行政院國家科學委員會專題研究計畫 期中進度報告

Exploring the role of inflammation and immune reaction in mediating Helicobacter pylori-independent--探討發炎與免疫相關基因多形性與幽門桿菌治療後胃黏膜相關淋巴組織淋巴瘤各種不同腫瘤緩解反應的關係(子計畫二)(2/3)

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行政院國家科學委員會團隊研究計畫期中報告

主計畫：探討發炎與免疫相關基因多形性與幽門桿菌治療後胃黏膜相關淋巴組織淋巴瘤各種不同腫瘤緩解反應的關係 (2/3)

Main Project: Exploring the role of inflammation and immune reaction in mediating Helicobacter pylori-independent transformation of gastric mucosa-associated lymphoid tissue lymphoma (2/3)

子計畫二：探討發炎與免疫相關基因多形性與幽門桿菌治療後胃黏膜相關淋巴組織淋巴瘤各種不同腫瘤緩解反應的關係

Sub-Project 2: Determination of the relationship between polymorphisms in inflammation and immune regulation genes and various tumor response of gastric mucosa-associated lymphoid tissue lymphoma to H. pylori eradication therapy

計畫類別：□個別型計畫　■整合型計畫

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□兩年後可對外提供參考
(必要時，本會得展延發表時限)

執行單位：台大醫學院一般醫學科

中華民國 97 年 5 月 28 日

中文摘要

雖然大多數的胃黏膜相關淋巴組織淋巴瘤(簡稱 MALToma)皆有幽門桿菌感染，但是在所有感染的患者中只有極小部分會產生 MALToma，意謂除了幽門桿菌外尚有其它決定感染結果的重要因子。此外，以抗生素除菌只能使 60~70%的 MALToma 患者的腫瘤完全緩解，表示仍有一些非幽門桿菌依存性轉形的 MALToma。最近，吾人發現某些 MALToma 患者在除菌後腫瘤必須到 2 年後才完全消失，這些對除菌效果"延遲反應"但仍具幽門桿菌依存性的患者可能被誤認為幽門桿菌非依存性而接受如手術、化療、放射治療等不須要的額外治療。因此找出決定幽門桿菌依存或非依存性及不同治療反應的決定性因子對於 MALToma 致病機轉的了解和 MALToma 進一步的治療與預防相當重要。

目前認為幽門桿菌感染和宿主免疫反應間的失調是幽門桿菌感染後有不同預後及致病機轉的基本原因，故誘發和調控發炎反應的因子可能決定感染的預後。胃黏膜異常發炎反應與發生和發炎細胞素/趨化素(chemokine)的表現有關，不同個人間細胞素/趨化素(chemokine)的表現與分泌差異頗大，而且可能由基因上的單核苷酸多型性(簡稱 SNP)調控，這些 SNP 可以影響細胞素整體表現與分泌，此種個人遺傳體質在發炎基因上的不同表現可以部分解釋感染疾病為何有不同的預後。

為了探討趨化素 SNPs 和胃 MALToma 的關係，我們以病案對照方式選取 67 例 MALToma 和 290 例對照，抽取周邊白血球的 geomic DNAs，8 個基因(CCR6, CCL20, CCR9, CCL25, CXCR5, CXCL13, CX3CR1, CX3CL1)上的 84 個 SNPs 進行分析，結果發現，其中 4 個 SNPs 測試良好並且在病案和對照組間 P 值有明顯統計上的差別。並中 3 個 SNPs 屬於 CXCR5 基因(51530 在 coding region, 51050 和 51079 在 3'UTR region)，1 個 SNP (51075 在 promoter region)屬 CX3CL1 基因。目前我們正進行功能分析和比對治療反應，期別，幽門桿菌感染狀態以進一步釐清這 4 個 SNPs 的臨床病理意義。

關鍵詞：單一核苷酸多形性、臨床預後、胃黏膜相關淋巴組織淋巴瘤

Abstract

Although Helicobacter pylori (HP) is present in the vast majority of MALToma, only small subsets of infected individuals develop this malignancy, suggesting other factors must be involved in determining the fate after exposure to HP. Furthermore, eradication of HP by antibiotics induces durable tumor remission in 60-70% of patients, indicating existence of HP-independent mechanisms of transformation. Recently, we have found that some HP-dependent gastric MALToma may persist for a long time, even up to two years after HP eradication, before it eventually remits. It is conceivable that some of those delayed responders may have been classified as HP-independence and unnecessary modalities of treatment such as surgery, chemotherapy and radiotherapy may be given. Therefore, identification of determining factors associated with HP-dependent and –independent transformation and the variable treatment response is crucial not only for a better understanding of the pathogenesis of gastric MALToma but also for future prevention and treatment of this special malignancy.

It was assumed that the deregulation between The HP infection and the host's immune response is essential for pathogenesis and responsible for the various outcomes after H. pylori infection. Accordingly, factors involved in the initiation and regulation of the inflammatory response would dictate the disease outcomes. Inappropriately regulated inflammation of the gastric mucosa is orchestrated by sequential elaboration of pro-inflammatory cytokine/chemokines and the in vitro maximal capacity to produce different cytokines/chemokines varies among different individuals. Such individual differences are genetically determined and can be attributed to several molecular mechanisms, including single nucleotide polymorphisms in the coding or promoter regions of cytokine or cytokine receptor genes. These polymorphisms may affect the overall expression and secretion of cytokines. The observed genetically determined differences in inflammatory response might thus account for some of the heterogeneity of infectious diseases.

To determine the relationship between chemokine polymorphisms and gastric MALToma, we enrolled 67 cases and 290 controls. Genomic DNAs from peripheral leukocytes were extracted and 84 SNPs from 8 genes (CCR6, CCL20, CCR9, CCL25, CXCR5, CXCL13, CX3CR1, CX3CL1) were tested in these subjects. Among them, 4 SNPs with good assay and significant p-value were found. Three of them belong to CXCR5 gene (51530 at coding region, 51050 and 51079 at 3'UTR region) and one from CX3CL1 gene (51075 at promoter). Functional evaluation and correlation with parameters including treatment response, stage, and H. pylori infection will be performed and in progress to further elucidate the clinicopatholoic significance of these SNPs.

Keywords: single nucleotide polymorphisms, clinical outcome, mucosa-associated lymphoid tissue lymphoma

前言

Background & significance

HP infection & gastric MALToma

Mucosa-associated lymphoid tissue (MALT) lymphoma is a histologically distinct malignancy characterized by lymphoepithelial lesions infiltrated by monoclonal B-cells.[23] It usually arises from MALT that has been acquired as a result of chronic inflammatory conditions at sites normally devoid of MALT, such as the stomach, salivary gland, lung, thyroid and ocular adnexa.[24] Gastric MALT lymphoma (MALToma) accounts for at least 50% of primary gastric lymphoma.[23] The current model of MALT lymphomagenesis assumes that one or more neoplastic clones with characteristics of marginal zone B cells arise from background of organized MALT, colonize, and displace the original follicles, and eventually destroy the gastric glands and form lymphoepithelial lesions.[25] MALT lymphoma is generally considered an indolent tumor because of its slow growth and low propensity for spread. However, a portion of cases undergo high-grade transformation, thereby limiting treatment options and resulting in unfavorable disease outcomes. Recently, several lines of epidemiologic, clinical and laboratory evidence have suggested that the gastric MALT lymphoma is linked to chronic Helicobacter pylori (HP) infection.[24,25] It is now generally accepted that the antigenic stimulation required to initiate the lymphoproliferative processes of gastric MALT lymphomagenesis is provided by HP.

Variable outcomes of HP infection and different treatment responses of MALToma

HP is a micro-aerophilic, spiral-shape bacterium that is estimated to infect at least half of the world's population. Chronic persistent infection by HP may cause gastroduodenal diseases, such as gastritis, peptic ulcer or even adenocarcinoma. Although abundant studies have documented that HP infection is strongly linked to the development of gastric MALToma, only less than 0.01% of patients with HP gastritis progress to gastric MALToma.[26] Such variable outcomes after infection are dependent on the interaction of HP and immune reaction of the host. It was assumed that additional microbial virulence, environmental cofactors, and host genetic makeup might contribute to the process of gastric lymphomagenesis.[27]

Despite the fact that gastric MALToma represents the first described neoplasia susceptible to regression following antibiotic therapy, the treatment response after eradication of HP varies greatly. It is well known that eradication of HP induces durable tumor remission in 60~70% of the patients. By histologic criteria, gastric MALToma is classified into high-grade and low-grade subtypes. Traditionally, high-grade MALToma was treated by systemic chemotherapy. However, our colleagues first discovered that a substantial portion of early-stage high-grade MALToma remain HP dependent and can be cured by HP eradication.[18] Furthermore, with long-term follow-up, we found that some HP-dependent gastric MALToma may persist a long time before it eventually remits.[19] As shown in Fig.1 and Table 1, complete pathologic remission rate at 6 months was 78.6% and 45.8% for high-grade and low-grade gastric MALToma respectively. At 12 months, the remission rate was 92.9% and 75% respectively for high-grade and low-grade gastric MALToma.

Some HP-dependent gastric MALToma, especially low-grade patients, may persist even up to two years after HP eradication. It is conceivable that these "delayed" responder may be misclassified as HP-independence and received unnecessary modalities of treatment such as surgery, chemotherapy and radiotherapy. Collectively, it is imperative to understand the determining factors and underlying mechanisms of HP-dependence and independence for optimal management of gastric MALToma.

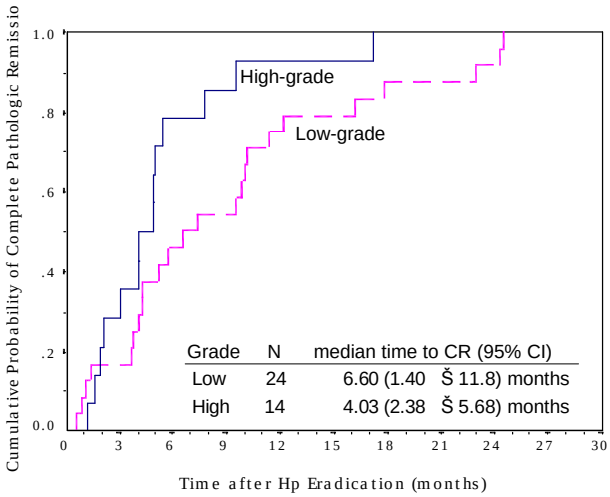


Figure 1. Time to complete pathologic remission after Hp eradication therapy in patients with Hp-dependent low-grade and high-grade MALT lymphoma.

Table 1. Complete pathologic remission rate at 3-month intervals after Hp eradication therapy in patients with Hp-dependent low-grade and high-grade MALT lymphoma.

	N	3 months	6 months	9 months	12 months
High-grade	14	28.6%	78.6%	85.7%	92.9%
Low-grade	24	26.7%	45.8%	54.2%	75%

Extensive inflammatory and immune cell infiltration is a constant characteristic of HP infection. Gastric inflammation is also a prerequisite for the development of various HP-related gastroduodenal diseases. The disease phenotypes of HP-infection are linked to the extent and severity of gastritis, duodenal ulcer being closely associated with antral gastritis whereas gastric cancer follows pangastritis and gastric atrophy.[28] For gastric MALToma, follicular gastritis and secondary inflammatory lymphoid tissues called mucosa-associated lymphoid tissues (MALTs) develop in the stomach after HP infection.[24] Ongoing exposure of the lymphoid cells of the MALT to HP might trigger their perpetual proliferation in order to eliminate HP. During this inflammatory process, increased release of reactive oxygen species might induce acquisition of genetic defects, which deregulate apoptosis and lead to malignant cells.[25] In addition, HP elicit a specific T-cell response in infected individuals with gastric MALToma.[29] In vitro co-culture studies showed these HP-induced T-cells exhibit abnormal help for autologous B-cell proliferation.[31] In animal models of Helicobacter-induced MALToma, immunophyenotyping revealed tumor B-cell proliferation was driven by T helper (Th) 2-polarized, immunocompetent, and activated cells. Furthermore, tumors were also densely colonized by follicular dendritic cells, whose numbers were closely associated

with and predictive of treatment outcome.[31] Taken together, HP infection can mount different inflammatory and immune responses, which may influence susceptibility to clinical diseases and the treatment outcomes.

Microbial characteristics & host's immune responses to clinical outcomes

In the past decades, the microbe itself stood at the heart of pathogenesis of infectious diseases. Considerable evidence has pointed out that strain variation may in part explain different outcomes of HP infection. Most strains of HP can be divided into two distinct phenotypes based on the presence or absence of a vacuolating toxin (vacA toxin) and the products of the cag pathogenicity island (cag PI), a large chromosomal region that encodes virulence genes. Some investigators believe that people infected with strains of HP with cagPI have more severe mucosal damage and are more likely to have adverse outcomes.[32,33] However, in Taiwan where HP infects most of the population, cag PI strains of HP are present in almost an infected people but only a few develop clinical disease.[7] Additionally, the majority of HP-reactive Th clones derived from MALToma proliferated to HP crude extract only but not to cagA or vacA.[34] One idea is that some still undefined but important antigens of HP are involved in driving T-cell activation and related B-cell proliferation in MALToma. Another possibility is that differences in host responses are critical. In this context, recent investigations have demonstrated exposure to the same HP strain may have different consequences in animals and human populations because of their different genetic background.[35,36] Moreover, studies of twins, familial clustering, ethnic differences and HLA genes have also provided strong evidence that host genetic factors play a significant role in the pathogenesis of HP related gastroduodenal disorders.[37,38] Specifically, the host response to environmental triggers, rather than bacteria or environmental factors per se, may confer different outcomes against HP infection.[39] It implicate that the delicate balance of host-bacteria interaction probably holds the key to understand underlying differences in immune response.

The critical balance between pro-inflammatory and anti-inflammatory cytokines

The extent and severity of mucosal inflammation after HP infection might be medicated by the interaction of pro-and anti-inflammatory cytokines. Studies of knockout animals have provided evidence that gastric inflammation to HP is more severe in IL-4 or IL-10 deficient mice.[40,41] In vitro and in vivo studies also indicated that HP could induce production of various cytokines and chemokines, such as interleukin (IL)-1, IL-4, IL-6, IL-7, IL-8, IL-10, interferon-γ, and tumor necrosis factor-α (TNF-α).[42] Such altered expression of cytokines may modulate the balance between Th1/Th2 responsiveness, and influence susceptibility for autoimmune disorders, infectious diseases and cancer. As regards to lymphoma, lymphoid development and differentiation of Th1/Th2 balance are regulated in part by key cytokines including IL-1, IL-2, IL-6, IL-10, TNF-α and lymphotoxin α.[43] Deregulated concentrations of several cytokines (e.g. IL-6, IL-10 and TNF-α) have also been detected in patients with lymphoma and were associated with an adverse outcome.[44] It is probable that abnormal production of an array of cytokines might promote activation and survival of B cells. Accordingly, it is the critical balance between pro- and anti-inflammatory cytokines that is responsible for disease progression and dictate the disease outcome after HP infection. The development and progression of gastric MALToma may thus be assumed to be facilitated in a

permissive cytokine environment by combination of different upregulate or down-regulated cytokine gene expression.

Genetic determinations of cytokine & chemokine production

The in vitro maximal capacity to produce different cytokines and chemokines varies among different individuals. Family studies indicate that much of this variability is genetically determined.[45] Such inter-individual differences can be attributed to several molecular mechanisms, including single nucleotide polymorphisms (SNPs) in the coding or promoter regions of cytokine or chemokine genes. These polymorphisms may affect the overall expression and secretion of cytokines or chemokines. Altered function arising from SNPs offers a possible explanation for variability to disease and pattern of disease progression.[46] In other words, different host cytokine or chemokine responses, determined at the genetic (transcription) level may explain why there is a variety of individual susceptibility to a given microbial infection or environmental toxins. These developments allow us the opportunity to investigate the role of SNPs in key immunoregulatory molecules (cytokines, chemokines, B cell and T cell receptors etc.)on the relative risk of gastric MALToma for a given individual.

The crucial role of genetic determinants of inflammatory and immune responses

A genetic basis for inter-individual variation in susceptibility to HP-related diseases has been indicated by an increasing number of genetic association studies.[47] These studies have implicated SNPs of cytokine genes act as host genetic factors influencing the outcomes of HP infection. For example, a recent breakthrough in the investigation of HP-related gastric adenocarcinoma is the identification that SNPs of IL-1β are strongly linked to the occurrence of hypochlorhydria and gastric cancer.[48] On the other hand, SNPs of TNF-α enhance the risk of HP-related duodenal ulcer.[49] As compared to other subtypes of non-Hodgkin's lymphoma, data regarding the role of SNPs in gastric MALToma are relatively few. There were reports demonstrating that IL-1β, TNF-α and toll-like receptor 4 polymorphisms were associated with, the risk of gastric MALToma.[50-52] Such researches, although still at the beginning, might account for some of the heterogeneity of infectious diseases and provide clues to fundamental questions about the pathogenesis of HP and gastric MALToma. If clarified, they may potentially allow more accurate prediction of variable outcomes of gastric inflammation and appropriate adjustment of treatment strategies, as well as indicating novel areas for future studies of lymphomagenesis.

Controversial and incomprehensive results of SNPs in gastric MALToma

Apart from possible associations of cytokine SNPs with pathogenesis or prognosis of gastric MALToma, their association with severe immune and inflammatory reactions may have an impact on treatment responses. However, previous studies lacked consideration of HP-dependence and independence and whether these polymorphisms are related to treatment outcomes remains to be determined.[50-52] Furthermore, some controversial results exist. Rollinson et al. first reported that both IL-1 RN2/2 and GST T1 null genotypes enhanced the risk of gastric MALToma.[50] However, Hellmig and colleagues could not confirm these results.[51] Therefore, further studies in this field are mandatory.

Our previous studies and preliminary results relevant to this proposal

Our colleagues have recently demonstrated that nuclear translocation of NF-κB and Bcl-10 of the tumor cells is closely associated with HP-independent status of gastric MALToma.[20,21] Activation of TNF-α signals is sufficient to recapitulate the scenario of nuclear translocation of both NF-κB and Bcl-10 in the tumor cells,[22] suggesting that HP-independence transformation may be related to altered signals of the inflammatory machinery of the hosts. Furthermore, they have observed that thalidomide, an anti-TNF-α agent, induced tumor remission in around 70% of HP-independent MALToma (unpublished data). Together, these lines of evidence suggest that HP-independence transformation of MALToma may occur in a later phase of the disease process when either inflammation in the tumor microenvironment or inflammation-related signal transduction of the tumor cells is so altered that the signals of the inflammation-related cytokines, such as TNF-α, continue to operate in the absence of HP.

We have previous experiences in the study of host genetic polymorphisms and gastric adenocarcinoma. Our group has demonstrated certain HLA alleles, polymorphisms of E-cadherin, cytokine and xenophobic-metabolizing enzyme genes were associated with the risk of gastric adenocarcinoma.[12-17] We also reported the role of TNF-α and IL-1β genotypes in gastric MALToma.[53,54] To be launched for this project, we have analyzed T cell regulatory gene (CTLA4, CD28, ICOS) polymorphisms, clinicopathologic characteristics and HP dependent status in a cohort of 62 gastric MALToma and 250 controls. Our preliminary results revealed CTLA449 G/G genotype was associated with a six-fold increased risk of developing MALToma in patients infected with HP. These results indicate genetic link of CTLA4 gene polymorphism to development of gastric MALToma and indirectly support the crucial role of host activated T cell in the MALT lymphomagenesis. [55]

Purpose of the investigation

Our team and other researchers have recently documented that subdivision of gastric MALToma into HP-dependent and -independent is crucial for diagnosis and treatment of this special malignancy. Specifically, our colleagues have first demonstrated that a substantial portion of early-stage high-grade gastric MALToma, traditionally treated by systemic chemotherapy, remain HP-dependent and can be cured by HP eradication.[18,19] In addition, we have found that some HP-dependent gastric MALToma may persist for a long time, even up to two years after HP eradication, before it eventually remits.[19] It is conceivable that some of those delayed responders may have been classified as HP-independence and unnecessary modalities of treatment such as surgery, chemotherapy and radiotherapy may be given. We have also uncovered several lines of evidence which indicate that altered signal transduction of inflammation or polymorphisms in immune reaction genes of the host would dictate the disease outcome of chronic HP infection.[20-22] Collectively, elucidation of the underlying mechanisms and identification of predictive markers for HP-dependence and-independence are crucial for management of gastric MALToma. Thus, the overall objective of this integrated research program is to explore the role of inflammation and immune reaction in mediating HP-independent transformation of gastric MALToma. In this subproject, we will address the issue whether genetic determinants of the immune and inflammatory response are linked to the variable infectious outcomes and treatment responses of gastric MALToma.

Study Subjects

Since January 1998, blood samples have been prospectively collected from individuals who participated in the national project on risk factors and natural history of gastric MALToma. Study protocol was approved by the Department of Health, Executive Yuan, Taiwan and the Taiwan Cooperative Oncology Group for gastric MALToma. A full verbal explanation of the study was given to all participants. They consented to participate on a voluntary basis. Patients with newly diagnosed gastric MALToma were enrolled from inpatient units and outpatient cancer clinics of 4 major medical centers in Taiwan. Inclusion and exclusion criteria along with the diagnostic criteria for tumor grading and staging were defined previously.[18] In brief, high-grade MALToma was defined as the presence of confluent clusters or sheets of large cells resembling centroblasts or lymphoblasts within predominantly low-grade centrocyte-like cell infiltrate, or predominance of high-grade lymphoma with only small, residual, low-grade foci and/or the presence of lymphoepithelial lesions. Patients with primary pure large-cell lymphoma, without evidence of a low-grade component, of the stomach were excluded. All patients were Han Chinese, and none had a family history of gastric malignancy. For the control group, we randomly screened subjects from health-examination clinics. They did not have malignancy or any autoimmune / immune-mediated disease such as type 1 diabetes, Graves' disease, autoimmune hypothyroidism. The main project will provide the biospecimens and necessary follow-up and treatment of these subjects.

HP infection was screened with serology test using a standard ELISA and confirmed by histologic examination, biopsy urease test, or bacterial culture. Patients were scheduled for regular follow-up as stated previously. Complete histologic remission was defined as a Wotherspoon's score of 2 or less on every histologic section of the biopsy specimens. Systemic chemotherapy would be given for those with grossly stable or progressive disease during follow-up courses.

DNA isolation and genotyping of candidate genes

Peripheral blood samples are collected after obtaining informed consent from each subject. Genomic DNA will be extracted from peripheral blood leukocytes using a DNA purification kit (QIAamp DNA Blood Mini kit; Qiagen, USA) according to the manufacturer's instructions. We will analyze SNPs of pre-selected genes including CTLA4, BAFF, FASL etc. based on their potential roles in inflammation and immune regulation. All PCRs are performed on a Perkin Elmer GeneAmp 9700 system and the presence of amplicons is checked on agarose gels. A single nucleotide primer extension assay will be carried out to analyze SNP using a SNaPshort Kit (Applied Biosystems, USA). The extended primers are analyzed on an ABI 3100 (Applied Biosystems). Initial denaturation will performed at 95℃ for 2min, followed by 35 cycles each consisting of denaturation at 95℃ for 30s, annealing at 60℃ and extension at 72℃ for 1 min, followed by final extension at 72℃ for 8 min. All laboratory assays will be conducted and interpreted blind, without the knowledge of case or control status.

Statistical Analysis

Hardy-Weinberg equilibrium will be tested among controls in the population under

investigation. The data will be analyzed using the SPSS 10.0 statistical software. The relative association between patients and controls for genotype or allele prevalence will be assessed by the χ^2 test or Fisher's exact test when necessary. The effect of tumor stage to anti-helicobacter therapy will be compared by the Kruskal-Wallis H test. Odds ratios and 95% confidence intervals (CIs) for relative risks are calculated. Statistical significance was set at the standard 5% level. Significant SNP associations will be also correlated with specific genomic alterations found in subproject 4.

結果與討論

1st SNP-typing

- Genotype: 44 SNPs (6 candidate gene regions: CX3CR1, CX3CL1, CXCL13, CXCR5, CCR6, CCL20)
- Phenotype: MALToma with different grades & response to treatment

Haplotype Analysis

Gene	Assay_id	SNP Marker	Case vs. control				
			Haplotype	Frequency		P-value	Global p-value
				Control	case		
CX3CL1	51075	rs223815	GCG	0.34429	0.29545	0.28692	0.00305
	51085	rs12928117	GCT	0.63668	0.62879	0.86488	
	51070	rs4151117	CCT	0.01903	0.07576	0.00078	
CXCR5	51049	rs630923	CCG	0.07283	0.01493	0.00981	0.04162
	51050	rs3922	CCC	0.21425	0.1791	0.34759	
	51079	rs676925	ACG	0.07014	0.05224	0.43646	
			CTC	0.63703	0.75373	0.01154	

2nd SNP-typing

- Total tested genes: 7
  CCR6 (15), CXCR5 (5), CXCL13 (2), CX3CR1 (2), CX3CL1 (1), CCR9 (5), CCL25 (10)
- Total tested SNPs: 40
- Unsuccessful SNP-typing: 5
  CCR6 (2), CXCR5 (0), CXCL13 (1), CX3CR1 (0), CX3CL1 (0), CCR9 (0), CCL25 (2)
- Cause: Low successful rate (No call):
  3 Calls from negative control (primer dimer): 2

Gene	SNP-ID	Primer_ ID	P value of Single point association test							
			case	control	Pro Genotype	Prob Allele	Prob Trend	XP2 FISH	Case aa:ab:bb	Control aa:ab:bb
CCR6	rs3093019	51515	288	65	0.0350	0.0352	0.0350	0.1841	288:0:0	64:1:0
CXCR5	rs598207	51530	289	63	0.0033	0.0013	0.0014	0.0028	21:115:153	2:13:48

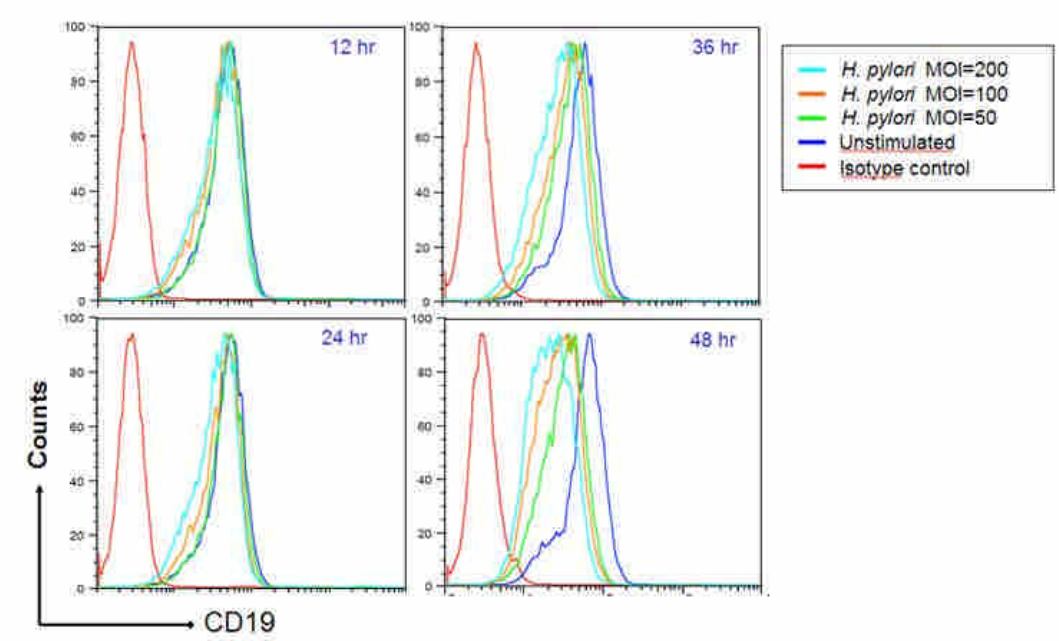
Summary

- We have tested 84 SNPs from 8 genes (CCR6, CCL20, CCR9, CCL25, CXCR5, CXCL13, CX3CR1, CX3CL1) in total
- 4 SNPs with good assay and significant p-value were found. Three of them belong to CXCR5 gene (51530 at CDS region, 51050 and 51079 at 3'UTR region) and one from CX3CL1 gene (51075 at promoter)
- Several potential SNPs from CCR6, CX3CR1, CCL25 were also found

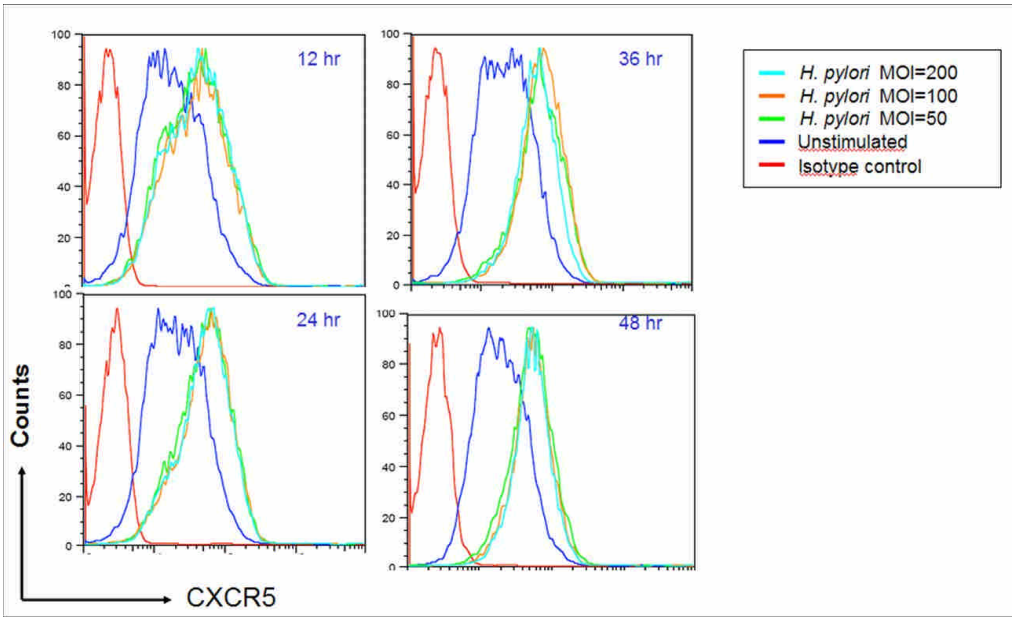
333 bp
88 bp

	rs2230319	chr11:118269592	84	G/T	Coding exon	G/G
	rs2230320	chr11:118270291	699	C/T	Coding exon	H/H
	rs2230321	chr11:118270297	6	C/T	Coding exon	V/V
	rs12795395	chr11:118270441	144	C/T	Coding exon	G/G
51530 →	rs598207	chr11:118270477	36	C/G	Coding exon	T/T
	rs665648	chr11:118270493	16	C/T	Coding exon	G/S
51050 →	rs3922	chr11:118270810	317	C/T	Exon	-
	rs1053881	chr11:118270870	60	A/G	Exon	-
51079 →	rs676925	chr11:118270898	28	C/G	Exon	-
	rs3741331	chr11:118271266	368	A/G	Exon	-
	rs1063929	chr11:118271417	151	G/T	Exon	-
	rs3741330	chr11:118271466	49	A/C	Exon	-
	rs3136698	chr11:118271578	112	A/G	Exon	-
	rs1053879	chr11:118271681	103	C/G	Exon	-
	rs1053877	chr11:118272054	373	A/C	Exon	-
	rs14811	chr11:118272070	16	A/G	Exon	-

CD19 Expression is Down-regulated by H. Pylori in Romas B-cell



CXCR5 Expression is Up-regulated by H. Pylori in Romas B-cell



Significant SNP found

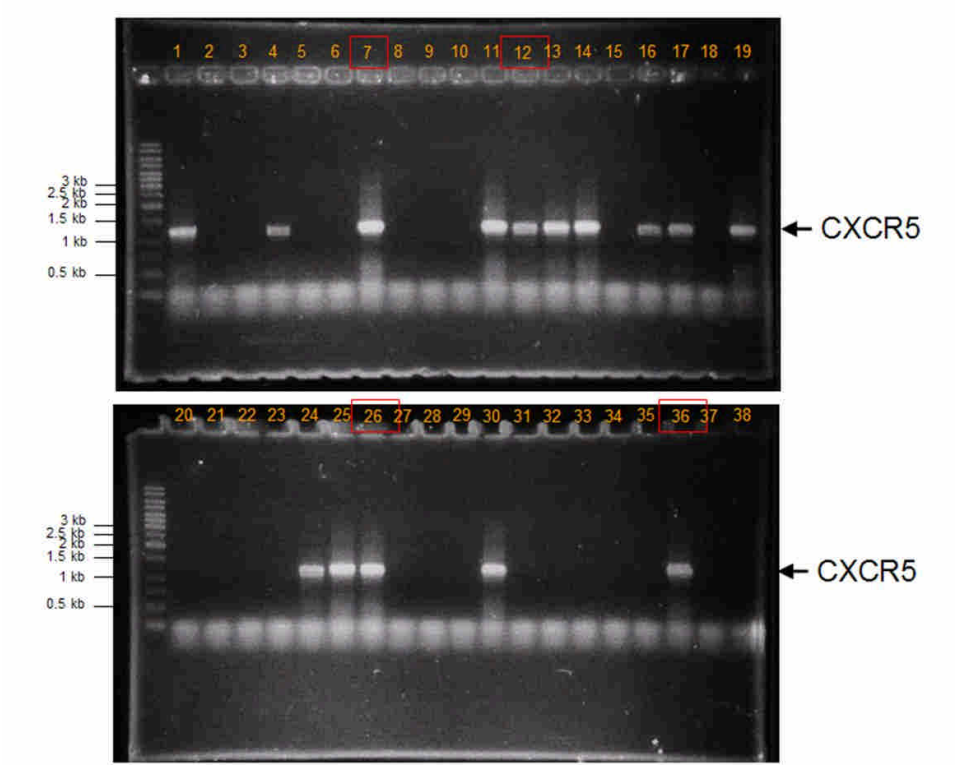
in MALToma Typing

SNP rs_ID	Gene	alleles	role	Amino acid change
rs598207	CXCR5	C/G	Coding region	T/T
rs3922	CXCR5	C/T	3’ UTR	-
rs676925	CXCR5	C/G	3’ UTR	-

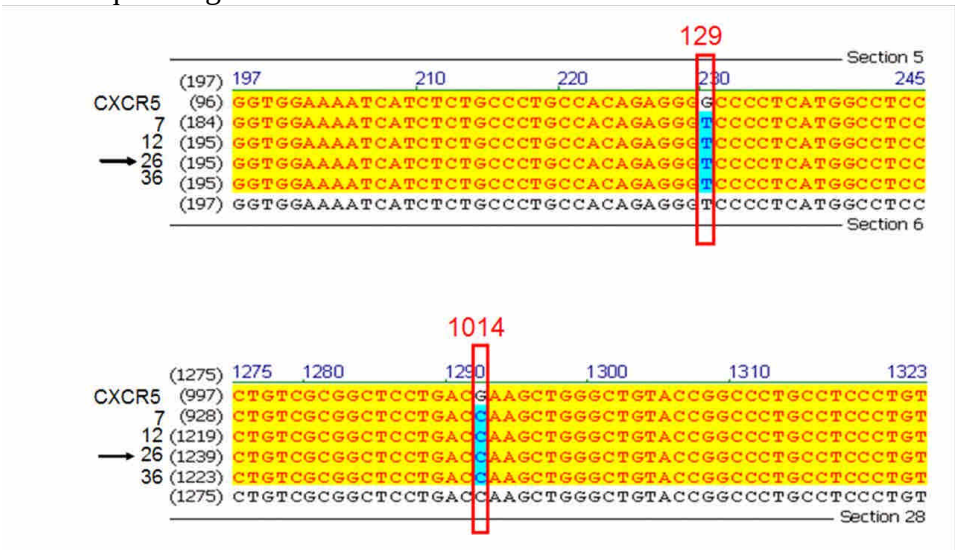
Cloning vector and insert

- Vector：pIRES2-EGFP
- Insert ：CXCR5 (PCR product from Raji cDNA)

Screening CXCR5 positive colony by colony PCR



DNA sequencing results of CXCR5



Genotype in Nigerian and Taiwanese

Nigerian

129G/T	Polymorphism	rs_2230319
1014C/G	Polymorphism	rs_598207

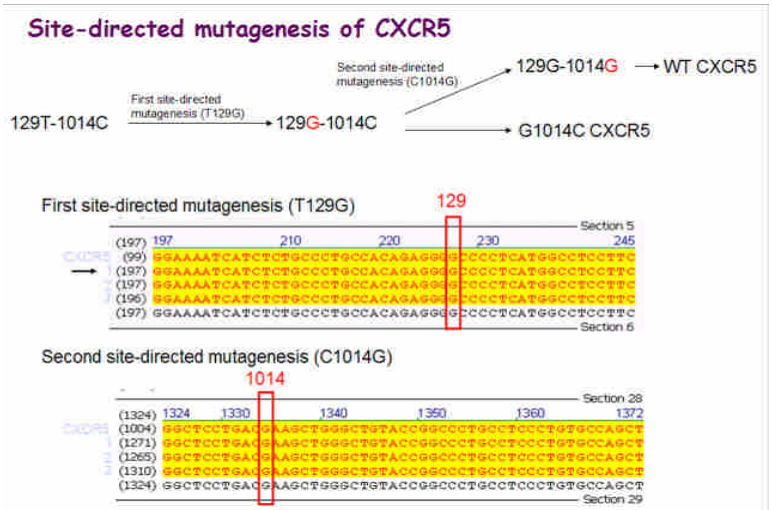
CXCR5 PCR product from Raji cDNA: 129T-1014C

Taiwanese

129G	No polymorphism	
1014C/G	Polymorphism	rs_598207

WT CXCR5 : 129G-1014G

G1014C CXCR5 : 129G-1014C



On-going work

Transfect WT CXCR5/pIRES2-EGFP or G1014C CXCR5/pIRES2 EGFP into HEK 293 cells.

- Compare the level of mRNA expression between WT and G1014C CXCR5 by RT-PCR.
- Compare the level of protein expression between WT and G1014C CXCR5 by Flow cytometry.
- Compare the protein conformation between WT and G1014C CXCR5 by trypsin digestion.
- Compare the biological function between WT and G1014C by calcium flux and chemotaxis/migration assay.

On-going work

MicroRNA

- Search the possible miRNA(s) that target to the 3'UTR of CXCR5.

	RNAhybrid	Mirnada
rs_3922	75	6
rs_676925	11	9

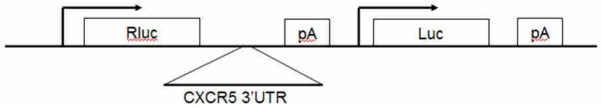
Potential candidate

hsa-miR-382

```
3' GCUUAGGUGGUGCUUGUUGAAG 5'
   | ||| | | |  |||||||||
5' CCAATGCTCAAGAAACAACTTC 3'
```

NM_001716

- Use luciferase reporter assay to exam whether rs3922 and rs676925 affect miRNA targeting.



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