/gut.46.5.601]
- 14 **Rossez Y**, Gosset P, Boneca IG, Magalhães A, Ecobichon C, Reis CA, Cieniewski-Bernard C, Joncquel Chevalier Curt M, Léonard R, Maes E, Sperandio B, Slomianny C, Sansonetti PJ, Michalski JC, Robbe-Masselot C. The laeDINAc-specific adhesin LabA mediates adhesion of Helicobacter pylori to human gastric mucosa. *J Infect Dis* 2014; **210**: 1286-1295 [PMID: 24755437 DOI: 10.1093/infdis/jiu239]
- 15 **Petersen AM**, Krogfelt KA. Helicobacter pylori: an invading microorganism? A review. *FEMS Immunol Med Microbiol* 2003; **36**: 117-126 [PMID: 12738380 DOI: 10.1016/s0928-8244(03)00020-8]
- 16 **Odenbreit S**, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Science* 2000; **287**: 1497-1500 [PMID: 10688800 DOI: 10.1126/science.287.5457.1497]
- 17 **Backert S**, Clyne M, Tegtmeier N. Molecular mechanisms of gastric epithelial cell adhesion and injection of CagA by Helicobacter pylori. *Cell Commun Signal* 2011; **9**: 28 [PMID: 22044679 DOI: 10.1186/1478-811X-9-28]
- 18 **Comelli EM**, Lariani S, Zwahlen MC, Fotopoulos G, Holzwarth JA, Cherbut C, Dorta G, Corthésy-Theulaz I, Grigorov M. Biomarkers of human gastrointestinal tract regions. *Mamm Genome* 2009; **20**: 516-527 [PMID: 19711126 DOI: 10.1007/s00335-009-9212-7]
- 19 **Galamb O**, Györfy B, Sipos F, Dinya E, Krenács T, Bercezi L, Szöke D, Spisák S, Solymosi N, Németh AM, Juhász M, Molnár B, Tulassay Z. Helicobacter pylori and antrum erosion-specific gene expression patterns: the discriminative role of CXCL13 and VCAM1 transcripts. *Helicobacter* 2008; **13**: 112-126 [PMID: 18321301 DOI: 10.1111/j.1523-5378.2008.00584.x]
- 20 **Galamb O**, Sipos F, Györfy B, Molnár B, Tulassay Z. Identification of helicobacter pylori and antrum erosion-specific gene expression patterns in gastric biopsy samples by whole genomic microarray analysis. *Helicobacter* 2005; **10**: 465-466
- 21 **Galamb O**, Sipos F, Molnár B, Szöke D, Spisák S, Tulassay Z. Evaluation of malignant and benign gastric biopsy specimens by mRNA expression profile and multivariate statistical methods. *Cytometry B Clin Cytom* 2007; **72**: 299-309 [PMID: 17366642 DOI: 10.1002/cyto.b.20189]
- 22 **Galamb O**, Sipos F, Molnár B, Tulassay Z. Diagnosis of malignancy and benign alterations in gastric biopsy specimen by mRNA expression profiling and multivariate statistical methods. *Gastroenterology* 2004; **126**: A408-A408
- 23 **Hornsby MJ**, Huff JL, Kays RJ, Canfield DR, Bevins CL, Solnick JV. Helicobacter pylori induces an antimicrobial response in rhesus macaques in a cag pathogenicity island-dependent manner. *Gastroenterology* 2008; **134**: 1049-1057 [PMID: 18395086 DOI: 10.1053/j.gastro.2008.01.018]
- 24 **Resnick MB**, Sabo E, Meitner PA, Kim SS, Cho Y, Kim HK, Tavares R, Moss SF. Global analysis of the human gastric epithelial transcriptome altered by Helicobacter pylori eradication in vivo. *Gut* 2006; **55**: 1717-1724 [PMID: 16641130 DOI: 10.1136/gut.2006.095646]
- 25 **Tsai CJ**, Herrera-Goepfert R, Tibshirani RJ, Yang SF, Mohar A, Guarner J, Parsonnet J. Changes of gene expression in gastric preneoplasia following Helicobacter pylori eradication therapy. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 272-280 [DOI: 10.1158/1055-9965.EPI-05-0632]
- 26 **Toyoda T**, Tsukamoto T, Yamamoto M, Ban H, Saito N, Takasu S, Shi L, Saito A, Ito S, Yamamura Y, Nishikawa A, Ogawa K, Tanaka T, Tatematsu M. Gene expression analysis of a Helicobacter pylori-infected and high-salt diet-treated mouse gastric tumor model: identification of CD177 as a novel prognostic factor in patients with gastric cancer. *BMC Gastroenterol* 2013; **13**: 122 [PMID: 23899160 DOI: 10.1186/1471-230X-13-122]
- 27 **Ikeno T**, Ota H, Sugiyama A, Ishida K, Katsuyama T, Genta RM, Kawasaki S. Helicobacter pylori-induced chronic active gastritis, intestinal metaplasia, and gastric ulcer in Mongolian gerbils. *Am J Pathol* 1999; **154**: 951-960 [PMID: 10079274 DOI: 10.1016/s0002-9440(10)65343-6]
- 28 **El-Etr SH**, Mueller A, Tompkins LS, Falkow S, Merrell DS. Phosphorylation-independent effects of CagA during interaction between Helicobacter pylori and T84 polarized monolayers. *J Infect Dis* 2004; **190**: 1516-1523 [PMID: 15378446 DOI: 10.1086/424526]
- 29 **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**: 1385-1396 [PMID: 20238406 DOI: 10.3748/wjg.v16.i11.1385]
- 30 **Eftang LL**, Esbensen Y, Tannæs TM, Bukholm IR, Bukholm G. Interleukin-8 is the single most up-regulated gene in whole genome profiling of H. pylori exposed gastric epithelial cells. *BMC Microbiol* 2012; **12**: 9 [PMID: 22248188 DOI: 10.1186/1471-2180-12-9]
- 31 **Schneider S**, Carra G, Sahin U, Hoy B, Rieder G, Wessler S.

- Complex Cellular Responses of Helicobacter pylori-Colonized Gastric Adenocarcinoma Cells. *Infect Immun* 2011; **79**: 2362-2371 [DOI: 10.1128/iai.01350-10]
- 32 **Behrens I**, Stenberg P, Artursson P, Kissel T. Transport of lipophilic drug molecules in a new mucus-secreting cell culture model based on HT29-MTX cells. *Pharm Res* 2001; **18**: 1138-1145 [PMID: 11587485 DOI: 10.1023/A:1010974909998]
- 33 **Alemka A**, Clyne M, Shanahan F, Tompkins T, Corcionivoschi N, Bourke B. Probiotic colonization of the adherent mucus layer of HT29MTXE12 cells attenuates Campylobacter jejuni virulence properties. *Infect Immun* 2010; **78**: 2812-2822 [PMID: 20308300 DOI: 10.1128/IAI.01249-09]
- 34 **Navabi N**, McGuckin MA, Lindén SK. Gastrointestinal cell lines form polarized epithelia with an adherent mucus layer when cultured in semi-wet interfaces with mechanical stimulation. *PLoS One* 2013; **8**: e68761 [PMID: 23869232 DOI: 10.1371/journal.pone.0068761]
- 35 **Dolan B**, Naughton J, Tegtmeyer N, May FE, Clyne M. The interaction of Helicobacter pylori with the adherent mucus gel layer secreted by polarized HT29-MTX-E12 cells. *PLoS One* 2012; **7**: e47300 [PMID: 23056622 DOI: 10.1371/journal.pone.0047300]
- 36 **Naughton JA**, Mariño K, Dolan B, Reid C, Gough R, Gallagher ME, Kilcoyne M, Gerlach JQ, Joshi L, Rudd P, Carrington S, Bourke B, Clyne M. Divergent mechanisms of interaction of Helicobacter pylori and Campylobacter jejuni with mucus and mucins. *Infect Immun* 2013; **81**: 2838-2850 [PMID: 23716616 DOI: 10.1128/IAI.00415-13]
- 37 **van Amsterdam K**, van der Ende A. Nutrients released by gastric epithelial cells enhance Helicobacter pylori growth. *Helicobacter* 2004; **9**: 614-621 [PMID: 15610074 DOI: 10.1111/j.1083-4389.2004.00272.x]
- 38 **Cottet S**, Corthésy-Theulaz I, Spertini F, Corthésy B. Microaerophilic conditions permit to mimic in vitro events occurring during in vivo Helicobacter pylori infection and to identify Rho/Ras-associated proteins in cellular signaling. *J Biol Chem* 2002; **277**: 33978-33986 [PMID: 12058029 DOI: 10.1074/jbc.M201726200]
- 39 **Smyth GK**. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 2004; **3**: Article3 [PMID: 16646809 DOI: 10.2202/1544-6115.1027]
- 40 **Edgar R**, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* 2002; **30**: 207-210 [PMID: 11752295 DOI: 10.1093/nar/30.1.207]
- 41 **Dennis G Jr**, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. *Genome Biol* 2003; **4**: P3 [PMID: 12734009 DOI: 10.1186/gb-2003-4-9-r60]
- 42 **Vandesompele J**, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002; **3**: RESEARCH0034 [PMID: 12184808]
- 43 **Gaudier E**, Forestier L, Gouyer V, Huet G, Julien R, Hoebler C. Butyrate regulation of glycosylation-related gene expression: evidence for galectin-1 upregulation in human intestinal epithelial goblet cells. *Biochem Biophys Res Commun* 2004; **325**: 1044-1051 [PMID: 15541394 DOI: 10.1016/j.bbrc.2004.10.141]
- 44 **Hennebicq-Reig S**, Lesuffleur T, Capon C, De Bolos C, Kim I, Moreau O, Richet C, Hénon B, Recchi MA, Maës E, Aubert JP, Real FX, Zweibaum A, Delannoy P, Degand P, Huet G. Permanent exposure of mucin-secreting HT-29 cells to benzyl-N-acetyl-alpha-D-galactosaminide induces abnormal O-glycosylation of mucins and inhibits constitutive and stimulated MUC5AC secretion. *Biochem J* 1998; **334** (Pt 1): 283-295 [PMID: 9693131]
- 45 **Guo JM**, Zhang Y, Cheng L, Iwasaki H, Wang H, Kubota T, Tachibana K, Narimatsu H. Molecular cloning and characterization of a novel member of the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase family, pp-GalNAc-T12. *FEBS Lett* 2002; **524**: 211-218 [PMID: 12135769 DOI: 10.1016/s0014-5793(02)03007-7]
- 46 **Gomes C**, Almeida A, Ferreira JA, Silva L, Santos-Sousa H, Pinto-de-Sousa J, Santos LL, Amado F, Schwientek T, Lavery SB, Mandel U, Clausen H, David L, Reis CA, Osório H. Glycoproteomic analysis of serum from patients with gastric precancerous lesions. *J Proteome Res* 2013; **12**: 1454-1466 [PMID: 23312025 DOI: 10.1021/pr301112x]
- 47 **Campos D**, Freitas D, Gomes J, Magalhães A, Steentoft C, Gomes C, Vester-Christensen MB, Ferreira JA, Afonso LP, Santos LL, Pinto de Sousa J, Mandel U, Clausen H, Vakhrushev SY, Reis CA. Probing the O-glycoproteome of gastric cancer cell lines for biomarker discovery. *Mol Cell Proteomics* 2015; **14**: 1616-1629 [PMID: 25813380 DOI: 10.1074/mcp.M114.046862]
- 48 **Gomes J**, Marcos NT, Berois N, Osinaga E, Magalhães A, Pinto-de-Sousa J, Almeida R, Gärtner F, Reis CA. Expression of UDP-N-acetyl-D-galactosamine: Polypeptide N-acetylgalactosaminyltransferase-6 in gastric mucosa, intestinal metaplasia, and gastric carcinoma. *J Histochem Cytochem* 2009; **57**: 79-86 [DOI: 10.1369/jhc.2008.952283]
- 49 **Marcos NT**, Cruz A, Silva F, Almeida R, David L, Mandel U, Clausen H, Von Mensdorff-Pouilly S, Reis CA. Polypeptide GalNAc-transferases, ST6GalNAc-transferase I, and ST3Gal-transferase I expression in gastric carcinoma cell lines. *J Histochem Cytochem* 2003; **51**: 761-771 [PMID: 12754287 DOI: 10.1177/00215540305100607]
- 50 **Magalhaes A**, Marcos-Pinto R, Nairn AV, dela Rosa M, Ferreira RM, Junqueira-Neto S, Freitas D, Gomes J, Oliveira P, Santos MR, Marcos NT, Wen XG, Figueiredo C, Oliveira C, Dinis-Ribeiro M, Carneiro F, Moremen KW, David L, Reis CA. Helicobacter pylori chronic infection and mucosal inflammation switches the human gastric glycosylation pathways. *Biochimica Et Biophys Acta-Molecular Basis Dis* 2015; **1852**: 1928-1939 [DOI: 10.1016/j.bbdis.2015.07.001]
- 51 **Nairn AV**, Aoki K, dela Rosa M, Porterfield M, Lim JM, Kulik M, Pierce JM, Wells L, Dalton S, Tiemeyer M, Moremen KW. Regulation of glycan structures in murine embryonic stem cells: combined transcript profiling of glycan-related genes and glycan structural analysis. *J Biol Chem* 2012; **287**: 37835-37856 [PMID: 22988249 DOI: 10.1074/jbc.M112.405233]
- 52 **Williams SJ**, Wreschner DH, Tran M, Eyre HJ, Sutherland GR, McGuckin MA. Muc13, a novel human cell surface mucin expressed by epithelial and hemopoietic cells. *J Biol Chem* 2001; **276**: 18327-18336 [PMID: 11278439 DOI: 10.1074/jbc.M008850200]
- 53 **Shimamura T**, Ito H, Shibahara J, Watanabe A, Hippo Y, Taniguchi H, Chen Y, Kashima T, Ohtomo T, Tanioka F, Iwanari H, Kodama T, Kazui T, Sugimura H, Fukayama M, Aburatani H. Overexpression of MUC13 is associated with intestinal-type gastric cancer. *Cancer Sci* 2005; **96**: 265-273 [PMID: 15904467 DOI: 10.1111/j.1349-7006.2005.00043.x]
- 54 **Linden SK**, Sutton P, Karlsson NG, Korolik V, McGuckin MA. Mucins in the mucosal barrier to infection. *Mucosal Immunol* 2008; **1**: 183-197 [PMID: 19079178 DOI: 10.1038/mi.2008.5]
- 55 **Smith SM**, Moran AP, Duggan SP, Ahmed SE, Mohamed AS, Windle HJ, O'Neill LA, Kelleher DP. Tribbles 3: a novel regulator of TLR2-mediated signaling in response to Helicobacter pylori lipopolysaccharide. *J Immunol* 2011; **186**: 2462-2471 [PMID: 21220698 DOI: 10.4049/jimmunol.1000864]
- 56 **Mandell L**, Moran AP, Cocchiarella A, Houghton J, Taylor N, Fox JG, Wang TC, Kurt-Jones EA. Intact gram-negative Helicobacter pylori, Helicobacter felis, and Helicobacter hepaticus bacteria activate innate immunity via toll-like receptor 2 but not toll-like receptor 4. *Infect Immun* 2004; **72**: 6446-6454 [PMID: 15501775 DOI: 10.1128/IAI.72.11.6446-6454.2004]
- 57 **Yokota S**, Ohnishi T, Muroi M, Tanamoto K, Fujii N, Amano K. Highly-purified Helicobacter pylori LPS preparations induce weak inflammatory reactions and utilize Toll-like receptor 2 complex but not Toll-like receptor 4 complex. *FEMS Immunol Med Microbiol* 2007; **51**: 140-148 [PMID: 17645528 DOI: 10.1111/j.1574-

- 695X.2007.00288.x]
- 58 **Uno K**, Kato K, Atsumi T, Suzuki T, Yoshitake J, Morita H, Ohara S, Kotake Y, Shimosegawa T, Yoshimura T. Toll-like receptor (TLR) 2 induced through TLR4 signaling initiated by *Helicobacter pylori* cooperatively amplifies iNOS induction in gastric epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G1004-G1012 [PMID: 17855767 DOI: 10.1152/ajpgi.00096.2007]
- 59 **Ishihara S**, Rumi MA, Kadowaki Y, Ortega-Cava CF, Yuki T, Yoshino N, Miyaoka Y, Kazumori H, Ishimura N, Amano Y, Kinoshita Y. Essential role of MD-2 in TLR4-dependent signaling during *Helicobacter pylori*-associated gastritis. *J Immunol* 2004; **173**: 1406-1416 [PMID: 15240737]
- 60 **Borén T**, Falk P, Roth KA, Larson G, Normark S. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. *Science* 1993; **262**: 1892-1895 [PMID: 8018146 DOI: 10.1126/science.8018146]
- 61 **Iiver D**, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Borén T. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* 1998; **279**: 373-377 [PMID: 9430586 DOI: 10.1126/science.279.5349.373]
- 62 **Odenbreit S**, Swoboda K, Barwig I, Ruhl S, Borén T, Koletzko S, Haas R. Outer membrane protein expression profile in *Helicobacter pylori* clinical isolates. *Infect Immun* 2009; **77**: 3782-3790 [PMID: 19546190 DOI: 10.1128/IAI.00364-09]
- 63 **Pachathundikandi SK**, Tegtmeyer N, Backert S. Signal transduction of *Helicobacter pylori* during interaction with host cell protein receptors of epithelial and immune cells. *Gut Microbes* 2013; **4**: 454-474 [DOI: 10.4161/gmic.27001]
- 64 **Pinto M**, Appay MD, Simonassmann P, Chevalier G, Dracopoli N, Fogh J, Zweibaum A. Enterocytic differentiation of cultured human-colon cancer-cells by replacement of glucose by galactose in the medium. *Biol Cell* 1982; **44**: 193-196
- 65 **Huet C**, Sahuquillo-Merino C, Coudrier E, Louvard D. Absorptive and mucus-secreting subclones isolated from a multipotent intestinal cell line (HT-29) provide new models for cell polarity and terminal differentiation. *J Cell Biol* 1987; **105**: 345-357 [PMID: 3611191 DOI: 10.1083/jcb.105.1.345]
- 66 **Heiskala K**, Andersson LC. Reg IV is differently expressed in enteroendocrine cells of human small intestine and colon. *Regul Pept* 2013; **183**: 27-34 [PMID: 23499801 DOI: 10.1016/j.regpep.2013.03.007]
- 67 **Mitani Y**, Oue N, Matsumura S, Yoshida K, Noguchi T, Ito M, Tanaka S, Kuniyasu H, Kamata N, Yasui W. Reg IV is a serum biomarker for gastric cancer patients and predicts response to 5-fluorouracil-based chemotherapy. *Oncogene* 2007; **26**: 4383-4393 [PMID: 17237819 DOI: 10.1038/sj.onc.1210215]
- 68 **Vaishnava S**, Yamamoto M, Severson KM, Ruhn KA, Yu X, Koren O, Ley R, Wakeland EK, Hooper LV. The antibacterial lectin RegIII γ promotes the spatial segregation of microbiota and host in the intestine. *Science* 2011; **334**: 255-258 [PMID: 21998396 DOI: 10.1126/science.1209791]

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