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胃癌致病機轉:以 cytokine 和 chemokine 基因單核甘酸多型性探討宿主敏感性角色(2/3)

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行政院國家科學委員會專題研究計畫成果報告

胃癌致病機轉：以cytokine和chemokine基因單核甘酸多型性探討宿主敏感性角色(2/3)

Gastric carcinogenesis: the role of host susceptibility studied by single muclectide polymorphisms of cytokine and chemokine genes (2/3)

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中 文 摘 要

胃癌為全世界第二常見惡性腫瘤，在台灣則每年有 2000 以上患者死於胃癌，佔癌症死因第四位。胃癌致病機轉複雜且主要經由遺傳敏感性和環境因子交互作用。過去多數研究著重於胃癌環境危險因子，有不少報告指出幽門桿菌感染和抽煙為主要致病因素，但是只有少數暴露這些因子的人得到胃癌，而且最近新的証據也顯示個體的遺傳因子也佔重要角色，更精確而言是宿主對環境因素的反應，而非細菌和環境因子本身，是造成較易或較不易發生胃癌的主因。

在胃癌的多步驟改癌過程中，胃發炎是胃癌發生的必經之路。因此誘使和調控發炎反應的因子可能在預後的決定上有關鍵作用，而遺傳因子和其它的環境因子也可能是透過胃炎的層次來影響。在此觀點中，cytokines 和 chemokines 由於本身在微生物免疫和發炎反應有一定角色，是最有可能控制胃炎預後的主要因子。故假設不同促發炎和抗發炎 cytokines 間的平衡決定了胃炎的後果，而胃癌可能是由不同 cytokine 基因的表現增加和減少組合成特定的 cytokine 環境下產生。

幽門桿菌和抽煙皆能誘發胃的 cytokine 和 chemokine 表現。而不同個體間 cytokine 產生能力差異頗大。這種個體差異主要是由於 cytokine 基因上的單核甘酸多形性(SNPs)造成。因此，個體在基因(或 transcription)層次上所導致的不同 cytokine 反應或許可解釋對感染或環境毒素的個體敏感性的差異。這些發現促使吾人得以探討 cytokines,chemokines 和其受体等免疫調控主要分子的 SNPs 在個人胃癌相對危險的角色。最近兩個研究也指出 IL-1β 的 SNPs 與胃癌危險性有關。雖然如此，由於基因型受種族影響，這些研究必須考慮種族的差異性而有所不同，而且基因－基因和基因－環境的交互作用在這些研究中也付之闕如。

在第一年的計劃裏，吾人收集 220 例胃癌及 230 例健康對照，抽取白血球 DNA，分析 TNF-α，IL-1，IL-4 和 IL-10 等基因上的 11 處多型性(表一)，並將基因型與 H. pylori 感染和抽煙等環境因子比較，結果顯示 H. pylori 感染 [odds ratio (OR) 1.7, 95% confidence interval (CI) 1.19~2.56]，抽煙 (OR:2.02, 95% CI 1.38~2.95)和高 IL-10 製造基因型 (OR:2.67, 95%ci 1.29~5.50)明顯增加胃癌發生的危險性。進一步分析不同亞型發現瀰漫型胃癌 (OR:1.64, 95%CI 1.01~2.61)或賁門癌 (OR:2.44, 95%CI 1.13~2.67)與

IL-4 (-590)的 CT/CC 基因型有關，而高 IL-10 基因型則與賁門癌(OR:3.21, 95% CI 1.06~9.73)或進行性胃癌(OR:2.29, 95%CI 1.14~2.52)有關，至於 IL-1 和 TNF-α基因型則和胃癌危險性無關。以迴歸分析則顯示 H. pylorh 感染(OR:1.7, 95%CE 1.14~2.52)，抽煙(OR:1.81, 95%CI 1.27~2.96)和 IL-10 基因型(OR:2.54, 95%CI 1.24~5.61)為胃癌發生的獨立危險因子。上述結果明白顯示胃癌的發生受到宿主和環境因子等共同影響。

在第二年的計劃裏，利用 cDNA microarray 分析不同菌株(潰瘍、胃癌、淋巴瘤)對上皮細胞的基因表現影響，初步已發現一些重要基因的改變在不同幽門螺旋桿菌疾病有所不同。另外，持續收集的病案與對照例數已超過 250 對。而在已知基因多型性的分析方面，吾人則進行 TNF-α(-238, -308, -857, -863, -1031)，TNF-α受器基因 TNFR1(-383)及 TNFR2(codon 196)，CD14(-159)與 Toll-like receptor 4(TLR4 Asp299Gly, Thr399ILe)，和 IL-8(-251)等基因多型性的對照病例研究，發現 TNF-α –857C→T 與胃黏膜相關淋巴瘤的危險性有關(OR=0.33，95%CI：0.15~0.75)，但與胃癌則並無相關性，這些結果顯示胃癌與胃黏膜相關淋巴瘤致病機轉上之明顯不同。

在第三年的計劃裏，吾人將收集 300 例不同表現型胃癌的病例以做為進一步分析之用，並利用 cDNA microarray 之結果，探討具 biologic significance 且為 novel 的 candidate gene 多形性於宿主敏感性之角色。

關鍵詞：胃癌，幽門螺旋桿菌，基因型，宿主易感性

Abstract

Gastric cancer (GC) is the second most common malignancy in the world. In Taiwan, GC remains the fourth leading cause of cancer death with more than 2000 persons dying of this devastating disease annually. The pathogenesis of GC is complex and consists of two interacting elements: genetic susceptibility and environment factors. In the past decades, much attention has been paid to the environmental risks of GC. Considerable evidence has pointed out that H. pylori infection and smoking are among the major determinants of GC. However, only a small portion of patients exposed to environmental stimuli develop GC. Recently, new evidence has emerged suggesting that host genetic factors also play a critical role. Specifically, the host response to environmental triggers, rather than bacteria or environmental factors *per se*, may confer susceptibility to or protection against GC.

In the multistage model of gastric carcinogenesis, gastric inflammation is a prerequisite for the development of GC. Therefore, factors involved in initiation and regulation of the inflammatory responses may play a pivotal role in determining outcomes and genetic factors, like various H. pylori strains or environmental toxins, may operate at the level of gastric inflammation. In this respect, the intrinsic role of cytokines and chemokines as modulators of anti-microbial immunity and inflammatory responses makes them attractive candidates as a key regulator influencing the outcome of gastritis. It was assumed that the critical balance between pro- and anti-inflammatory cytokines would dictate the outcomes of gastric inflammation and development of GC might be facilitated in a permissive cytokine environment by combination of different unregulated or down regulated cytokine gene expression.

Both H. pylori infection and cigarette smoking could induce cytokine and chemokine expression in the stomach. The in vitro maximal capacity to produce different cytokines varies among different individuals. Such inter-individual differences can be attributed to single nucleotide polymorphisms (SNPs) in the coding or promoter regions of cytokine genes. SNPs may affect the overall expression and secretion of cytokines. Therefore, different host cytokine 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, and their receptors on the relative risk of GC for a given individual. Two recent studies have indeed provided evidence that SNPs of IL-1β are associated with the risk of GC. However, such study was performed mainly in Caucasians and the

genotype status varies greatly between different ethnic backgrounds. Furthermore, studies concerning gene-environment and gene-gene interactions remain lacking.

In the first year grant period, we analyzed 11 functional polymorphisms in tumor necrosis factor-α (TNF-α), interleukin (IL)-1, IL-4 and IL-10 genes (Table 1) in 220 Taiwanese Chinese with GC and in 230 healthy controls. The risk of genotype was adjusted with confounding environmental risks. Our results revealed that the frequency of Helicobacter pylori infection [odds ratio (OR) 1.7, 95% confidence interval (CI) 1.19~2.56], cigarette smoking (OR:2.02, 95%CI 1.38~2.95) and high IL-10 producer genotype (OR:2.67, 95%CI 1.29-5.50) was significantly increased in the entire GC patients. Among different subtypes of GC, a higher risk of developing diffuse type (OR:1.64, 95%CI 1.01~2.67) or cardia cancer (OR:2.44, 95%CI 1.13~2.67) was observed for the CT/CC genotype of IL-4 at the position –590, whereas the high IL-10 producer genotype was significantly linked with the risk of cardia cancer (OR:3.21, 95%CI 1.06-9.73) or advanced stage (OR:2.29, 95%CI 1.12-4.64). No association was noted between GC and controls in the distribution of IL-1 and TNF-α genotypes. Logistic regression analyses revealed that H. pylori infection (OR:1.7, 95%CI 1.14~2.52), cigarette smoking (OR:1.87, 95%CI 1.27-2.96) and IL-10 genotype (OR:2.54, 95%CI 1.24~5.61) are independent risks for GC. Independent efects of IL-10 genotype, H. pylori infection and cigarette smoking indicate that carcinogenesis of GC is influenced by a variety of host and environmental factors.

In the second year grant period, analyses of gene expression in AGS induced by different strains of H. pylori has been performed by cDNA microarray. We have found some differences among bacterial strains from various disease (ulcer vs. cancer vs. maltoma). In addition, case and control enrollment keeps continuing and is more than 250 in numbers. Genetic polymorphisms in TNF-α (-238, -308, -857, -863, -1031), TNFR1 (-383), TNFR2 (cadon 196), CD14 (-159), TLR4 (Asp299 Gly and Thr 399 Ile) and IL-8 (-251) have been investigated in GC, maltoma and controls. Our results demonstrated TNF-α –857 C→T was significantly underrepresented in maltoma compared to controls (6.4% vs. 14.3%, p=0.018), conferring a 3-fold decreased in risk (OR=0.33, 95%CI 0.15-0.75). Comparison of allele frequencies between GC and controls failed to show any statistical significance. The differences in genetic background as well as divergent clinicopathologic features between GC and multoma supports the notion that fundamental mechanistic differences exist in these 2 well-defined H. pylori-associated maliguancies.

In the third year grant period, we will collect at least 300 cases of GC

with different but well-characterized phenotypes for further genotypic analyses. The novel genes with biologic significance in H. pylori-associated diseases disclosed by cDNA microarray will be tested for their roles in host susceptibility of gastric carcinogenesis.

Keywords: Gastric cancer, Helicobacter pylori, Genotype, Host Susceptibility

Table I. Primer sequences and methods used for detection of cytokine gene polymorphisms

Gene	Primers	PCR condition	Detection method
TNF-α (-238,-308)	5'-CAAACACAGGCCTCAGGACTC-3' 5'-AGGGAGCGTCTGCTGGGCTG-3'	95℃ 40s 65℃ 1min 72℃ 30s 35 cycles	direct sequencing with primer 5'-TCTGGAAGTT AGAAGGAAAC-3'
TNF-α (-857,-863, -1031)	5'-GCTTGTGTGTGTGTCTGG-3' 5'-GGACACACAAGCATCAAGG-3'	94℃ 1min 55℃ 2min 72℃ 3min	Direct sequencing with primer 5'-TGTGGCCATATCTTCTTAAA-3'
TNFR1 (-383)	5'-TTATTGCCCCTTGGTGTTTGGTTG-3' 5'-GGAGGGGAAGAGTGAGGCACTGTT-3'	95℃ 30sec 65℃ 30sec 72℃ 30sec 35 cycles	BgIII digestion, 2.5% agarose gel electrophoresis
TNFR2 (codon 196)	5'-ACTCTCCTATCCTGCCTGCT-3' 5'-TTCTGGAGTTGGCTGCGTGT-3'	95℃ 30sec 72℃ 30sec 35 cycles	NlAIII digestion 4% agarose gel electrophoresis
IL-1β (-31,-511)	5'-CTCAGAGGCTCCTGCAATTG-3' 5'-AGATAAGCAGTATCCATTCCC-3'	95℃ 45s 55℃ 1min 72℃ 1min 35 cycles	direct sequencing with primer 5'-TCGTTCTGCAG TTGATGTCCA-3'
IL-1RN (intron 2 VNTR)	5'-CTCAGCAACACTCCTAT-3' 5'-TCCTGGTCTGCAGGTAA-3'	95℃ 1min 58℃ 1min 72℃ 1min 35 cycles	size fractionation in 2.5% agarose gel electrophoresis
IL-4(-590)	5'-ACTAGGCCTCACCTGATACG-3' 5'-GTTGTAATGCAGTCCTCCTG3'	95℃ 30s 55℃ 30s 72℃ 30s 35 cycles	Bsm FI digestion, 6% polyacrylamide gel electrophoresis
IL-4R (Ile50 Val)	5'-GGCAGGTGTGAGGAGCATCC-3' 5'-GCCTCCGTTGTTCTCAGGTA-3'	95℃ 30s 55℃ 30s 72℃ 1min 35 cycles	Rsa I digestion, 3% agarose & 1% NuSieve agarose gel electrophoresis
IL-4R(Q576R)	5'-GCCCCCACCAGTGGCTACC-3' 5'-GCCTTGTAACCAGCCTCTCCT-3'	94℃ 30s 55℃ 30s 72℃ 1min 40 cycles	Msp I digestion, 6% polyacrylamide gel electrophoresis
IL-10(-592,-819,-1082)	5'-ATCCAAGACAACACTACTAA-3' 5'-TAAATATCCTCAAAGTTCC-3'	94℃ 40s 54℃ 45s 72℃ 30s 35 cycles	direct sequencing with primer 5'-TAAATATCCTCA AAGTTCC-3' and 5'-TTGGCCTTAGAG TTTCTTTT-3'

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