行政院國家科學委員會專題研究計畫成果報告

計畫名稱：中文：評估微小衛星不穩定性、喪失異合子性以及其他分子生物標記在大腸直腸多發性良性息肉的致癌以及切除後再發的角色

英文：Evaluating the microsatellite instability, loss of heterozygosity and other molecular markers in the tumorigenesis and postpolypectomy relapse of multiple sychronous polyps

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主持人姓名：王世名 教授

執行機構：台大醫院　外科部

摘要:

微小衛星不穩定（MSI）主要發生於年輕成人，本研究主要在於了解這些早發性癌症的臨床病理表徵。我們收集在 1993 年 1 月至 2000 年 6 月，總共 126 位年輕型大腸癌病患（小於 40 歲），當作本研究的實驗組，而另外 323 位老年型病患（大於 60 歲）當作對照組。當評估這些癌症的分子生物學表徵時，我們分析 126 對 TNM 期別相同的年輕型與老年型大腸直腸癌的 p53 overexpression，DCC gene 的喪失異合子性(Loss of heterozygosity)，以及 BAT25，BAT26，D5S346，D2S123，以及 D17S250 等基因位置的微小衛星不穩定性，在臨床病理上，我們發現年輕型大腸直腸癌傾向於分化較差，黏液產生，發生同時性和異時性大腸直腸癌的比例較高，腫瘤較為晚期，以及手術死亡率較低（$P<0.05$）。在生物特性上，年輕型和老年型大腸直腸癌在 K-ras 基因第 12 與 13 codon 的突變率，以及 DCC 基因的 LOH 比例並無顯著差別。年輕型大腸直腸癌出現 MSI-H 的比例遠較老年型者高（29.37% vs. 6.35%）。具 MSI-H 之腫瘤傾向位於右側大腸，黏液產生，p53 表現正常，以及出現較高比例的同時性和異時性腫瘤。然而，大部分具 MSI-H 的年輕型腫瘤（81.08%, 30/37）在整個追蹤過程並不具家族遺傳傾向。存活分析顯示整體而言，年輕型大腸直腸癌的存活率較差。不過，當同 TNM 期別的腫瘤互相做比較時，第 I 、II 、III期之年輕型與老年型大腸直腸癌在存活率方面可以說沒有差別；而就第IV期的腫瘤而言，則年輕型者存活率較佳。對所有 126 對年輕型與老年型大腸直腸癌所做之多變數分析顯示腫瘤細胞分化不良、黏液產生、CEA $\geq$ 3.5 ng/ml 、第IV期腫瘤、p53 overexpression、以及 DCC 基因之 LOH 為不良預後因子（$p<0.05$）。反之，年齡小於 40 歲、MSI-L、以及 MSI-H 則為正面之預後因子。總之，年輕型之大腸直腸癌具獨特之病理與預後因子，明瞭這些臨床病理與分子生物學特徵有助於臨床病患的

治療。

關鍵字：大腸直腸癌、年輕、微小衛星不穩定

Abstract:

Background & aims: Microsatellite instability (MSI) was reported to be largely present in the colorectal cancers of young patients. The present study aims to further clarify the characteristics of these early-onset colorectal cancers, based on Taiwanese data.

Methods: Between January 1, 1993 and June 30, 2000, a total of 142 consecutive young colorectal cancer patients with age less than 40 years were collected and constituted the study group. The control group consisted of 346 patients at the age of 60 or over, randomly selected from our data base of colorectal cancers treated in the same period. Until June 30, 2001, 126 of younger and 323 of older group of patients completed the follow-up. The clinicopathologic data were compared between these two groups of patients. In evaluating the molecular biologic characteristics, 126 pairs of tumors of the younger and older patients, matched by UICC/TNM stage, were processed and compared for the p53 overexpression, loss of heterozygosity (LOH) of DCC gene, and MSI in the following chromosomal loci: BAT 25, BAT 26, D5S346, D2S123, and D17S250.

Results: Clinicopathologically, the younger colorectal cancer patients were more likely to have poor differentiation and mucin production in tumor histology, higher incidence of synchronous and metachronous colorectal cancers, more advanced in tumor stage, and lower operative mortality ($p<0.05$). Biologically, there was no significant difference between the younger and older patients in codon 12 and 13 mutations of K-ras gene and LOH of DCC gene ($p>0.05$). In contrast, the early-onset tumors tended to have normal p53 expression ($p<0.05$). Moreover, the tumors of younger patients presented with significantly higher percentage of high-frequency MSI (MSI-H), with 29.37% in comparison to 6.35% in those of older patients ($p<0.05$). MSI-H tumors in these young patients tended to be located at right colon, and to have mucin production, normal p53 expression, and higher incidence of synchronous and metachronous colon cancers. Paradoxically, however, most young patients with MSI-H tumors (81.08%, 30/37) did not develop a positive family history of colorectal or extracolonic cancers during the whole follow-up periods. Survival analysis indicated that on the whole, the younger patients' cancer-specific survival was poorer than that of older patients ($p<0.05$, log-rank test). However, when stage-to-stage comparisons were made, there was no significant difference of cancer-specific survival between the younger and older patients in their respective stage Ⅰ, Ⅱ, and Ⅲ disease ($p>0.05$). In contrast, in stage Ⅳ cancer, the cancer-specific survival time of the younger patients was significantly longer ($p<0.05$). Multivariate analysis of the 126 pairs of stage-matched younger and older patients indicated that poor differentiation, mucin production, CEA $\geq$ 3.5 ng/ml, stage Ⅳ tumor,

p53 overexpression, and LOH of DCC were independent poor prognostic factors ($p<0.05$). In contrast, age <40 years, MSI-L, and MSI-H were favorable prognostic factors ($p<0.05$).

Conclusions: Young colorectal cancer patients presented with distinct clinicopathologic and molecular biologic features. Recognition of these features may have an impact on the management of these early-onset colorectal cancers. However, the mechanisms underlying these features, especially the inconsistency of MSI-H and the development of family history of cancer, deserves further investigation.

Key words: colorectal cancer, young age, microsatellite instability (MSI)

緣由及目的：

Colorectal cancer is currently the third leading cause of cancer-related death in the general population of Taiwan. During the last 3 decades, there were remarkable increase in the incidence of colorectal cancer in Taiwan and the cause was generally considered as the increasingly Westernized life-style. In 1999, 3128 Taiwanese died of colorectal cancer, representing the mortality rate of 14.21 per 100,000 population [1]. Similar to those reported in the Western countries, most colorectal cancer patients in Taiwan were in their sixth to seventh decade of life, with the mean age of 62 years [1,2]. In Taiwan, the treatment policy for colorectal cancer has been according to NIH consensus in 1990 [3]. However, most colorectal surgeons in this country were impressed that colorectal cancers occurring in young adults or even in adolescents represented a subset of cancers with poor prognosis and should be treated more aggressively. Colorectal cancers of the young patients below than 40 years of age accounted for about 2 to 8 percent of all cases [4-6]. As a matter of fact, the natural history and prognosis of colorectal cancers in the younger patients have been controversial, with many studies reporting a worse prognosis [7-19], whereas other studies reported that prognosis was better than or the same as in older patients [5,6,20-31]. Therefore, the clinicopathologic features of colorectal cancers in the younger patients remain inconclusive and deserve further investigation.

On the other hand, the genetic basis and developmental biology of colorectal cancers in the younger patients are poorly understood, although most researchers tend to consider this subset of diseases as overwhelming caused by hereditary factors [32-34]. Based on molecular genetics, colorectal cancers can be classified into two major categories: sporadic cancers and hereditary nonpolyposis colorectal carcinomas (HNPCCs). The carcinogenesis of sporadic colorectal cancers is via the adenoma-carcinoma sequence, which involves the sequential alterations of multiple oncogenes such as K-ras, and tumor suppressor genes including APC, p53, and DCC [35]. In contrast, the molecular

mechanism responsible for HNPCCs was the germline mutations of mismatch repair genes including hMSH2, hMLH1, hPMS1, and hPMS2 [36]. The defects in mismatch repair genes could result in DNA replication error (RER) which manifested as microsatellite instability (MSI). Remarkably, recent investigations have shown that a subset of sporadic colorectal cancers also harbor DNA RER [37-40]. In this study, we defined the molecular biologic features of colorectal cancer in the young patients in Taiwan by comparing the genetic alterations of K-ras, p53, and DCC genes, and the status of MSI between the younger and older groups of patients . We believe that further exploration of the clinicopathologic and molecular biologic features of colorectal cancer in the young patients of Taiwan, and with reference to those of the Western counterpart, will facilitate not only the better understanding of the carcinogenesis and evolution of colorectal cancer but also the development of more effective treatment strategies, or even the chemopreventive measures [41-45].

結果與討論：

Compared to older patients, the younger colorectal cancer patients were characterized by poor differentiation and mucin production in histology, increased incidence of synchronous and metachronous colorectal cancers, more advanced in tumor stages, and lower surgical mortality. There was no significant difference between younger and older patients in the clinicopathologic variables including gender, predisposing conditions (familial adenomatous polyposis, and chronic ulcerative colitis), duration of symptoms, tumor location, tumor gross type, tumor size, vascular and/or lymphatic invasion of tumor cells, lymphocyte infiltration within the tumor, the incidence of synchronous or metachronous extracolonic cancers, and serum level of carcinoembryonic antigen. In evaluating the molecular biologic features, we found that there was no significant difference between the younger and older patients in codon 12 and 13 mutations of K-ras gene, and LOH of DCC gene. In contrast, the percentage of p53 overexpression was significantly lower in the tumors of young patients. Remarkably, the tumors of younger patients presented with significantly higher precentage of MSI-H, as compared to those of the older patients (29.37% vs. 6.35%, $p<0.05$). During the whole intervals of follow-up, there was no significant difference between the younger and older groups of patients in the development of a positive family history of cancer (17.46% vs. 14.24%, $p>0.05$). Compared to MSS tumors, the MSI-H tumors of the young patients tended to be right-sided colon cancer, and to have mucin production, higher incidence of synchronous or metachronous colorectal cancers, and normal p53 expression (Table 1). Remarkably, most young patients with MSI-H tumors (81.08%, 30/37) did not develop a positive family history of cancer. Survival analysis indicated that on the whole, the cancer-specific survival of younger patients was significantly poorer than that of older patients ($p=0.0140$, log-rank test). However, when

stage-to-stage comparisons were made, the younger and older patients were comparable in the cancer-specific survival of their respective stage Ⅰ, Ⅱ, and Ⅲ disease ($p>0.05$). In contrast, in stage Ⅳ cancer, the younger patients survived significantly longer than the older patients ($p<0.0001$, log-rank test), with the mean cancer-specific survival time of 25.46 months (95% confidence interval: 22.50 to 28.41 months) and 14.83 months (95% confidence interval: 12.61 to 17.05 months), respectively. Multivariate analysis of the 126 pairs of stage-matched younger and older patients indicated that poor differentiation, mucin production, CEA ≧ 3.5 ng/ml, stage Ⅳ disease, p53 overexpression, LOH of DCC gene were the significant poor prognostic factors. In contrast, age< 40 years, MSI-L and MSI-H were the favorable prognostic parameters.

計畫成果自評：

The clinical implications for this study were multiple. Firstly, the recognition of more biologically malignant nature of the colorectal cancers in the young patients should justify the use of more aggressive treatment modalities, which may result in better treatment outcome. Secondly, the realization of the increased of synchronous and metachronous colorectal incidence cancers in young patients should facilitate more active preoperative and postoperative colonic survey. And thirdly, the understanding of the tendency to more advanced tumor stages, more genomic instability, and potentially faster tumor progression should alert the physicians to the initial clinical manifestations, and even facilitate the changes of the current screening policy, thus preventing a delayed diagnosis of colorectal cancer in the younger population.

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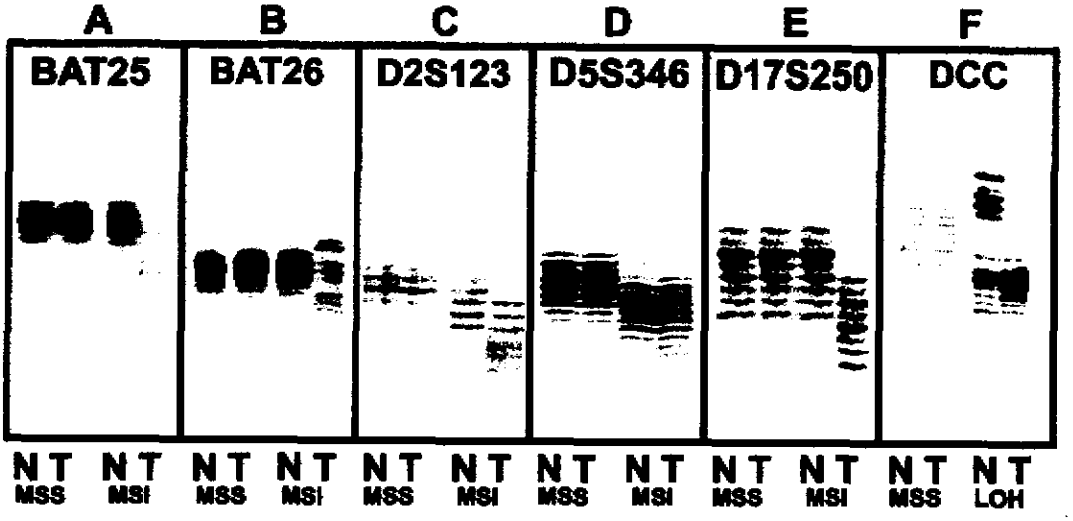
Table 1. Correlation of MSI Status with Various Clinicopathologic Features, p53 Overexpression, K-ras Gene Mutation, and LOH of DCC Gene in 126 Younger Colorectal Cancer Patients

	MSI-H (n=37)	MSI-L (n=14)	MSS (n=75)	p-value
Gender				
female	21	8	40	NS
male	16	6	35	
Tumor location				
right colon	26	4	11	
left colon	4	6	32	P<0.001
rectum	7	4	32	
Family history of cancer				
+	7	3	12	NS
−	30	11	63	
Stage				
I	2	1	5	
II	7	4	16	NS
III	12	5	25	
IV	16	4	29	
Gross Type				
ulcerative	19	8	38	
polypoid	18	6	37	NS
Differentiation				
well	6	1	16	
moderate	27	11	56	NS
poor	4	2	3	
Mucin production				
+	12	4	2	P<0.001
−	25	10	73	
Vascular/Lymphatic Permeation				
+	27	9	50	NS
−	10	5	25	
Lymphocyte infiltration				
+	22	8	46	NS
−	15	6	29	
Synchronous colorectal cancer	7	0	0	P<0.05
Synchronous extracolonic cancer	1	0	1	NS
Metachronous colorectal cancer	3	1	0	P<0.05
Metachronous extracolonic cancer	1	1	0	NS
CEA level (ng/ml)				
<3.5	8	4	20	
≥3.5	29	10	55	NS
p53 overexpression				
+	6	7	36	P=0.001
−	31	7	39	
K-ras mutation				
+	15	4	34	NS
−	22	10	41	
LOH of D.C.C.				
+	14	6	20	NS
−	23	8	55	

*p-value was calculated based on the comparison between MSI-H and MSS groups of patients



圖一　當正常組織與腫瘤在某些特定染色體標記的電泳圖出現型態不同時，即稱為微小衛星不穩定（ＭＳＩ）。F欄可以發現DCC基因位置的LOH。