Predominant mucosal IL-8 mRNA expression in non-cagA Thais is risk for gastric cancer

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学位論文の内容要旨

Predominant mucosal *IL-8* mRNA expression in non-*cagA* Thais is risk for gastric cancer

CagA 陰性 *H.pylori* 感染における胃粘膜 IL-8 優位な発現は
タイ胃癌の危険因子である

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Background: *Interleukine-8* (*IL-8*) gene expression was reported to be related to the *cagA* gene *in vitro* study. COX-2 expression was also reported to be higher in gastric cancer. There is no *in vivo* study demonstrated of how these genetic factors and their level related to the *cagA* genotype and serum pepsinogen I/II ratio. We aimed to study its level, risk related factors, and factors correlated with gastric cancer in Northern Thais and Japanese

Methods: Patient Characteristics and volunteer selection: We conducted an experimental based cross sectional study in the gastrointestinal surgery and endoscopy unit, Chiang Mai University hospital during year 2007-2010. Informed consent were obtained from 86 Thai gastric cancer patients who underwent NBI endoscopy and gastric surgery during year 2007-2010, and 134 Thai non-cancer volunteers who underwent NBI endoscopic examination during 2006-2008. We recruited 17 advanced stage Japanese gastric cancer and 12 non-cancer surveillance patients. All gastric cancer patients had locally advanced gastric cancer and underwent examinations by endoscopy before curative gastric resection. Peptic ulcer disease was excluded in this study. Gastric mucosal tissue samples were taken by endoscopy with three biopsy sites for pathology and bimolecular genetic tests before surgical treatment. The histopathology description of the tumor and histologic type were defined. The pathology of gastritis, metaplasia, and *H.pylori* were reported by a modified Sydney Score System.

Serum pepsinogen I and II level, and *H.pylori* IgG antibody test

The amounts of 5cc blood were used. The red blood cell and serum separation was done, and preserved at -20°C. The serum pepsinogen I, II, and *IgG* Antibody for *H.pylori* were tested by the standard ELISA technique. The standard cut off value used was a PGI level of more than 70 ng/ml or PGI/II ratio more than 3.0 for no atrophy or positive grade 1, PGI <70 ng /ml and PGI/II ratio <3.0 excluding severe atrophy for moderate atrophy or positive grade 2, and PGI <30 ng /ml and PGI/II ratio <2.0 for severe atrophy or positive

grade 3, respectively. All samples were tested twice for reliability confirmation (Toyobo, co, Ltd.)

Tissue *H.pylori* DNA extraction and *cagA* genotypting method

The tissue *H.pylori* DNA extracted from the lower antral position in the stomach was examined by PCR method, and genotyped for *cagA* mutation in by the author (Samples were also blinded double test by Toyobo, co, Ltd). The *H.pylori* positive control of *cagA* positive strain number 11638(Western), 26695(Western), and F57 (East Asian) were provided by the collaborative institute. The bacterial tissue DNA and genotyping method with primers used in this study were conducted as recently described. The specific oligonucleotide primers Forward (5'- AAAAGCGACCTTGAAAATTCC-3'; nucleotides 2299-2319), Reverse-1(5'-CTTCATTTTTTTGAGCTTGTTGAGC-3'; nucleotides 2488-2463) and Reverse- 2(5'-ATTAATGCGTATGTGGCTGTTAGTAGC-3'; nucleotides 3222-3195, were originally described by Azuma T, *et al.*

Cell line culture and gastric mucosal total mRNA extraction with reverse transcriptase reaction for cDNA synthesis: The AGS cell line was grown before cell collection for mRNA extraction at a cell count of 2-4 $x10^6$. Total mRNA extraction protocol was conducted. The technique followed was a reverse transcriptase reaction using a commercial high capacity RNA-to-cDNA kit (Applied Biosystems).

Gastric mucosal *IL-8* and COX-2 mRNA expression by Real time RT-PCR (Relative quantification real time RT-PCR)

We conducted the experiment from three positions gastric mucosal biopsies in both populations. All of gastric mucosal tissue samples were transformed to cDNA after total mRNA extraction. The analysis was substantially correctable by analysis both in raw relative quantitation (raw RQ) and log10 value for adjusted normal distribution curve. All human TaqMan probe primer express which was used in this study had 81base pairs of *IL-8* specific human primer Assay ID number Hs99999034_m1, 111 base pairs of COX- 2 Assay ID number Hs01573471_m1, and 121 based pair of specific human Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Hs99999905_m1 designed and supplied by Applied Biosystems, USA. The internal control was performed by GAPDH of a matched number template. The Real-Time relative quantitation value was measured by comparing to the base line value of AGS cell line subject control before making the analysis.

A student t-test was used for quantitative data, *IL-8* and COX-2 mRNA expression level, and pepsinogen level. The Chi squares test was used for qualitative data. The correlation study for pair factors was done in subgroup analysis in each group of ethnic, cancer and non-cancer populations. The multivariate analysis was used for risk study by STATA 11.0, USA and SPSS 16, USA were used for statistical analysis, and the p value of less than 0.05 was considered as statistically significant.

Results: There were 86 cases of advanced gastric cancer and 45(33.8%) normal control cases, 46(34.6 %) non-peptic disease benign lesions without recent history of any treatment, and 42(31.6%) chronic gastritis cases among 134 non-cancer control cases who included in the genetic expression experiment. Thai male and female cancer incidences are 60.5% (52/86) and 34% (39/86), respectively. Males are also the predominant gender in Japanese. Both nations have significant high incidence of gastric cancer at age 40 years old or above. The *H.pylori* infection prevalence is reported by combined histopathology, *H.pylori IgG* Ab level, and 23SrDNA results that have 77.1% and 97.4 % of sensitivity and specificity, respectively. Among Thai cancer patients and non-cancer volunteers, *H.pylori* prevalence was 72.1% and 71.6%, respectively. Meanwhile, Thai gastric cancer cases had a *cagA* genotype demonstrated in only 7/62(12.3%) in positive *H. pylori* infection patients that yields 6 cases of East Asian and 1 case of Western type.

In non-cancer Thais, there were 62/98 (63.9%) of positive *cagA* and 34/98(36.1%) of negative *cagA* genotyping in positive *H.pylori* infection cases that yielded 47.7 % of East Asian , 27.4% of Western, and 24.9% of Mix genotype. For the 6 years followed up of 18 cases of high grade chronic atrophic gastritis(CAG group) in non-cancer Thais who has long term *H.pylori cagA* East Asian type infection, no one develop to be gastric cancer.

There is a high non- *cagA* gene of 86.8 per cent in Thai gastric cancer although there are high yields of the East Asian type in the positive *cagA*.

For *IL-8* and COX-2 mRNA expression results, 86 Thai gastric cancers were tested successfully in comparison with 134 Thai non-cancer volunteers. The detection rates of *IL-8* mRNA expression were 77/86(89.5%) in Thai gastric cancer and 102/134 (74.6%) in Thai non-cancer volunteers. At the same stage of advanced gastric cancer, the mean level of *IL-8* mRNA expression in Thai cancer and Japanese cancer were 9,615.65 ($\log_{10}$= 2.62) and 1509.11 ($\log_{10}$=2.17), respectively, p= 0.014. For gastric cancer risk at cut off *IL-8* expression level by $\log_{10}$>2 in Thais and Japanese, Odds ratio= 7.97 (95%CI=3.75-16.97, p<0.001) and Odd ratios= 4 (95%CI=1.29-12.40), respectively. In the non-cancer group, we found that the *IL-8* mRNA expression level was lower than its level in cancer population with a significant difference, p<0.001. The total mean *IL-8* mRNA expression in non-cancer Thais was 2,262 ($\log_{10}$=1.49) while that in Japanese non-cancer was 10.79($\log_{10}$= 0.69), p<0.001. From comparison within the same ethnic group, the mean level of *IL-8* mRNA expression in Thai and Japanese cancer was higher than that in non-cancer, p = 0.05

For COX-2 mRNA expression, the detection rate was 65 per cent without significant rising level in both nations gastric cancer when compared to *IL-8* mRNA expression, although the level of expression was minimal higher in gastric cancer than normal gastric mucosal tissue.

The serum PGI/II ratio in gastric cancer is significantly lower than in the non-cancer group, p =0.045. The serum PGI/II ratio of less than 3.0 and *IL-8* mRNA expression ≥100 or $\log_{10}$ ≥2 are significant cut off risk differences between Thai cancer and non-cancer, p= 0.03 and p < 0.001, respectively. There is no direct correlation of *IL-8* mRNA expression level with the *cagA* gene mutation in Thai gastric cancer. In the multivariate analysis application, four factors found related to gastric cancer risk including *IL-8* mRNA expression in Thais. The high expression of *Il-8* gene demonstrated a poorer prognosis by stage and histology in signet ring cell type in this study.

Conclusion: In summary, *IL-8* mRNA expression had predominant level and trended to inverted correlation to PGI/II low ratio in Thai gastric cancer patients. The *IL-8* mRNA is a significant risk as well as *H.pylori* infection, and low pepsinogen I/II ratio for gastric cancer. However, there is no direct correlation of *IL-8* mRNA high expression with *cagA* mutation of *H.pylori* infection in Thai gastric cancer. The predominant *IL-8* mRNA expression level reflects individualized host stomach mucosal difference between Thai gastric cancer and non-cancer populations. The *IL-8* mRNA expression predominant level is related to poor prognostic cell type of gastric cancer in both nations. Gastric mucosal *IL-8* mRNA expression may be a feasible marker for future clinical gastric cancer research and treatment.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨			
受 付 番 号	乙 第2118号	氏 名	Sirikan Yamada
論 文 題 目 Title of Dissertation	Predominant mucosal *IL-8* mRNA expression in non-*cagA* Thais is risk for gastric cancer CagA 陰性 *H.pyliri* 感染における胃粘膜 IL-8 有意な発現はタイ胃癌の危険因子である		
審 査 委 員 Examiner	主 査 Chief Examiner 川端真人 副 査 Vice-examiner 林祥剛 副 査 Vice-examiner 平田健一		

（要旨は１，０００字～２，０００字程度）

Aim: We aimed to study the level of *Interleukine-8* (*IL-8*) gene expression , risk related factors such as the *cagA* genotype and serum pepsinogen I/II ratio, and factors correlated with gastric cancer in Northern Thais.

Results: In the genetic expression experiment, 86 cases of advanced gastric cancer and 134 non-cancer control cases were subjected. The *H.pylori* infection prevalence is reported by combined histopathology, *H.pylori IgG* Ab level, and 23SrDNA results that have 77.1% and 97.4 % of sensitivity and specificity, respectively. Among Thai cancer patients and non-cancer volunteers, *H.pylori* prevalence was 72.1% and 71.6%, respectively. Meanwhile, Thai gastric cancer cases had a *cagA* genotype demonstrated in only 7/62(12.3%) in positive *H. pylori* infection patients that yields 6 cases of East Asian and 1 case of Western type.

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