行政院國家科學委員會專題研究計畫　期中進度報告

宿主易感性與宿主－微生物交互作用在胃黏膜相關淋巴瘤發生與進展的角色(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

宿主易感性與宿主－微生物交互作用在胃黏膜相關之淋巴瘤發生與進展的角色(2/3)

Host susceptibility and host-microbe interaction in the development and progression of gastric mucosa-associated lymphoid tissus lymphoma

計畫類別：■個別型計畫　□整合型計畫

計畫編號： NSC92-2314-B-002-123

執行期間： 92年 8月 1日至 93年 7月 31日

個別型計畫：計畫主持人：林肇堂醫師

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子計畫主持人：

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中 文 摘 要

黏膜相關淋巴瘤(Maltoma)因其獨特的組織及臨床特徵而漸受矚目，胃部為 Maltoma 最好發的部位，而且在發生率逐漸增加的胃淋巴瘤中，佔了約 40~50%。與淋巴結發生的 B 細胞淋巴瘤不同的是，胃 Maltoma 在發現時期別較早且腫瘤也較慢散播出去。雖然由組織型態學、流行病學、實驗和臨床資料等証據顯示幽門螺旋桿菌(HP)和胃 Maltoma 息息相關，根除 HP 治療胃 Maltoma，仍然有 20~40%的胃 Maltoma 對抗生素無效。另外，只有極少數感染 HP 的患者發生胃 Maltoma。到底那些因素決定了治療成敗？為何只有少數敏感者易罹患胃 Maltoma？至今仍不清楚。雖然早期國外的研究認為高惡性度的胃 Maltoma 可能對治療無效，但是最近三年來，包括吾人和國外的研究皆顯示胃 Maltoma 的 HP 治療成效與組織學的惡性度無關。因此，如何區別治療有效與無效的病例？如何辨認 HP 是致病感染還是非致病感染？臨床醫師對於 HP 相關疾病和胃 Maltoma 如何作合理處置？仍是極大的挑戰。

目前認為胃炎的強度和胃 Maltoma 發生的危險性是 HP 慢性感染後宿主不適當反應的結果。胃炎可能和 HP 感染後的不同預後有關，控制微生物免疫和發炎的宿主基因也可能影響感染 HP 後的危險性。由家族、雙胞胎、動物和病案對照組等研究也證實發炎強度和胃黏膜損傷隨宿主反應的基因差異而定。顯示“ 多種基因的特殊結合決定宿主敏感性 ”是為何得到 HP 感染者只有一小部分發病的合理假設。因此研究基因差異和疾病表現型的相關性將有助於更精確地預測 HP 感染後的不同預後，適當調整治療策略，並提供 HP 進一步的研究方向。

在第一年的計劃中，吾人收集 70 例胃 Maltoma 和 210 例健康對照，抽取白血球中的 DNA，以 polymerase chain reaction (PCR)配合 direct sequencing 分析 IL-1β(-31, -511)及 TNF-α(-238, -308, -857, -863, -1031)的基因多型性，另外以 PCR 配合 restriction enzyme length polymorphism 分析 IL-6(-174)，TNFR1 (-383), TNFR2 (codon 196)等基因多型性。結果顯示在 TNF-α-857 的 C→T 變異，Maltoma 明顯低於對照(6.4% vs. 14.3%, p=0.018)，換算成勝算比(odds ratio)為 0.33 (95%依賴區間 0.15~0.75)。至於其它位置的基因多型性則與 Maltoma 之發生無關。由於 TNF-α-857T 具較低的 transcription activity，且 TNF-α在 lymphoma 發生扮演重要角色，因此吾人上述結果有利於 Maltoma 致病機轉的了解，並首次提供 host genetic

factor 在 Maltoma 發生有關的證據。

由於 glutathione S-transferase 和 interleukin-1β 的基因多形性最近有報告發現與 Maltoma 的發生有關係，因此吾人在第二年的計劃中以 75 位患者配合 321 位健康對照者分析 GSTM1、GSTP1、GSTP1、GSTT1、IL-1β 和 IL-1 receptor antagonist (IL-1RN)的基因變異，結果在對照組的變異基因型分佈為 GSTM1 null 52.4%, GSTT1 null 43.0%, GSTP1 105 val/val 7.4%, GSTP1 105 Ile/val 25.9%, IL-1β –511T/T 19%, -511 C/T 54.2%, IL-RN 1/2 & 2/2: 11.2%, 上述分佈與 Causasians 略有不同，但與過去華人報告相同。其中 GSTM1, GSTP1, IL-1β 和 IL-1RN 的基因型分佈與 Maltoma 並無統計上差異，但是 GST T1 null 型在 Maltoma 則高於對照組 (43/75 vs. 138/321, p=0.029; OR=1.8, 95%CI: 1.1~3.0)。上述結果支持 Maltoma 的宿主易感性可以經由對 carcinogen 活化與解毒能力的遺傳能力差異而達成，而且吾人資料也顯示基因多形性的研究有種族上的不同。

第三年的計劃中，吾人除了繼續以 candidate gene association study 方式分析一些在 lymphoma 形成過程中重要的 chemokine gene polymphism 外，也探討這些 genotype 與治療的關係。另外，吾人也將利用 cDNA microarray 的全基因分析找出一些 potential candidate 進行 polymorphism 及 association 的分析。

關鍵詞：宿主易感染性，細胞素基因多型性，胃黏膜相關淋巴瘤

Abstract

Gastric mucosa-associated lymphoid tissue lymphoma (Maltoma) have received increasing attention in recent years because of their unique histologic and clinical features. Stomach is the most common site of Maltoma and represent 40~50% of gastric lymphoma with increasing incidence. In comparson with nodal B-cell lymphoma, gastric Maltomas are usually at low stage when diagnosed and slow to disseminate. Several lines of evidence, including histomorphological, epidemiological,experimental and clinical data, suggest H. pylori infection is closely linked to gastric Maltoma. However, there remain a substantial portion (20~40%) of gastric Maltoma unresponsive to antibiotic treatment and only a minority of H. pylori-infected patients may develop gastric Maltomas. Which factors will determine the success or failure after eradication of HP and why only some susceptible patients suffered from gastric Maltoma remain unknown. Although high-grade transformation was initially assumed unresponsive to anti-H. pylori therapy, our study and others have recently shown the response rate is independent of histological grading. Therefore, to distinguish responsive and unresponsive cases and to separate the disease-causing infection from the silent infection remains a great challenge for optimizing management of H. pylori-related diseases and gastric Maltomas.

Enhanced gastric inflammation and risk of developing gastric Maltomas is a consequence of an inappropriate host response to the chronic presence of H. pylori within the gastric niche. Because gastric inflammation provides a common denominator that translates into the different outcomes of host-microbe interaction, genes involved in regulating antimicrobial immunity and inflammation may modulate the risk after exposure to H. pylori. Family, twin, animal and case-association studies provide evidence that the intensity of inflammation and degree of gastric epithelial lesions may vary with genetic differences in host response. On the basis of above assumption, particular combination of multiple genes may confer susceptibility and only a small number of individuals who encounter H. pylori infection will develop diseases according to different genetic background of the host. Therefore, relating genetic differences to disease phenotypes may potentially allow more accurate prediction of variable outcomes of H. pylori and appropriate adjustment of treatment stragegies, as well as indicating novel area for future studies of H. pylori-associated diseases.

In the first year grant period, we have enrolled 70 patients with maltoma and 310 healthy controls. Genomic DNAs were extracted from peripheral white blood cells. Polymorphism analyses for IL-1β (-31 C/T & -511 T/C), IL-6 (-174 G/C), TNF-α (-238 G/A, -308 G/A, -857 C/T, -863 C/A, -1031 T/C), TNFR1 (-383 A/C) and TNFR2 (codon 196 T/G) were performed with

polymerase chain reaction (PCR) followed by direct sequencing for IL-1β (-31, -511) and TNF-α (-238, -308, -857, -863, -1031), and PCR combined with restriction enzyme length polymorphism for IL-6, TNFR1 and TNFR2. Genotype frequencies showed no differences between patients with Maltoma and controls for IL-1β, IL-6, TNFR1, or TNFR2. For TNFα, the TNF-α-857T variant, corresponding to low transcription activity, was significantly underrepresented in Maltoma compared to controls (6.4% vs. 14.3%, p=0.018), conferring a 3-fold decrease in risk (odds ratio:0.33, 95% confidence interval 0.15~0.75). Considering the low transcription activity of TNF-α-857T variant and the central role of TNF-α in development of lymphoma, our preliminary results are helpful for lymphomagenesis and provide first evidence that genetic host factors play a key role in Maltoma.

Genetic polymorphisms in glutathione s-transferase (GST) and interleukin (IL) -1β have been reported to influence interethnic and interindividual susceptibility to Maltoma, we tested whether polymorphic variations in GSTM1, GSTT1, GSTP1, IL-1β and IL-1 receptor antagonist (IL-1RN) confer susceptibility to Maltoma in the second year grant period. DNA samples were collected from 75 patients and 321 controls. Genotypes were determined by PCR-based techniques. Prevalences of variant genotypes in the control group were GSTM1 null 52.4%, GSTT1 null 43.0%, GSTP1 105 val/val 7.4%, GSTP1 105 Ile/val 25.9%, IL-1β –511 T/T 19%, -511 C/T 54.2%, and IL-1RN 1/2 & 2/2 11.2%, as expected for Chinese but somewhat different from those reported for Caucasians. The rates of GSTM1, GSTP1, IL-1β and IL-1RN genotypes did not differ between patients and controls. However, GSTT1 null genotypes were significantly more common in Maltoma patients (43/75 vs. 138/321, p=0.029; OR=1.8, 95% CI:1.1~3.0) as compared to controls. These data support the notion that interindiviaual differences in susceptibility to H. pylori-associated Maltoma may be mediated in part through inherited variability in the inactivation and detoxification of carcinogens. Furthermore, our results suggest the effects of these genotypes show interethnic variations.

In the following third year grant period, we will extend our study to investigate chemokine gene polymorphisms in the development and progression of Maltoma. Furthermore, the genotypes will be correlated with treatment response. Besides, genomewide host profiling with cDNA microarray to search for some potential novel candidate genes and polymorphism will be performed and launched for association studies.

Keywords: Host susceptibility, cytokine polymorphism, mucosa-associated lymphoid tissue lymphoma

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