行政院國家科學委員會專題研究計畫成果報告

胃癌基因變化與幽門螺旋桿菌及EB病毒感染之間的關係

(第一年及第二年計劃)

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中 文 摘 要

Epstein-Barr 病毒(EBV)與胃癌之關連性至今仍有許多不明瞭之處，它們的相關性在不同的族群及不同的組織類型差異甚大。胃癌當中較為罕見之淋巴上皮細胞狀癌(簡稱 LELC)多半可以找到 EBV，但 EBV 也可能在少數的非 LELC 之普通胃癌組織當中被發現。這類與 EBV 相關之 LELC 究竟有何臨床病理特徵值得探討，另外有關 EBV 陽性或陰性之胃癌與幽門螺旋桿菌之關係也罕有研究報告。

吾人在 1998 年至 1996 年之期間內，一共研究 379 例胃癌病人。利用 PCR 及 in situ hybridization 方法，共發現 22 例胃癌，在其癌組織內可找到 EBV 的 DNA(表一)。另外根據組織學檢查發現 6 例之組織形態為 LELC，其中女性 5 例，男性 1 例，年齡自 51 歲至 75 歲不等，平均年齡 61.5 歲。3 例之腫瘤位在下三分之一之胃，另 3 例之腫瘤位在上或中三分之一胃；2 例之腫瘤侵犯至漿膜層，4 例只限於粘膜下層及肌肉層。所有腫瘤均可在腫瘤細胞找到 EBV-RNA 之核訊，但不在其週邊之淋巴基層與非癌組織。有 4 例可找到幽門螺旋桿菌；1 例在術後一年發現腫瘤復發，其餘 5 例均在腫瘤緩解期。吾人發現 LELC 多好發於女性，預後較佳，與 EBV 之相關性極高(Hepato-Gastroenterology 1999;46:1214-9)。

另外為了進一步探討台灣之 EBV 相關胃癌之臨床病理特徵及遺傳變異，與幽門螺旋桿菌之相關性，吾人利用 PCR，RNA in situ hybridization 來評估 130 例胃癌(其中 8 例為 LELC)之 EBV-DNA 狀態。另外用血清學方法來偵測胃癌病人之幽門螺旋桿菌 IgG 抗體，以免疫組織化學反應偵測胃癌組織之 p53，c-erbB-2，E-cadherin，同時利用 10 組 primer 之 PCR 反應來偵測胃癌及非癌組織之微衛星體不穩性(microsatellite instability)。其結果顯示：在所有 8 個 LELC 之組織中，全部(8/8，100%)可以找到 EBV-DNA，在其餘的 122 個普通胃癌當中，僅有 16 例(13.1%)可以找到 EBV-DNA。LELC 較一般的 EBV-陰性胃癌容易侵犯近端胃部，具瀰漫型組織類型(表二)，p53 常有過度表現，E-cadherin 表現減少，淋巴結轉移較少見，過去較少曾感染幽門螺旋桿菌，c-erbB-2 之過度表現也屬罕見(表三)。相對的 EBV-陽性之非 LELC 胃癌與 EBV 陰性之胃癌兩者並無其他特別差別，三組胃癌之微衛星體不穩性也無差別。吾人之數據顯示，LELC 具備很特殊的臨床病理特徵及遺傳路徑，EBV 在這方面的角色較幽門螺旋桿菌為重。但 EBV 與幽門螺旋桿菌在常見的胃癌之組織學及遺傳特徵則仍需進一步研究。

關鍵語：淋巴上皮細胞狀癌，胃，Epstein-Barr 病毒，臨床病理特徵，幽門螺旋桿菌

表一：癌組織含有 EBV-DNA **之** 22 **例胃癌之臨床病徵**

No	Sex	Age	Location	Stage	LN	TNM	Histology	H.pylori	Classification
1	F	79	L	A	+	IIIb	I	-	III
2	M	74	L	A	+	IIIa	I	+	III
3	M	55	M	A	+	II	I	+	III
4	M	67	U	A	-	II	I	+	III
5	F	74	L	A	+	IV	D	-	II
6	M	65	U	A	-	Ib	I	+	III
7	F	46	U	A	+	IV	D	+	III
8	M	81	M	E	-	IA	I	+	Early Ia
9	M	75	U	A	+	II	I	-	III
10	M	80	L	E	-	Ia	I	+	Early I
11	M	50	L	A	-	Ib	D	+	II
12	F	82	L	A	+	IIIa	D	-	III
13	F	52	R	A	+	IV	I	-	III
14	M	70	L	A	+	IV	D	+	III
15	M	67	L	A	+	IV	D	+	III
16	F	45	L	A	+	IV	D	-	IV
17	F	52	L	E	+	Ia	I	-	Early III
18	M	60	M	A	+	IIa	I	+	II
19	F	51	U	A	+	Ia	I	+	I
20	F	65	L	A	-	IIa	I	+	III
21	F	66	M	E	+	Ib	D	-	Early III
22	F	75	L	A	+	IIIb	D	-	III

表二：130 例胃癌病人之 EBV 感染狀況及臨床病理特徵

	EBV-positive (n=24)			EBV-negative
	LELC (n=8)	non-LELC (n=16)	Total (n=24)	(n=106)
Age (Mean ±SD)	62.3±8.2	66.4±12.9	65.0±11.5	60.9±10.9
Gender				
Male	3	11	14	67
Female	5	5	10	39
H. pylori infection				
Positive	4	11	15	69
Negative	4	5	9	37
Tumor location				
Cardia/body	6*	8	14	45
Antrum	2	8	10	61
Tumor stage				
Early	2	2	4	23
Advanced	6	14	20	83
Histologic subtype				
Diffuse	8#	8	16	43
Intestinal	0	8	8	63
Lymph node metastasis				
Present	1$	11	12	64
Absent	7	5	12	42

LELC: lymphoepithelioma-like carcinoma; SD: standard deviation
* p=0.1 vs. EBV-negative gastric cancer, #p<0.001 vs. EBV-negative gastric cancer
$p<0.05 vs. EBV-negative gastric cancer

表三：130 例胃癌病人之 EBV 感染狀況及遺傳變異

	EBV-positive (n=24)			EBV-negative
	LELC (n=8)	non-LELC (n=16)	Total (n=24)	(n=106)
p53 overexpression				
Positive	8*	9	17	50
Negative	0	7	7	56
c-erbB-2 overexpression				
Positive	0#	11	11	27
Negative	8	5	13	79
E-cadherin expression				
Reduced	8$	8	16	60
Normal	0	8	8	46
Microsatellite instability (MSI)				
Present	1	3	4	12
Absent	7	13	20	94

LELC: lymphoepithelioma-like carcinoma

* $p<0.01$ vs. EBV-negative gastric cancer, [#]$p=0.2$ vs. EBV-negative gastric cancer
[$]$p<0.05$ vs. EBV-negative gastric cancer

Abstract

The association of Epstein-Barr virus (EBV) and gastric carcinomas (GC) has been demonstrated to vary among different populations and certain histologic subtypes. EBV has been found in most cases of rare gastric lymphoepithelioma-like carcinomas (LELC) and a small but significant proportion of common gastric adenocarcinomas. Few studies have addressed the status of Helicobacter pylori (H. pylori) infection and genetic alterations in these EBV-positive or-negative GC.

To evaluate the clinicopathological features of lymphoepithelioma-like carcinoma of stomach in Taiwan, 379 patients with gastric adenocarcinoma from 1993 to 1996 were studied. EBV-DNA has been found in 22 samples of gastric cancer tissues by PCR and in situ hybridization (Table 1). Among them, lymphoepithelioma-like gastric cancer was detected by histology in 6 patients. These 6 patients with LELC of stomach were retrospectively studies. Five patients were females and one patient was male. Their age ranged from 51 to 75 years and with a mean age of 61.5 years. Endoscopically two patients were initially diagnosed as early gastric cancer and the other four were diagnosed as advanced gastric cancer. Three patients had tumors locating in lower third of the stomach, while the other three tumors were located in the middle and upper third. Two tumors invaded into serosal layer and the other four lesions were confined at submucosal and muscular layers. Using in situ hybridization method, all six patients (100%) had positive nuclear EBV-encoded small RNA signals in the tumor cells but not in the surrounding lymphoid stroma and non-neoplastic gastric mucosa. Helicobacter pylori was found in four (66.7%) of the cases. The mean follow-up period of the six patients was 27.0 months. Five patients were free of the disease. Lymph node involvement and mesenteric implantation was noted in one patient in which cancer recurred one year after gastrectomy. Lymphoepithelioma-like carcinoma of stomach in this study revealed a female predominance, preferential localization in proximal part of stomach, better prognosis, and a high association with Epstein-Barr virus infection (Hepato-Gastroenterology 1999;46:1214-9).

To investigate the clinicopathologic characteristics of EBV-associated GCs in Taiwan, and their relation to H. pylori infection and genetic alterations, eight cases of gastric LELC and 122 cases of common non-LELC were evaluated for the presence of EBV-DNA using polymerase chain reaction (PCR) and RNA in situ hybridization. The status of H. pylori infection was determined by anti-H. pylori IgG in preoperative sera. Immunostaining for p53, c-erbB-2 and E-cadherin was performed by a standard avidin-biotin-complex detection system. Microsatellite instability was analyzed by PCR using ten primers. EBV was detected in eight (100%) LELC and in 16 (13.1%) of 122 common GC. Compared with EBV-negative GC, gastric LELC tended to have a relatively higher frequency of proximal location, diffuse histologic subtype, p53 overexpression, and reduced E-cadherin expression but a lower frequency of lymph node metastasis, previous H. pylori infection and c-erbB-2 overexpression (Table 2,3). In contrast, no significant difference of clinicopathologic and genetic profiles was observed between EBV-positive non-LELC GC and EBV-negative GC. No correlation of microsatellite instability was found among these three subsets of GC. Our data indicate that a distinct clinicopathologic and genetic pathways exist in gastric LELC in which EBV may play a more important role than H. pylori infection. The impact of EBV and H. pylori on histologic and genetic features of common GC remains to be further clarified.

Key Words: Lymphoepithelioma-like carcinoma, stomach, Epstein-Barr virus, clinicopathological features, H. pylori

參 考 文 獻

1. Osato T, Imai S. Epstein-Barr virus and gastric carcinoma. Semin Cancer Biol 1996;7:175-182.
2. Iezzoni JC, Gaffey MJ, Weiss LM. The role of Epstein-Barr virus in lymphoepithelioma-like carcinomas. Am J Clin Pathol 1995;103:308-315.
3. Shibata D, Weiss LM. Epstein-Barr virus-associated gastric adenocarcinoma. Am j Pathol 1992;140:769-774.
4. Leoncini L, Vindigni C, Megha T, et al. Epstein-Barr virus and gastric cancer: Data and unanswered questions. Int J Cancer 1993;53:898-901.
5. Imai S, Koizumi S, Sugiura M, et al. Gastric carcinoma: Monoclonal epithelial malignant cells expressing Epstein-Barr virus latent infection protein. Proc Natl Acad Sci USA 1994;91:9131-9135.
6. Harn HJ, Chang JY, Wang MW, et al. Epstein-Barr virus-associated gastric adenocarcinoma in Taiwan. Hum Pathol 1995;26:267-271.
7. Shih WS, Kang MW, Kang JH, et al. Epstein-Barr virus-associated gastric adenocarcinomas among Koreans. Am J Clin Pathol 1996;105:174-181.
8. Tokunaga M, Land CE, Uemura Y, et al. Epstein-Barr virus in gastric carcinoma. Am J Pathol 1993;143:1250-1254.
9. Gulley ML, Pulitzer DR, Eagan PA, et al. Epstein-Barr virus infection is an early event in gastric carcinogenesis and is independent of bcl-2 expression and p53 accumulation. Hum Pathol 1996;27:20-27.
10. Lin JT, Wu MS, Shun CT, et al. The occurrence of microsatellite instability in gastric carcinoma is associated with enhanced expression of erbB-2 oncoprotein. Cancer Res 1995;55:1428-1430.
11. Anagnostopoulos I, Hummel M. Epstein-Barr virus in tumors. Histopathology 1996;29:297-315.
12. Watanabe H, Enjoji M, Imai T. Gastric carcinoma with lymphoid stroma: its morphologic characteristics and prognostic correlations. Cancer 1976;38:232-243.
13. Nakamura S, Ueki T, Yao T, et al. Epstein-Barr virus in gastric carcinoma with lymphoid stroma: special reference to its detection by the polymerase chain reaction and in situ hybridization in 99 tumors, including a morphologic analysis. Cancer 1994;73:2239-2249.
14. Tsai WS, Chang MH, Chen JY, et al. Seroepidemiological study of Epstein-Barr virus infection in children in Taipei. Acad Paed Sin 1989;30:81-86, 1989.
15. Takano Y, Kato Y, Saegusa M, et al. The role of the Epstein-Barr virus in the oncogenesis of EBV (+) gastric carcinomas. Virchous Arch 1999;434:17-32.
16. Shinohara K, Miyazaki K, Noda N, et al. Gastric diseases related to Helicobacter pylori and Epstein-Barr virus infection. Microbiol Immunol 1998;42:415-421.
17. Hagashi K, Teramoto N, Akagi T, et al. In situ detection of Epstein-Barr virus in the gastric glands with intestinal metaplasia. Am J Gastroenterol 1996;91:1481.
18. Niedobitek G, Agathanggelou A, Barber P, et al. P53 overexpression and Epstein-Barr virus infection in undifferentiated and squamous cell nasopharyngeal carcinomas. J Pathol 1993;170:457-461.
19. Zhang Q, Gutsch D, Kenney S. Functional and physical interaction between p53 and BZLF1: Implications for Epstein-Barr virus latency. Mol Cell Biol 1994;14:1929-1938.
20. Yonemura Y, Ninomiya I, Yamaguchi A., et al. Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer. Cancer Res 1991;51:1034-1038.

已發表之論文

1. Wang HH, Wu MS, Shun CT, Wang HP, Lin CC, Lin JT. Lymphoepithelioma-like carcinoma of the stomach: A subset of gastric carcinoma with distinct clinicopathological features and high prevalence of Epstein-Barr virus infection (Hepato-Gastroenterology 1999;46:1214-1219)

已送審查之論文(Submitted to Cancer Research)

1. Wu MS, Shun CT, Wu CC, Hsu TY, Lin MT, Wang HP, Lin JT. Clinicopathologic characteristics of Epstein-Barr-virus-associated gastric carcinomas in Taiwan: relation to Helicobacter pylori infection and genetic alterations.