行政院國家科學委員會補助專題研究計畫成果報告

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※ 評估具微小衛星不穩定之大腸直腸息肉之下游基因 ※

※ （包括 TGFβRⅡ, BAX, hMSH3, hMSH6,IGFⅡR, ※

※ BLM 基因）之變化 ※

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計畫類別：■個別型計畫　□整合型計畫

計畫編號：NSC 90－2314－B－002－337－

執行期間：90 年 8 月 1 日至 91 年 7 月 31 日

計畫主持人：王世名 教授

執行單位：國立台灣大學醫學院附設醫院外科部

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中文摘要：

在本研究中，我們探討具 Microsatellite instability 之大腸直腸的臨床病理表徵，及其下游基因變化。我們發現，具 MSI 之大腸直腸癌傾向於發病年齡較輕，右側大腸病變，腫瘤產生黏液，以及 p53 基因的表現正常。具 microsatellite instabiliey 的腫瘤，其對化學治療（HDFL:5-FU 2600mg/m^2, leucororin 300mg/m^2）的化療敏感度較佳，預後較佳。而具 microsatellite instability 的腫瘤，約有 63.5%（33/52）會有 TGFβⅡR 突變的現象，而具 TGFTGFβⅡR 基因變化的腫瘤，其化學治療效果及存活情況亦普較佳。

英文摘要：

The influence of microsatellite instability (MSI) on treatment outcome of colorectal cancers remains unclear and deserves further investigation. Between January 1, 1994 and June 30, 1999, we recruited 244 patients with stage IV sporadic colorectal cancers for our study, based on appropriate eligibility criteria. The patients were nonrandomly allocated to two treatment groups of either with or without high-dose 5-fluorouracil plus leucovorin chemotherapy (HDFL: 5-Fu: 2600 mg/m^2, leucovorin 300 mg/m^2, maximum 500mg). Each treatment group was further divided into two subgroups according to the status of high-frequency microsatellite instability (MSI-H). MSI-H was defined as the appearance of MSI in at least 2 of the 5 examined chromosomal loci (BAT-25, BAT-26, D5S346, D2S123, D17S250). We compared the clinicopathologic parameters, p53 overexpression, and overall survival among the allocated patient groups. Totally, four subgroups of patients were allocated in our study and were designated as: MSI-H (+) HDFL (+), n=35; MSI-H (−) HDFL (+), n=134; MSI-H (+) HDFL (−), n=17; MSI-H (−) HDFL (−), n=58. There was no significant difference of background clinicopathologic data between the HDFL (+) and HDFL (−) treatment groups (p>0.05). Survival analyses indicated that the patients of subgroup MSI-H (+) HDFL (+) survived significantly longer than those of subgroup MSI-H (−) HDFL (+), with median survival time (95% confidence interval [CI]) of 24.0 (20.2-27.9) and 13.0 (11.6-14.4) months, respectively (p=0.0001, log-rank test). In contrast, in patients without chemotherapy, the prognosis was poor irrespective of their MSI status, with median survival time (95% CI) of 7.0 (4.6-9.4) and 7.0 (6.1-7.9) months in MSI-H(+) HDFL (−) and MSI-H (−) HDFL (−) subgroups of patients, respectively (p=0.8205, log-rank test). Cancers of MSI-H responded significantly better to HDFL (p=0.001), with mean response rate (95% CI) being 65.71% (49.98%-81.44%) in subgroup MSI-H (+) HDFL (+), as compared to 35.07% (26.99%-43.15%) in subgroup MSI-H (−) HDFL (+). The toxicity to HDFL was similarly minimal between MSI-H(+) and MSI-H (−) patients (p>0.05). Remarkably, the MSI-H cancers tended to be young age in tumor onset, to be located at right colon, to present with mucin production in histology, and to have normal p53 expression (p<0.05). Multivariate analysis of the whole patients indicated that poor differentiation, mucin production, level of carcinoembryonic antigen (CEA) > 100ng/ml, and p53 overexpression were the significant independent poor prognostic factors. In contrast, MSI-H and chemotherapy were the favorable prognostic parameters (p<0.05). We thus concluded that the better prognosis of stage IV sporadic colorectal cancers with MSI-H was possibly associated with their better chemosensitivity rather than the less aggressiveness in biologic behavior.

計劃緣由及目的：

Microsatellite instability (MSI) resulted from genetic mutation or epigenetic silencing of mismatch repair (MMR) genes. It was reported that approximately 15% of sporadic colorectal cancers harbored this genomic alterations. Remarkably, numerous investigators have attempted to correlate this important molecular marker with clinical outcome of colorectal cancers. However, results to date have been inconclusive. Many researchers have advocated the association of MSI with better prognosis, but the others did not find this relation. The inconsistency of the clinical relevance of MSI in different studies might result from different methodology and interpretation criteria used for the assessment of MSI, different clinical treatment modalities used for patients, and the variations in clinicopathologic characteristics of the included patients, in particular in regard of pTNM, stage grouping, and residual tumor (R) classification. Furthermore, biologically, tumor prognosis is determined by intrinsic aggressiveness and/or potential sensitivity to chemotherapy. However, currently, although some authors strongly advocate that colorectal cancers with MSI benefit from adjuvant chemotherapy, we are not fully convinced whether this is due to their associated better chemotherapeutic sensitivity and/or less biologic invasiveness. On the other hand, the recent in vitro study have indicated the conflicting results that colorectal cancer cells with deficient MMR genes were more resistant to 5-fluorouracil-based treatment. Therefore, the clinical implications of MSI remain obscure and deserve further investigation. Further clarification of the prognostic significance of MSI status will rely on the implementation of large, population-based studies and prospective clinical trials. In this study, we further determine the clinical relevance of high-frequency microsatellite instability (MSI-H) in stage IV sporadic colorectal cancer, based on a nonrandomized, prospective study. This study was focused on exploring whether MSI-H was associated with chemosensitivity and/or biologic aggressiveness in predicting the clinical outcome of stage IV sporadic colorectal cancers. We believe that the clinical significance of MSI-H in colorectal cancer will be better clarified through this study.

結果及討論：

Between January 1, 1994 and June 30, 1999, a total of 244 patients were enrolled onto this study, with n=35 in group MSI-H (+) HDFL (+), n=134 in group MSI-H (−) HDFL (+), n=17 in group MSI-H (+) HDFL (−), and n=58 in group MSI-H (−) HDFL (−), respectively. All patients were followed up until October 2001. There was no significant difference of background clinicopathologic data between HDFL (+) and HDFL (−) groups of patients ($p>0.05$). Multivariate analysis for the whole patients indicated that poor differentiation, mucin production, CEA level > 100 ng/ml, and p53 overexpression were the significant independent poor prognostic factors for survival. In contrast, MSI-H and chemotherapy were the significant favorable prognostic factors ($p<0.05$). The patient survival was not significantly affected by the age, gender, tumor location, performance status, lymphatic/vascular permeation, and the number of organ metastasized ($p>0.05$). Remarkably, the MSI-H tumors (n=52, 21.3%) were significantly associated with young-age of tumor onset, right-sided colon cancers, mucin production in histology, and normal p53 expression ($p<0.05$). Kaplan-Meier survival curves indicated that the patients of group MSI-H (+) HDFL (+) survived significantly longer ($p=0.0001$, log-rank test) than those of group MSI-H (−) HDFL (+), with median survival time (95% confidence interval [CI]) of 24.0 (20.2-27.9) and 13.0 (11.6-14.4) months, respectively (Figure1,2). In contrast, there was no significant difference of survival ($p=0.8205$, log-rank test) between group MSI-H (+) HDFL (−) and group MSI-H (−) HDFL (−), with median survival time (95% CI) of 7.0 (4.6-9.4) and 7.0 (6.1-7.9) months, respectively. These findings implied that in patients with HDFL therapy, MSI-H (+) group had better survival than MSI-H (−) group. In contrast, in patients without HDFL therapy, the prognosis was similarly poor regardless of their MSI-H status. This can translate into

that the prognostic significance of MSI-H for stage IV sporadic colorectal cancers possibly lies in its prediction of better chemosensitivity rather than the less biologic aggressiveness. The better chemosensitivity in cancers with MSI-H was further demonstrated by the direct evidence that the response rate to HDFL was significantly higher (p=0.001) in group MSI-H (+) HDFL (+) (mean 65.71%, 95% CI: 49.98%-81.44%) than in group MSI-H (−) HDFL (+) (mean 35.07%, 95% CI: 26.99%-43.15%) (Table 4). Remarkably, the data also showed that patients with chemotherapy survived significantly longer than patients without chemotherapy, irrespective of their MSI-H status [MSI-H (+) HDFL (+) vs. MSI-H (+) HDFL (−), p< 0.0001; MSI-H (−) HDFL (+) vs. MSI-H (−) HDFL (−), p<0.0001, log-rank test]. This finding can translate into that both MSI-H (+) and MSI-H (−) patients seem to benefit from the HDFL chemotherapy. On the other hand, we found that the toxicity to HDFL was minimal and there was no correlation of chemotherapeutic toxicity with MSI-H status (p>0.05).

In colorectal cancers with MSI-H, 63.5% were with the mutation of TGFβIIR gene. Tumors with mutations of TGFβIIR gene in tanden repeats also tend to have better survival and better chemosensitivity(Figure 3).

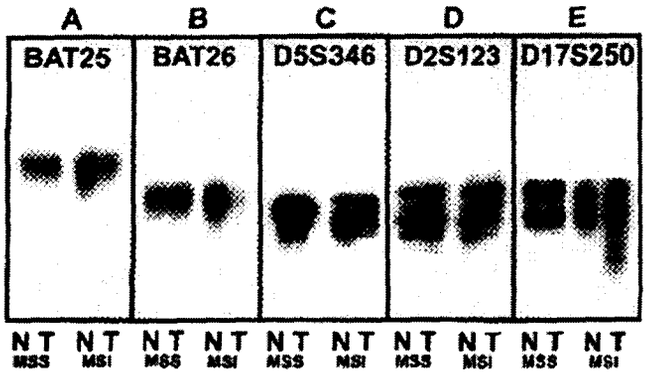
計劃成果自評：

本文不但對 microsatellite instability 在大腸直腸癌致癌角色更進一步闡明，再者，MSI-H 與 chemosensitivity 的關係亦得到解答，相信本研究可在臨床上實際應用。本研究已在 Int. J. Cancer 發表。

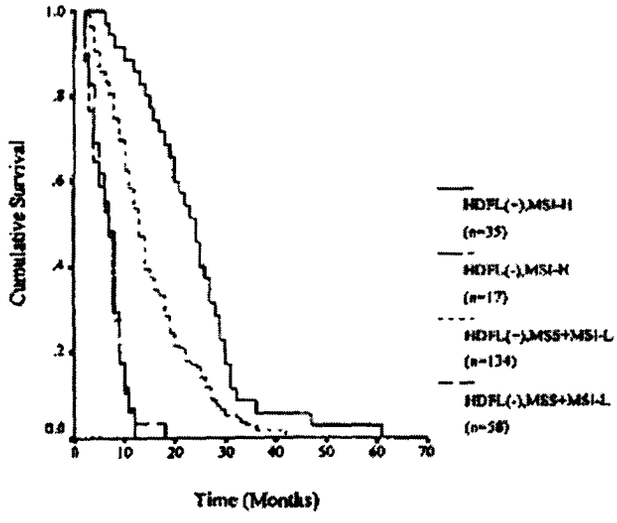
參考資料：

Liang JT, Huang KC, Lai HS, et al.High-Frequency Microsatellite Instability Predicts Better Chemosentsitivity to High-Dose 5-Fluorouracil Plus Leucovorin Chemotherapy for Stage IV Sporadic Colorectal Cancer After Palliative Bowel Resection.Int. J. Cancer.2002;101:519-525

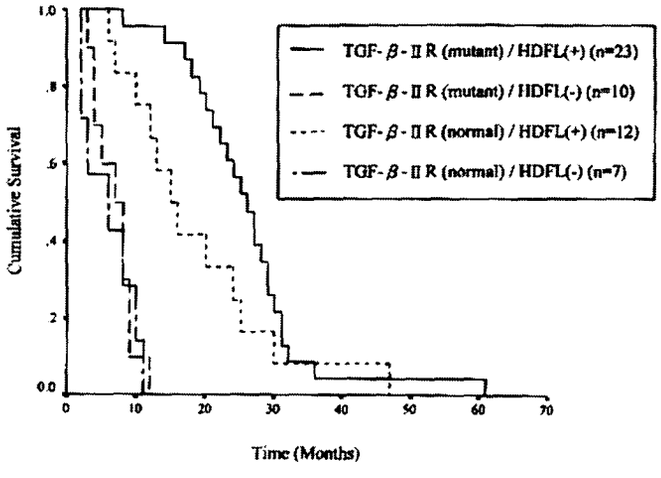
圖一


A
B
C
D
E
BAT25
BAT26
D5S346
D2S123
D17S250
N T N T N T N T N T N T N T N T N T N T
MSS MSI MSS MSI MSS MSI MSS MSI MSS MSI

圖二


Cumulative Survival
Time (Months)
HDFL(-),MSI-H
(n=35)
HDFL(-),MSI-N
(n=17)
HDFL(-),MSS+MSI-L
(n=134)
HDFL(-),MSS+MSI-L
(n=58)

圖三


Cumulative Survival
Time (Months)
TGF-β-II R (mutant) / HDFL(+) (n=23)
TGF-β-II R (mutant) / HDFL(-) (n=10)
TGF-β-II R (normal) / HDFL(+) (n=12)
TGF-β-II R (normal) / HDFL(-) (n=7)