Pathol. Oncol. Res. (2017) 23:173–179
DOI 10.1007/s12253-016-0122-1

ORIGINAL ARTICLE

Fucosyltransferase-4 and Oligosaccharide Lewis Y Antigen as potentially Correlative Biomarkers of *Helicobacter pylori* CagA Associated Gastric Cancer

Faisal Aziz[1] · **Wei Gao**[1] · **Qiu Yan**[1]

Received: 30 November 2015 / Accepted: 4 October 2016 / Published online: 18 October 2016

Abstract *H. pylori* cytotoxin associated antigen A (CagA) plays a significant role in the progression of gastric cancer but their effect on fucosylation to develop gastric cancer is unknown. Fucosyltransferase IV (FUT4) is the key enzyme for synthesis of LewisY (LeY) carried by glycoproteins and glycolipids on the cell membrane. Herein, we compare the expression of CagA, p-EGFR, FUT4 and LeY in gastritis (n = 128, 176), gastric ulcer (n = 174, 213), and gastric cancer (n = 323, 261) tissue and serum samples, respectively by IHC and ELISA. Moreover, we investigated the potential correlation of CagA with FUT4 and LeY overexpression through EGFR activation. IHC and ELISA results showed higher positive cases of *H. pylori* CagA (83, 86 %), p-EGFR (81, 72 %), FUT4 (91, 97 %) and LeY (93, 92 %) in gastric cancer, compared to gastritis and gastric ulcer, *H. pylori* CagA (58, 67 & 59, 73 %), p-EGFR (52, 63 & 35, 47 %), FUT4 (68, 78 & 67, 82 %) and LeY (62,76 & 65, 85 %), respectively. We found a significant high expression (H-Value) of CagA (1.79, 1.66), p-EGFR (1.53, 1.58), FUT4 (2.14, 1.66) and LeY (1.69, 1.61) in gastric cancer tissues and serum, respectively as compared to chronic gastritis and gastric ulcers, CagA (0.64,1.14), p-EGFR (0.856, 0.678), FUT4 (0.949,1.197) and LeY (0.68,1.008) ($P < 0.0001$), respectively. Furthermore, *H. pylori* CagA showed significant correlation with p-EGFR (R–0.62, −0.74), FUT4 (R–0.81, −0.76) and LeY (R–0.82, −0.70) in gastric tissues and serum ($P < 0.0001$). *H. pylori* CagA plays key role in the development of gastric cancer with overexpression of FUT4/LeY, serve as potentially correlative biomarkers of *H. pylori* CagA associated gastric cancer.

Keywords Correlative biomarkers · Fucosyltransferase IV · Gastritis · Gastric ulcer · Gastric cancer · *H. pylori* CagA · Lewis Y · P-EGFR

Abbreviations

H. pylori	*Helicobacter pylori*
CagA	Cytotoxin associated antigen A
CAG	Chronic atrophic gastritis
EGFR	Epidermal growth factor receptor
FUTs	Fucosyltransferases
GED	Gastric epithelial dysplasia
GC	Gastric cancer
GIs	Chronic gastritis
GU	Gastric ulcer
IHC	Immunohistochemistry
IM	Intestinal metaplasia
LeY	Lewis Y
p-EGFR	Phosphorylated epidermal growth factor receptor

Introduction

Helicobacter pylori is a causative agent of gastritis and gastric ulcer diseases, which leads to the development of gastric cancer. Hence, it is classified as class I carcinogen by the World Health Organization [1, 2]. More than 50 % of the world population is colonized by *H. pylori*, but few of them suffer from active disease because of different factors such as age, gender, crowding, hyperacidity, smoking habit and poor socioeconomic status. Developing

✉ Qiu Yan
yanqiu63@126.com

[1] Department of Biochemistry and Molecular Biology, Liaoning Provincial Core Laboratory of Glycobiology and Glycoengineering, Dalian Medical University, Dalian, People's Republic of China

countries had a prevalence rate of (80–90 %) as compared to developed countries (20–50 %) [3].

H. pylori carries several virulence factors, such as urease, flagella, vacuolating cytotoxin A (VacA), cytotoxin associated gene A (CagA), and sialic acid–binding adhesin (SabA) proteins that play an important role in invasion, colonization, and cell proliferation [4, 5]. CagA is one of the most important virulence factor produced by *H. pylori* [6] that enters the host cell through the type IV secretion system, gets phosphorylated and interrupts various cellular cascades, resulting in epithelial damage [7, 8]. It is identified as the first bacterial oncoprotein, which associated with gastric cancer by inducing abnormal cell proliferation, inflammation, apoptosis, metastasis and poor prognosis [9–11].

Glycosylation is essential in many of the physiological and pathological processes [12, 13]. Bacterial infections induce alterations in the host cancer cells glycosylation [14]. Fucosylation is one of the most frequently reported types of glycosylation in carcinogenesis. It is a process of fucose transferred to the precursor oligosaccharide by the catalyzation of fucosyltransferases (FUTs) [15, 16]. High expression of FUT4 promotes cell proliferation through augmenting the synthesis of LeY. LeY is difucosylated oligosaccharide that belongs to A, B, H and Lewis blood group family with the chemical structure [Fucα1 → 2Galβ1 → 4(Fucα1 → 3) GlcNAcβ1 → R]. The α1, 3- fucosylation of LeY is catalyzed by FUT4 [17]. LeY is type 2 blood group Lewis antigens, which expressed at high level in the pathological and physiological process such as bacterial infection, tumor invasion, fertilization, embryo implantation, inflammation and cancer metastasis [18–21].

H. pylori CagA plays a significant role in the progression of gastric cancer but their effect on fucosylation to develop gastric cancer is still unknown. The effect of CagA on fucosylation with potential role of LeY antigen through epidermal growth factor receptor (EGFR) activation has not been studied. In this study, we examined the potential correlation of CagA, p-EGFR, FUT4 and LeY in human gastric patient's tissues and blood samples by IHC and ELISA. In addition, we evaluated the correlation between the cagA status of *H. pylori* with the expression of FUT4, LeY and p-EGFR. This study indicates significant correlation of *H. pylori* CagA with FUT4, LeY overexpression through EGFR activation in gastric cancer tissues and serum samples.

Material and Methods

Antibodies and Reagents

Rabbit anti-human FUT4 was purchased from Proteintech Group. Mouse anti-human LeY antibody (BG-8), was purchased from Abcam (Cambridge, USA). Anti-mouse p-EGFR antibodies were obtained from Transduction Laboratories, US. Mouse anti-CagA antibody was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). HRP-conjugated anti-mouse and rabbit IgM, IgG were purchased from Proteintech group, USA. Commercial ELISA kits of p-EGFR were purchased from Abcam, (Cambridge, US) while CagA and FUT4 ELISA Kits were purchased from (Cusabio, US). Immunocytochemical avidin-biotin peroxidase complex kit was obtained from ZSGB-BIO, Beijing, China and DAPI was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA).

Gastric Tissue and Blood Samples Collection

This study involved a total of 625 human tissue and 650 blood samples from gastric patients (2011 to 2013), which were characterized as non-neoplastic gastric epithelium of chronic gastritis (GIs), chronic atrophic gastritis (CAG), gastric ulcer (GU), intestinal metaplasia (IM), gastric epithelial dysplasia (GED) and gastric cancer (GC). The paraffin-embedded human gastric tissues were selected after histological review. Sample collection from the patients and all research protocols were approved by the Institutional Review Board of Dalian Medical University and were in accordance with the established guidelines.

Immunohistochemistry

Paraffin-embedded gastric tissues were analyzed for the expression of CagA, p-EGFR, FUT4, and LeY by immunohistochemistry (IHC). Serial sections (4–6 mm each) were prepared from paraffin-embedded tissues. The sections were heated at 60 °C for 1 h, deparaffinized in xylene and rehydrated in a graded alcohol. Slides were microwaved for 20–40 min in citrate buffer to expose antigen and were washed with PBS. Slides were then incubated in 3 % H_2O_2 for 10 min at room temperature to block endogenous peroxidase activity. After washing in PBS, sections were blocked with goat serum at room temperature for 30 min to eliminate non-specific binding and then incubate overnight at 4 °C with mouse IgM LeY (1:100), mouse IgG CagA (1:100), mouse IgG p-EGFR (1:50) and rabbit IgG FUT4 (1:100). Next day, sections were rinsed with PBS and subsequently incubated with their respected secondary antibody for 30 min at room temperature. The signal was visualized with peroxidase-labeled streptavidin-complexes DAB and the sections were briefly counterstained with hematoxylin. Yellowish-brown stain indicated a positive result. The negative control was generated by replacing the primary antibody with isotype IgM or IgG. Slides were mounted and visualized at ×10 magnifications on an inverted microscope (Nikon Ti-DS, Japan). Immunostaining intensity was evaluated by using a semi-quantitative method. The immunohistochemical staining was evaluated and scored by at least

two independent pathologists. Each sample was given a score according to the intensity of the staining as follows, none staining =0; low staining =1; medium staining =2; strong staining =3. The data was statistically analyzed. The immunohistochemical localization pattern was also recorded by digital imaging.

Enzyme-Linked Immunosorbent Assay

Serological assessment of *H. pylori* CagA, p-EGFR, FUT4 and LeY were measured by enzyme-linked immunosorbent assay (ELISA). Commercial ELISA kits were used to analyze serological values of CagA, p-EGFR and FUT4 according to manufacturer's instructions. LeY serological assessment was detected by in-house ELISA as; 96-well plates (Costar, US) were coated with mouse anti-LeY monoclonal antibody (1:500, 100 μl/well) for 36 h at 4 °C. Plates were blocked with 3 % bovine serum albumin (BSA) for 1 h at 37 °C. After washing, 100 μl (1:100) of patient serum was added into consecutive wells along with negative and positive controls, and incubated at 37 °C for 1 h. HRP-labeled *Ulex europaeus* (UEA) lectins (2.5 μg/ml) were added and incubated for 2 h at 37 °C. 100 μl of substrate (Xiamen Technology Co., Ltd.) was added and placed in dark place for 30 min at room temperature. The reaction was stopped by adding 0.3 M H_2SO_4 and color intensity was measured at 450 nm. A431 cell lysates (1:50) were used as positive control of LeY. A standard curve was constructed and analysis by using antibody concentration in the range of 1000–15.625 ng/ml. ELISA results for CagA, p-EGFR and FUT4 were calculated and analyzed according to the manufacturer's instructions. Results were analyzed by constructed a standard curve by plotting mean absorbance for each standard on the Y-axis against the concentration on the X-axis, and a best fit curve drawn for the regression analysis.

Statistical Analysis

Statistical differences between test groups were analysed by paired Student's *t*-test and one-way analysis of variance (ANOVA) followed by (post hoc) Newman-Keuls multiple comparison tests. Linearity of correlation was calculated by the Pearson's coefficient correlation method. $P < 0.05$ and $P < 0.01$ were considered as significant. The statistical software GraphPad prism 5.03 was used for analyzing the data.

Results

Gastric Patients History

This study was designed to determine the frequency and correlation of CagA, p-EGFR, FUT4 and LeY expression level in chronic gastritis (GIs), chronic atrophic gastritis (CAG), gastric ulcer (GU) intestinal metaplasia (IM), gastric epithelial dysplasia (GED) and gastric cancer (GC) patients. A total of 625 gastric tissues and 650 serum samples, ranging in age from 10 to 80 years, were included in the study. Among 625 gastric tissues, 174 (27.84 %) samples were regarded as GU, 323 (51.68 %) as GC and 128 (20.48 %) were diagnosed as GIs, further sub-grouped into CAG; 81 (63.28 %), IM; 29 (22.65 %) and GED; 18 (14.06 %), while 650 blood serum samples were characterized as GU; 213 (32.76 %), GC; 261 (40.15 %), GIs; 176 (27.07 %), CAG; 117 (66.47 %), IM; 37 (21.02 %) and GED; 22 (12.5 %), (Table 1).

Detection of the *H. pylori* CagA, p-EGFR, FUT4 and LeY Expression Level and Clinical Manifestations by IHC

Among 625 gastric tissues, we found wide range of positive cases of CagA, p-EGFR, FUT4 and LeY in (CAG), (IM), (GED), GU and GC. Among 323 (51.68 %) GC tissues, 269 (83.28 %) were positive for CagA; 262 (81.11 %) for p-EGFR; 294 (91.02 %) for FUT4; and 301 (93.18 %) for LeY, whereas, respective percentages in CAG 81 (63.28 %), IM 29 (22.65 %), GED 18 (14.06 %), and GU 174 (27.84 %) tissue samples had significantly low number of positive cases for CagA; 49 (60.49 %), 15 (51.72 %), 11 (61.11 %) & 116 (66.81 %), p-EGFR; 37 (45.67 %), 23 (79.31 %), 7 (38.88 %) & 110 (63.18 %), FUT4; 62 (76.54 %), 16 (55.17 %), 9 (50.0 %) & 135 (77.72 %) and LeY; 53 (65.43 %), 17 (58.62 %), 10 (55.55 %) & 132 (75.90 %), respectively (Table 1).

Detection of the *H. pylori* CagA, p-EGFR, FUT4 and LeY Serological Titer and Clinical Manifestations by ELISA

We found that GC patients; 261 (40.15 %) had high number of positive cases for CagA; 224 (85.82 %), p-EGFR; 189 (72.32 %), FUT4; 252 (96.66 %) and LeY; 241 (92.22 %). In comparative analysis of CAG 117 (66.47 %), IM 37 (21.02 %), GED 22 (12.50 %), and GU 213 (32.76 %), significantly low positive cases were observed in CagA; 71 (60.68 %), 19 (51.35 %), 14 (63.63 %) & 156 (73.33 %), p-EGFR; 42 (35.89 %), 11 (29.72 %), 8 (36.36 %) & 101 (47.28 %), FUT4; 81 (69.23 %), 21 (56.75 %), 15 (68.18 %) & 175 (82.22 %) and LeY; 72 (61.53 %), 31(83.78 %), 11(50.00 %)& 180 (84.44 %), respectively (Table 1).

Comparative Analysis of *H. pylori* CagA, p-EGFR, FUT4 and LeY Expression Level in Gastric Tissues and Blood Samples

Gastric tissue collected from patients of GIs, GU and GC were showed different level of immunoreactivity for CagA, p-EGFR, FUT4 and LeY. GC tissues were showed high expression level of CagA (1.790 ± 0.18), p-EGFR (1.533 ± 0.22).

Table 1 Analysis of CagA, p-EGFR, FUT4, and LeY expression in human gastric tissues and bloods by IHC and ELISA

Characteristics	IHC $n = 625$					ELISA $n = 650$			
	Male	Female	Age (years)		*p*-value	Male	Female	Age (years)	*p*-value
	226 (36.16)	399(63.84)				215 (33.07)	435(66.92)		
Chronic Gastritis (GIs)	51(22.56)	77(19.29)	53.95 ± 4.80			72(33.48)	104(23.90)	46.30 ± 3.896	
Gastric Ulcer (GU)	66(29.20)	108(27.06)	60.07 ± 2.615		$P < 0.0001$	55(25.58)	158(36.32)	56.17 ± 2.489	$P < 0.0001$
Gastric Cancer (GC)	109(48.23)	214(53.63)	60.85 ± 2.9966			88(40.93)	173(39.77)	61.43 ± 2.339	
Positive number of Cases (%)									
H. pylori & Host factors	IHC					ELISA			
	GIs		GU	GC		GIs		GU	GC
	128 (20.48)		174(27.84)	323(51.68)		176(27.07)		213(32.76)	261(40.15)
	CAG	IM	GED			CAG	IM	GED	
	81(63.28)	29(22.65)	18(14.06)			117(66.47)	37(21.02)	22(12.50)	
CagA	49(60.49)	15(51.72)	11(61.11)	116(66.81)	269(83.28)	71(60.68)	19(51.35)	14(63.63)	156(73.33)	224(85.82)
p-EGFR	37(45.67)	23(79.31)	7(38.88)	110(63.18)	262(81.11)	42(35.89)	11(29.72)	8(36.36)	101(47.28)	189(72.32)
FUT4	62(76.54)	16(55.17)	9(50.0)	135(77.72)	294(91.02)	81(69.23)	21(56.75)	15(68.18)	175(82.22)	252(96.66)
LeY	53(65.43)	17(58.62)	10(55.55)	132(75.90)	301(93.18)	72(61.53)	31(83.78)	11(50.00)	180(84.44)	241(92.22)

FUT4 (2.146 ± 0.08) and LeY (1.695 ± 0.16) ($P < 0.0001$) as compared to expression level in GIs and GU tissue samples of CagA (0.644 ± 0.12 & 1.145 ± 0.16), p-EGFR (0.856 ± 0.11 & 0.678 ± 0.15), FUT4 (0.949 ± 0.14, 1.197 ± 0.13) and LeY (0.687 ± 0.07 & 1.008 ± 0.13), respectively ($P < 0.0001$) (Fig. 1 and Table 2). Moreover, ELISA was used to determine serological titer of CagA, p-EGFR, FUT4 and LeY in gastric tissues of GIs, GU and GC. Among 650 gastric serum samples, GIs showed significant weak expression of CagA (0.821 ± 0.060), p-EGFR (0.673 ± 0.070), FUT4 (0.680 ± 0.026) and LeY (0.717 ± 0.084) ($P < 0.0001$) by Newman-Keuls multiple test (ANOVA). However, GC showed significantly high expression levels of CagA (1.660 ± 0.100), p-EGFR (1.587 ± 0.070), FUT4 (1.662 ± 0.087) and LeY (1.610 ± 0.094) ($P < 0.0001$) as compared to GIs and GU blood samples (Fig. 1 and Table 2).

Correlation between CagA, P-EGFR, FUT4 and LeY in Human Gastric Tissues and Blood Samples

Correlative study was performed between CagA, p-EGFR, FUT4 and LeY expression level in gastric tissue and serum samples by Pearson's coefficient correlation method. We found a significant correlation of *H. pylori* CagA with p-EGFR (*R*—0.624), FUT4 (*R*—0.814) and LeY (*R*—0.822) ($P < 0.0001$) (Table 3). Moreover, CagA titer also showed significant correlation with p-EGFR (*R*—0.744), FUT4 (*R*—0.767) and LeY (*R*—0.705) ($P < 0.0001$), and FUT4 and LeY (*R*—0.680) had significant correlation ($P < 0.0001$) (Table 3).

Discussion

This is consecutive study from Northeast China on molecular characterization and correlation of *H. pylori* CagA with different host factors, which play an important role in the development of gastric cancer [4, 22]. China has high prevalence of gastric cancer and alone accounts 42 % of all gastric cancer cases worldwide [23, 24]. *H. pylori* CagA positive *H. pylori* strains are more virulent causing higher levels of gastric mucosal inflammation in gastritis and gastric cancer [6, 25]. In this study, we found significant high expression of CagA in the gastric cancer (83.28 %) as compared to chronic atrophic gastritis (60.49 %), intestinal metaplasia (51.72 %), gastric epithelial dysplasia (61.11 %) and gastric ulcer tissues (66.81 %) by immunohistochemistry (Fig. 1 and Table 1). Furthermore, the serological results of CagA by ELISA were consistent as gastric cancer showed high titer (85.82 %) compared to chronic atrophic gastritis (60.68 %), intestinal metaplasia (51.35 %), gastric epithelial dysplasia (63.163 %) and gastric ulcer (73.33 %) (Table. 1). Our results showed comparable prevalence rates, with those from other reported data; [Figueroa et al. (26)] reported high CagA prevalence of 100 % in gastric cancer, 96 % for ulcer and 82 % for gastritis in Chile [26]. Adnan et al. (2013) reported higher expression of CagA in gastric cancers (80 %) compared to ulcer (74 %) and gastritis (48 %) in Pakistan [27]**.** Recently (2014), we also analyzed cagA and vacA genotyping among gastric patients and reported higher level of cagA gene expression in gastric cancers (63 %) compared to gastric ulcer (50 %), chronic

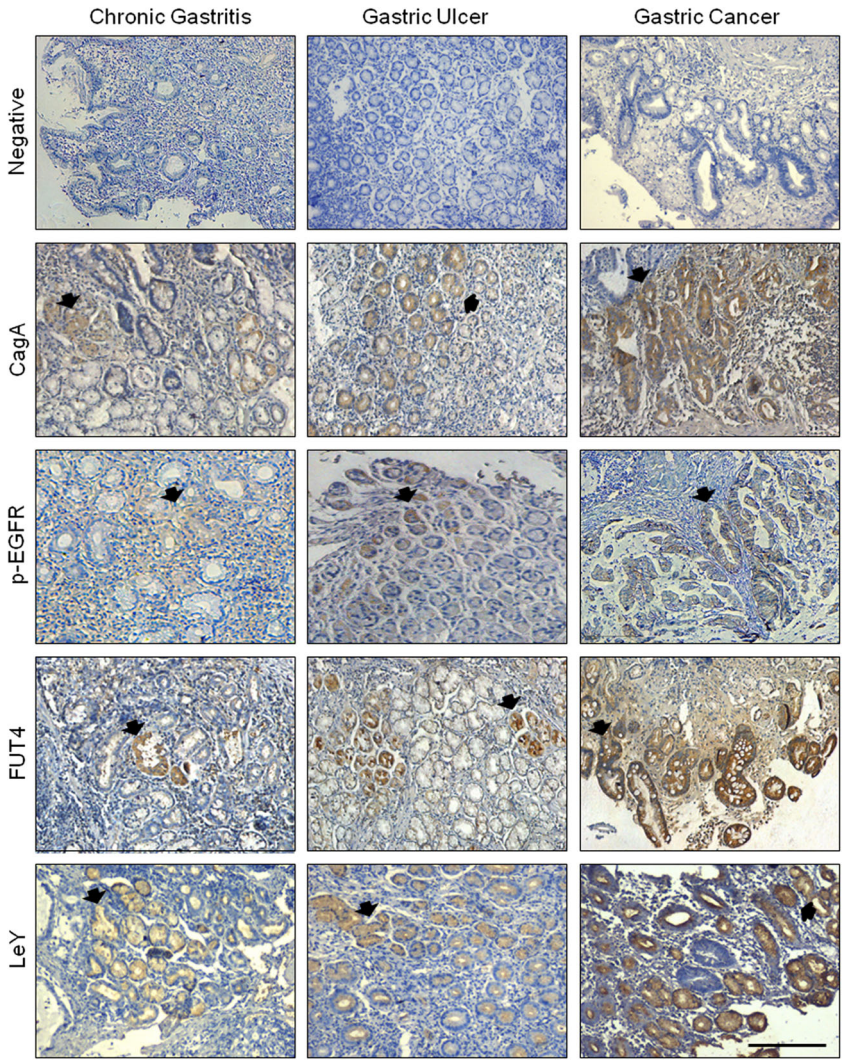

Fig. 1 Analysis of CagA, p-EGFR, FUT4 and LeY expression in human gastric tissues by immunohistochemistry. CagA protein level were positively correlated with the p-EGFR, FUT4 and LeY in paraffin-embedded gastritis (GIs), gastric ulcer (GU) and gastric cancer (GC) tissue samples. Immunohistochemical analysis were carried out with anti-CagA, anti-p-EGFR, anti-FUT4 and anti-LeY antibodies and revealed by staining with diaminobenzene. The panels were magnified ×10 (Bar =100 μm). Positive staining is indicated by the arrows

gastritis (41 %) and normal (11 %) tissues sample in Northeast China [4]. These results suggest that *H. pylori* CagA expression may have potential application as a marker for the prognosis of CagA associated gastric cancer.

Gastric cancer's diagnostic and treatment are carried out by targeting a specific biological gastric marker protein, which plays an important role in the progression of gastric cancer, such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), vascular endothelial growth factor receptor 2 (VEGFR2) and Cyclooxygenase-2 (COX-2) [22, 23]. *H. pylori* CagA could regulate the process of gastric cell proliferation with EGFR activation [28, 29]. In our study, we found significantly high EGFR activation in gastric cancer as compared to chronic gastritis and gastric ulcer tissues and blood samples by immunohistochemistry and ELISA, respectively (Fig. 1 and Table 2). Moreover, p-EGFR

Table 2 Comparative analysis of CagA, p-EGFR, FUT4, and LeY expression in human gastric tissues and bloods by IHC and ELISA

Comparative analysis by Newman-Keuls multiple comparison test (ANOVA)							
Comparison H- value	GIs IHC	GU	GC	GIs ELISA	GU	GC	
CagA	0.644 ± 0.12	1.145 ± 0.16	1.790 ± 0.18	0.821 ± 0.060	0.838 ± 0.090	1.660 ± 0.100	$P < 0.0001$
p-EGFR	0.856 ± 0.11	0.678 ± 0.15	1.533 ± 0.22	0.673 ± 0.070	0.913 ± 0.040	1.587 ± 0.070	
FUT4	0.949 ± 0.14	1.197 ± 0.13	2.146 ± 0.08	0.680 ± 0.026	0.981 ± 0.063	1.662 ± 0.087	
LeY	0.687 ± 0.07	1.008 ± 0.13	1.695 ± 0.16	0.717 ± 0.084	0.892 ± 0.098	1.610 ± 0.094	

Table 3 Correlative analysis of CagA, p-EGFR, FUT4, and LeY expression in human gastric tissues and bloods by IHC and ELISA

Correlative analysis by Pearson's coefficient correlation method (*R*–value)			
	IHC	ELISA	
CagA			
p-EGFR	0.624	0.744	$P < 0.0001$
FUT4	0.814	0.767	
LeY	0.822	0.705	
p-EGFR			
CagA	0.624	0.744	$P < 0.0001$
FUT4	0.609	0.770	
LeY	0.725	0.719	
FUT4			
CagA	0.814	0.767	$P < 0.0001$
p-EGFR	0.609	0.770	
LeY	0.748	0.680	
LeY			
CagA	0.822	0.705	$P < 0.0001$
p-EGFR	0.725	0.719	
FUT4	0.748	0.680	

showed significant correlation with *H. pylori* CagA in gastric cancer samples (Table 3). Previously reported data from our lab showed that EGFR activation mediated cancer cell proliferation [30]. Hence, we suggested that CagA develops the gastric cancer by enhancing activation of EGFR.

H. pylori infection alters the host's glycosylation with the stimulation of specific glycosyltransferases and the sugar antigens, such as Lewis blood group antigen [22]. The host fucosylated blood group antigens facilitate the *H. pylori* to adhere with human gastric epithelial cells [31]. In this study, we found high expression of FUT4 in the gastric cancer tissues and blood samples (Fig. 1 and Table 2) among the gastritis and ulcer, which indicate a close relationship between CagA and FUT4 fucosylation. High expression of FUT4 was reported in different type of cancers in reported data. Kudo et al. reported the high FUT4 expression in colorectal cancer [32, 33], pancreatic [34], and lung cancer [35]. Recently (2015), we also reported significant high expression of FUT4 in breast cancer as compared to normal tissues and serum samples [36]. These results indicate a close linkage between FUT4 overexpression with *H. pylori* CagA.

Human Lewis antigens represent terminal modifications on the glycans. In human stomach, the LeY blood antigen is predominately expressed by the mucous, chief and parietal cells of gastric glands [12]. In our study, we found strong staining for LeY in gastric cancer by immunohistochemistry while gastric ulcer and the chronic gastritis showed low level (Fig. 1 and Table 2). Previous studies reported that LeY expressions were high in gastric cancer in the range of 15–85 % [37–39]. Escrevente et al. reported high expression of LeY and LeX in ovarian cancer with the elevation and progression of ovarian cancer cell proliferation [40]. These results indicate a role of CagA in promoting gastric cancer with the correlation of LeY. Our results indicate that *H. pylori* CagA can modify the intensity of the gastric Lewis antigen expression in gastric cancer. According to Wirth et al., high expression level of host Lewis antigen might be particularly adaptive in CagA strain of *H. pylori* [41].

Biomarkers such as sLeA (CA19–9), STn (CA724) or mucin glycoprotein such as MUC1 (CA15–3) and MUC16 (CA125) used as the source of potential prognostic biomarkers in gastric cancer [42–44]. Now the approach has shifted towards the discovery of group of biomarkers directly related to disease processes. In conclusion, our findings suggest that infection with *H. pylori* cagA^{+ve} strains results in abnormal FUT4 fucosylation, which leads to the development of gastric cancer. FUT4/LeY overexpression may serve as potential correlative biomarkers for the prognosis of *H. pylori* CagA associated gastric cancer.

Acknowledgments The project was supported by the China 973 grant no. 2012CB822100, National Natural Science Foundation of China Research grants no. 30672753 and 31270866.

Compliance with Ethical Standards

Conflict of Interests The authors declare that they have no conflict of interests.

References

1. Aziz F, Sherwani SK, Akhtar SS, Kazmi SU (2014a) Development of an in-house enzyme-linked immunosorbent assay based on surface whole cell antigen for diagnosis of helicobacter pylori infection in patients with gastroduodenal ulcer disease. World J Microbiol Biotechnol 30:305–315
2. Zhu Y, Zhong X, Zheng S, Du Q, Xu W (2005) Transformed immortalized gastric epithelial cells by virulence factor cagA of helicobacter pylori through Erk mitogen-activated protein kinase pathway. Oncogene 24:3886–3895
3. Aziz F, Taj Y, Kazmi SU (2013b) In-house enzyme-linked immunosorbent assay based on helicobacter pylori sonicate whole cell antigen for diagnosis of helicobacter pylori infection in Karachi. Pakistan Int J Microbiol Adv Immunol 1(4):24–31
4. Aziz F, Chen X, Yang X, Yan Q (2014b) Prevalence and correlation with clinical diseases of helicobacter pylori cagA and vacA genotype among gastric patients from Northeast China. Biomed Res Int 2014:142980
5. Yamaoka Y (2008) Increasing evidence of the role of helicobacter pylori SabA in the pathogenesis of gastroduodenal disease. J Infect Dev Ctries 1:174–181
6. Hatakeyama M, Higashi H (2005) Helicobacter pylori CagA: a new paradigm for bacterial carcinogenesis. Cancer Sci 96:835–843

7. Asahi M, Azuma T, Ito S, Ito Y, Suto H, Nagai Y et al (2000) Helicobacter pylori CagA protein can be tyrosine phosphorylated in gastric epithelial cells. J Exp Med 191:593–602
8. Odenbreit S, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R (2000) Translocation of helicobacter pylori CagA into gastric epithelial cells by type IV secretion. Science 287:1497–1500
9. Menaker RJ, Ceponis PJ, Jones NL (2004) Helicobacter pylori induces apoptosis of macrophages in association with alterations in the mitochondrial pathway. Infect Immun 72:2889–2898
10. De la Cruz-Herrara CF, Flores-Luna L, Guterrez-Xicotencatl L, Chihu-Amparan L, Sanchez-Aleman MA, Lazcano-Ponce E, Torres J, Ayala G (2013) IgG2 response and low IgG titer specific to helicobacter pylori CagA as serological markers for gastric cancer. J Med Microbiol 62:591–598
11. Aziz F, Wang X, Liu J, Yan Q (2016) Ginsenoside Rg3 induces FUT4-mediated apoptosis in H. Pylori CagA-treated gastric cancer cells by regulating SP1 and HSF1 expressions. Toxicol in Vitro 31:158–166
12. Moran AP, Gupta A, Joshi L (2011) Sweet-talk: role of host glycosylation in bacterial pathogenesis of the gastrointestinal tract. Gut 60:1412–1425
13. Reis CA, Osorio H, Silva L, Gomes C, David L (2010) Alteration in glycosylation as biomarkers for cancer detection. J Clin Pathol 63: 322–329
14. Delmotte P, Degroote S, Lafitte JJ, Lamblin G, Perini JM, Roussel P (2002) Tumor necrosis factor alpha increases the expression of glycosyltransferases and sulfotransferases responsible for the biosynthesis of sialylated and/or sulfated Lewis x epitopes in the human bronchial mucosa. J Biol Chem 277:424–431
15. Miyoshi E, Moriwaki K, Nakagawa T (2008) Biological function of fucosylation in cancer biology. J Biochem 14:725–729
16. Moriwaki K, Miyoshi E (2010) Fucosylation and gastrointestinal cancer. World J Hepatol 2:151–161
17. Yang X, Zhang Z, Jia S, Liu Y, Wang X, Yan Q (2007) Overexpression of fucosyltransferase IV in A431 cell line increase cell proliferation. Int J Biochem Cell Biol 39:1722–1730
18. Sheu BS, Sheu SM, Yang HB, Huang AH, Wu JJ (2003) Host gastric Lewis expression determines the bacterial density of helicobacter pylori in babA2 genopositive infection. Gut 52:927–932
19. Wang XQ, Zhu ZM, Fenderson BA, Zeng GQ, Cao YJ, Jiang GT (1998) Effects of monoclonal antibody directed to LeY on implantation in the mouse. Mol Hum Reprod 4:295–300
20. Dettke M, Palfi G, Pursch E, Fischer MB, Loibner H (2001) Increased expression of the blood group-related Lewis Y antigen on synovial fluid granulocytes of patients with arthritic joint diseases. Rheumatology (Oxford) 40:1033–1037
21. Madjd Z, Parsons T, Watson NF, Spendlove I, Ellis I, Durrant LG (2006) High expression of Lewis y/b antigens is associated with decreased survival in lymph node negative breast carcinomas. Breast Cancer Res 7:80–87
22. Aziz F, Yang X, Wang X, Yan Q (2014c) Anti-LeY antibody enhances therapeutic efficacy of celecoxib against gastric cancer by downregulation of MAPKs/COX-2 signaling pathway: correlation with clinical study. J Cancer Res Clin Oncol 141(7):1221–1235
23. Aziz F, Qiu Y (2014) The role of anti-LeY antibody in the downregulation of MAPKs/COX-2 pathway in gastric cancer. Curr Drug Targets 15:469–476
24. Lin Y, Ueda J, Kikuchi S, Totsuka Y, Wei WQ, Qiao YL, Inoue M (2011) Comparative epidemiology of gastric cancer between Japan and China. World J Gastroenterol 17:4421–4428
25. Björkholm B, Falk P, Engstrand L, Nyrén O (2003) Helicobacter pylori: resurrection of the cancer link. J Intern Med 253:102–119
26. Figueroa G, Troncoso M, Toledo MS, Faúndez G, Acuña R (2002) Prevalence of serum antibodies to helicobacter pylori VacA and CagA and gastric diseases in Chile. J Med Microbiol 51:300–304
27. Khan A, Farooqui A, Raza Y, Rasheed F, Manzoor H, Akhtar SS, Quraishy MS, Rubino S, Kazmi SU, Paglietti B (2013) Prevalence, diversity and diseases association of helicobacter pylori in dyspeptic patients from Pakistan. J Infect Dev Ctries 7:220–228
28. Liu JJ, Lin B, Hao YY, Li FF, Liu DW, Qi Y, Zhu LC, Zhang SL, Iwamori M (2010) Lewis(y) antigen stimulates the growth of ovarian cancer cells via regulation of the epidermal growth factor receptor pathways. Oncol Rep 23:833–841
29. Keates S, Sougioultzis S, Keates AC, Zhao D, Peek RM Jr, Shaw LM, Kelly CP (2001) cag + Helicobacter pylori induce transactivation of the epidermal growth factor receptor in AGS gastric epithelial cells. J Biol Chem 276:48127–48134
30. Zhang Z, Sun P, Liu J, Fu L, Yan J, Liu Y, Yu L, Wang X, Yan Q (2008) Suppression of FUT1/FUt4 expression by SiRNA inhibits tumor growth. Biochim Biophys Acta 1783:287–296
31. Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Borén T (1998) Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 279:373–377
32. Kudo T, Ikehara Y, Togayachi A, Morozumi K, Watanabe M, Nakamura M et al (1998) Up-regulation of a set of glycosyltransferases genes in human colorectal cancer. Lab Investig 78:797–711
33. Saito H, Hiraiwa N, Sawada-Kasugai M, Akamatsu S, Tachikawa T, Kasai Y et al (1997) Altered mRNA expression of specific molecular species of fucosyl- and sialyl-transferases in human colorectal cancer tissues. Int J Cancer 71:556–564
34. Taniguchi A, Suga R, Matsumoto K (2002) Expression and transcriptional regulation of the human alpha1,3-fucosyltransferase 4 (FUT4) gene in myeloid and colon adenocarcinoma cell lines. Biochem Biophys Res Commun 273(1):370–376
35. Martin-Satue M, Marrugat R, Cancelas JA, Blanco J (1998) Enhanced expression of α1,3-fucosyltransferase genes correlates with E-selectin-mediated adhesion and metastastic potential of human lung adnocarcinoma cells. Cancer Res 58:1544–1550
36. Yan X, Lin Y, Liu S (2015) Faisal Aziz, Qiu Yan, fucosyltransferase IV (FUT4) as an effective biomarker for the diagnosis of breast cancer. Biomed Pharmacother 70:299–304
37. Dohi T, Abe K, Ohshiba S (1989) Immunohistochemical study of carbohydrate antigen expression in gastric carcinoma. Gastroenterol Jpn 24:239–245
38. Sakamoto S., Watanabe T., Tokumaru T., Takagi H., Nakazato H. and Lloyd K.O (1989) Expression of Lewisa, Lewisb, Lewisx, Lewisy, siayl-Lewisa, and sialyl-Lewisx blood group antigens in human gastric carcinoma and in normal gastric tissue. Cancer Res 49:745–752.
39. Kobayashi K, Sakamoto J, Kito T, Yamamura Y, Koshikawa T, Fujita M, Watanabe T, Nakazato H (1993) Lewis blood group-related antigen expression in normal gastric epithelium, intestinal metaplasia, gastric adenoma, and gastric carcinoma. Am J Gastroenterol 88:919–924
40. Escrevente E, Machado C, Brito CA, Reis A, Stoeck S, Runz A, Marme P, Altevogt JC (2006) Different expression levels of alpha3/4 fucosyltransferases and Lewis determinants in ovarian carcinoma tissues and cell lines. Int J Oncol 29:557–566
41. Wirth HP, Yang M, Karita M, Blaser MJ (1990) Expression of human cell surface glycoconjugates Lewis x and Lewis y by helicobacter pylori isolates is related to CagA status. Infect Immun 64:4598–4605
42. Aziz F, Yang X, Wang X, Qiu Y (2013a) Helicobacter pylori infection leads to gastric cancer with the coexpression of Lewis Y and CA724. Glycoconj J 30:411
43. Aziz F, Yang X, Wen Q, Qiu Y (2015) A method for establishing human primary gastric epithelial cell culture from fresh surgical gastric tissues. Mol Med Rep 12(2):2939–2944
44. Gomes C, Almeida A, Ferreira JA, Silva L, Santos-Sousa H, Pinto-de-Sousa J, Santos LL, Amado F, Schwientek T, Levery SB, Mandel U, Clausen H, David L, Reis CA, Osório H (2013) Glycoproteomics analysis of serum from patients with gastric precancerous lesions. J Proteome Res 12:1454–1466