行政院國家科學委員會專題研究計畫　成果報告

基因修補之基因體變異及喪失於臨床治療之運用

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-002-254-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：國立臺灣大學醫學院外科

計畫主持人：李章銘

計畫參與人員：謝馨誼

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 11 月 17 日

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告 □ 期中進度報告

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成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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執行單位：台灣大學醫學院外科

中　華　民　國　　94 年　10 月　26 日

一、 中文摘要

對食道癌的病患接受術前化學與放射治療併用的臨床反應，目前仍存在著極大的個體差異，但是到目前為止，並沒有一個很好的因子，可以作為預測此反應之依據，本計畫在去年收集了 121 位食道癌接受術前化學與放射治療併用後接受食道切除之病患，我們研究了 EGFR 此基因之基因多型性與此種反應之關聯性，我們應用 PCR 的方式來偵測 EGFR 的基因型，此基因型之測定是由病患在術前抽取血球的 DNA 而後經過 PCR 放大反應測試，我們將病患分成兩種不同的族群，一個是良好的反應，也就是說病患在接受化學與放射治療併用之後，腫瘤已經完全消失了，或是在顯微鏡底下，看到殘存的癌組織；第二組是不良反應，也就是在肉眼就有明顯的腫瘤組織，或是腫瘤組織繼續在增長。在我們過去一年的研究當中，這些病患有 47 位佔所有病患的 38.8%歸類為好的反應，而另外 74 位病患(佔 61.2%)歸類為不良反應者，我們發覺 EGFR 的基因型是預測這些化學與放射治療反應唯一有意義的因子，跟其他基因型相對而言，如果 EGFR 病患擁有兩個以上的常見基因型者，也就是說，他有大於 19 個 CA 以上的基因型，則他的臨床反應就會比較傾向於不良反應(69.5%：53.2%；OR 值=2.03；95%信賴區間落在 0.97-4.23；p=0.061)

所以在我們的觀察，EGFR 基因多型性也許可以當做一個預測術前化學與放射治療在食道癌病患的反應預測因子，如此可以幫助我們在臨床上偵測哪些病患是不良反應者，而避免不必要的治療與副作用，而可以及早接受絕對的手術治療。

二、 英文摘要

Variation in response to concurrent neoadjuvant chemoirradiation exists for the patients of esophageal cancer. There are no reliable markers to predict the individual response for such patients. A polymorphic CA repeat was found at the 5'-regulatory sequence in the intron 1 of the EGFR gene, which influence the transcription activity of the gene. In this study, we investigate whether this genetic polymorphism can modulate the response to concurrent chemoirradiation for patients of esophageal cancer. We conducted a prospective study including 121 patients with esophageal cancer who received concurrent neoadjuvant chemoirradiation followed by esophagectomy in National Taiwan University Hospital between 1996 and 2002. The EGFR genotype was determined by polymerase chain reaction (PCR) amplification of leukocyte DNA obtained before surgery. The response was classified as 1):*good response* which included patients with pathologically complete remission or remained with microscopically residual tumor; and 2): *poor response* which include grossly visible or progressively growing tumor after treatment. Results: There were 47 (38.8 %) patients classified as good and 74 (61.2 %) as poor responders in this study. We found the EGFR genotype were the single one factor influencing the response to concurrent neoadjuvant chemoirradiation. As compared to patients with other genotypes, those who with two long allele (> 19 CA) were more likely to poorly respond to treatment (69.5% vs. 53.2%; OR (95% CI)= 2.03 (0.97-4.23); p=0.061). Conclusion: EGFR genetic polymorphism might serve as a valuable predictor for the response to concurrent chemoirradiation in patients of esophageal cancer, which help patients of esophageal cancer of poor responders to chemoirradiation avoid unnecessary treatment before surgery.

三、 報告內容(包括前言、研究目的、文獻探討、研究方法、結果與討論[含結論及建議])

前言

Esophageal cancer remains an important disease threatening the public health. Squamous cell carcinoma (SCC) is the predominant histologic type of esophageal cancer worldwide. However, the incidence rates of adenocarcinomas of the esophagus have been increasing since 1970 in several western countries (1-4). In Taiwan, esophageal cacncer was the 7th cancer death in the male population in the official documents in 2000 (Cancer Registry Annual Report, Department of Health, Republic of China, 1996). Most of the patients presents with advanced disease at the time of diagnosis. The treatment result for the esophageal cancer is still far from satisfactory with the 5-year survival rate ranging around 20% (5-10). To improve the result of surgical treatment, multimodality therapies have been investigatedby several authors, including combination of chemotherapy with or without radiotherapy followed by surgery.

Several non-randomized trials reported survival benefit that are more than two-fold higher than those reported in past studies for esophageal cancer alone(7, 9, 10). However, limited number of phase III trials on neoadjuvant therapy followed by surgery versus surgery alone have produced conflicting results, contributing little to justify the routine use of preoperative CRT (6, 11-13). Indeed, patients of esophageal cancer can enjoy a better survival once they had good response to the neoadjuvant therapy (14). However, for the individuals who did not respond well to neoadjuvant therapy, the survival may be compromised by neoadjuvant treatment-related toxicity and delay in definite surgical resection. Unfortunately, there is no reliable biological marker available in literature to predict the response to neoadjuvant therapy in literature.

Epidermal growth factor receptor (EGFR) belongs to a family of receptors known as the ErbB family (ErbB tyrosine kinase receptors) including EGFR (ErbB-1), HER-2/*neu* (ErbB-2), HER-3 (ErbB-3) and HER-4 (ErbB-4) which activate a cascade of multiple signaling pathways that facilitate tumor growth process. EGFR has been shown to be overexpressed in esophageal cancer patient and associate with a higher tendency of tumor invasiveness, local recurrence and poorer prognosis (15). Recently, a polymorphic variant with a simple sequence repeat (SSR) with 14-21 CA dinucleotides in the EGFR gene was identified which could significantly influence the transcription activity of EGFR (16). In colorectal cancer, lower number of CA repeats (both alleles <20) was found to associate a higher risk of local recurrence after treated(17). In this study, we investigated the association of CA repeat polymorphism in intron 1 of EGFR and the response to CCRT of esophageal cancer.

研究目的

To investigated the association of CA repeat polymorphism in intron 1 of EGFR and the response to CCRT of esophageal cancer.

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研究方法

Materials and Methods:

Study Population

This project was part of an ongoing cooperative study aimed at understanding the causes esophageal cancer in Taiwan. It included patients of esophageal squamous cell carcinoma receiving neoadjuvant chemoirradiation (CCRT) followed-by esophagectomy at National Taiwan University Hospital from 1996 to 2005. Institutional review board-approved informed consent was obtained from each patient prior to sample acquisition. Peripheral blood of the patients was drawn before surgery or chemoirradiation. Tumor specimen was also obtained from the patients via the endoscopy before CCRT. In our hospital, the tumors located at or above the middle third thoracic esophagus are routinely resected through right thoracotomy and reconstructed via retrosternal conduit and left cervical esophageal anastomosis. The stomach was the first choice of organ for esophageal reconstruction, while left side colon based on the left colic artery would be used if the stomach is unsuitable for reconstruction. For the tumor at the lower third thoracic esophagus or near the gastro-esophageal junction, the tumor wais resected through left thoracoabdominal incision and reconstructed via intrathoracic esophagogastrostomy or esophagojejunostomy. For the patients with locally advanced diseases (T3 at least), neoadjuvant CCRT was given with concurrent uasage of cisplatin(5mg/m2) plus 5-FU (225 mg/m2) (or cisplatin plus paclitaxol) with 3600 cGy to 4000 cGy of irradiation. All samples collected from the patients was maintained at -80^{o}C until subsequent analysis. The response was classified into 4 grades according to the pathological finding in the surgical specimen: **garde 1**: complete remission; **grade 2**: microscopic residual tumor; **grade 3**: grossly visible residual tumor; **grade 4**: progression of tumor.

DNA Extraction from the Blood

Genomic DNA was prepared from the leukocytes with serial extraction by phenol and chloroform, and then ethanol precipitation. The content of DNA of each sample was analyzed by spectrophotometry, and about 1 μg of DNA will be taken from each sample for PCR amplification.

Genotyping of EGFR polymorphism

The genotyping of the polymorphic gene EGFR was determined by PCR or PCR-RFLP. Determination of EGFR polymorphisms was conducted with polymerase chain reaction (PCR) amplification of leukocyte DNA obtained before surgery. Primers of 5'-GGCTCACAGCAAACTTCTC-3') & (5'-AAGCCAGACTCGCTCATGTT-3') were used in the PCR reaction. (Maria, Cancer Res, 2004;64: 9139). A 114- to 128-bp PCR fragment containing the polymorphic region was amplified and analyzed by the ABI377 genetic analyzer for allele length determination. Genotype of EGFR were defined as: Long allele: CA repeat longer than 118 BP (17 [CA] repeats) in the intron 1 polymorphic site

計畫進度及重要成果 (Progress and results of the project)

- **The clinical results of our patients:**
- **CCRT response:**
- Good response: 47 (38.8 %) patients
- Poor response: 74 (61.2 %) patients
- **OP complications:**

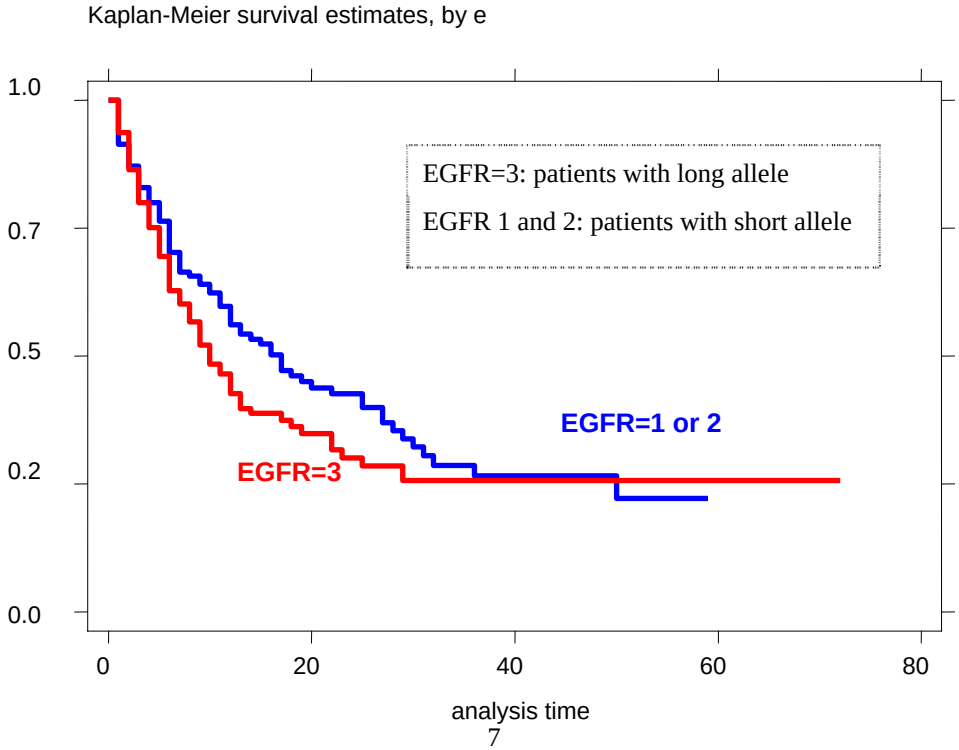
Leak: 39 (32%)

Pulmonary complications: 19 (15.7%)

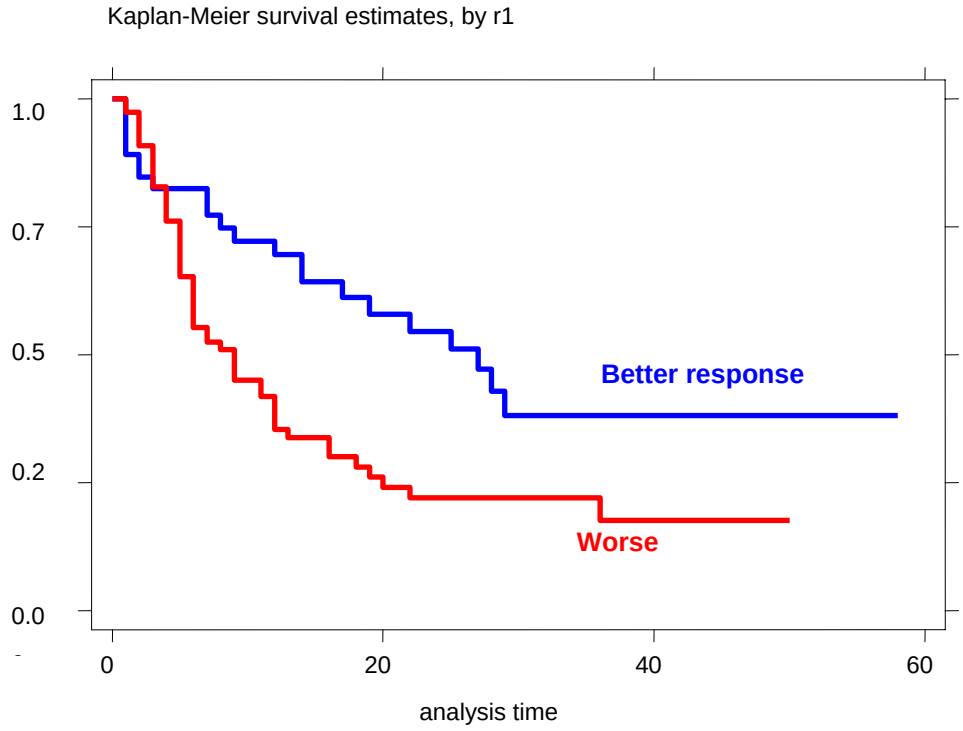
Other complications: 3 (2.4%)

- **One month hospital mortality**: 13 (10.7%)

Survival curves according to the EGFR genotype



Survival curves according to the CCRT response



Good response: Gr 1 or 2

Poor response: Gr 3 or 4

Table 1.1 Univariate analysis for descriptive data

Characteristics	Better response(%) N=47	Worse response(%) N=79	OR(95%CI)	p-value
Gender				
Female	3 (50.0)	3 (50.0)	1.0	
Male	44 (36.7)	76 (63.3)	1.73 (0.33-8.93)	0.51
Age				
<60yrs	20 (37.7)	33 (62.3)	1.0	
>=60yrs	27 (37.0)	46 (63.0)	1.03 (0.50-2.15)	0.93
Stage				
0,1,2	43 (61.4)	27 (38.6)	1.0	
3,4	4 (7.1)	52 (92.9)	20.7 (6.7-63.8)	<0.0001
Stage2				
0,1	25 (96.1)	1 (3.2)	1.0	
2	18 (40.9)	26 (59.1)	36.1 (4.5-291.1)	0.0008
3,4	4 (7.1)	52 (92.9)	324.9 (34.5->999.9)	<0.0001
T stage*				
0,1,2	39(67.2)	19(32.8)	1.0	
3,4	6(9.8)	55(90.2)	18.82(6.89-51.42)	<0.0001
N**				
0	35(63.6)	20(36.4)	1.0	
1	10(16.1)	52(83.9)	9.10(3.81-21.76)	<0.0001
Complication1#				
No	30(35.3)	55(64.7)	1.0	
Yes	17(43.6)	22(56.4)	0.71(0.33-1.53)	0.38
Complication2#				
No	44(41.9)	61(58.1)	1.0	
Yes	3(15.8)	16(84.2)	3.85(1.06-14.01)	0.041
Complication3#				
No	47(38.8)	74(61.2)		
Yes	0(0.0)	3(100.0)		0.29╳
EGFR				
1,2	29 (45.3)	35 (54.7)	1.0	
3	18 (29.0)	44 (71.0)	2.03 (0.97-4.23)	0.061

Better response: r=1 or 2; worse response: r=3 or 4

***：T-stage: 7 missing cases**

****：N: 9 missing cases**

#：Complication: 1 missing case

╳：Fisher's exact test two-sided p-value

EGFR=3: patients with homozygous long allele

EGFR 1 and 2: patients with heterozygote and homozygous short allele

Table 2.4 Multivariate analysis on EGFR effect of chemotherapy responses

Characteristics	OR (95% CI)	p-value
Gender		
Female	1.0	
Male	5.64(0.50-64.01)	0.16
Age		
<60 yrs	1.0	
>=60 yrs	0.94 (0.43-2.04)	0.88
Complication2		
Yes	1.0	
No	4.57(1.16-18.04)	0.030
EGFR		
1,2	1.0	
3	1.99(0.92-4.32)	0.080

Complication 2: pulmonary complication

EGFR=3:patients with long allele

EGFR 1 and 2: patient with short allele

Discussion:

A higher risk of pulmonary complications in the patients with poor response to CCRT , might be attributed to the poorer general condition by the cancer progression, and more extensive surgical trauma for a curative resection.

We also found a better prognosis noted in the group with good response to CCRT, probably attributed to tumor down staging by the treatment. There was a borderline significance for the association of CCRT response with EGFR genotype of homozygous long allele after adjustment with age, sex and postoperative pulmonary complications. Conclusion: The EGFR intron 1 (CA) repeat genetic polymorphism had a borderline significance influencing the CCRT response for esophageal cancer. Further clinical and in vitro confirmation was required.

四、 計畫成果自評

Due to the limited statistic power of our study, only a borderline significance we noted abort the EGFR polymorphism effect on CCRT response. Therefore, muse ease of study is still needed to confirm the result of our study.