行政院國家科學委員會專題研究計畫　成果報告

台灣地區乳癌患者存活率與病毒感染因子的關聯性及血清標記之研究

計畫類別：個別型計畫
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計畫主持人：楊繼江

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附件一

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

（計畫名稱）台灣地區乳癌患者存活率與病毒感染因子的關聯性及血清標記之研究

計畫類別：■ 個別型計畫　　□ 整合型計畫
計畫編號：NSC 94-2314-B-040-027
執行期間：2005 年 08 月 01 日至 2006 年 07 月 31 日

計畫主持人：楊繼江
共同主持人：
計畫參與人員：

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

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
■涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：中山醫學大學

中　華　民　國　95 年　10　月　23　日

中、英文摘要及關鍵詞(keywords)

根據我們先前的研究，是以聚合酶鏈鎖反應與南方雜交法偵測非家族性乳癌病人HPV、CMV、EBV、HSV-1、HSV-2 和 HHV-8之DNA，結果發現HHV-8與乳癌之間具有相關性存在(P<0.01)。因此，需要進一步調查乳癌淋巴結狀態與病毒感染之相關性。總共有62位乳癌病人參與此回溯性研究。以六個具有潛在致癌性病毒來探討乳癌淋巴結狀態和治療結果之間的相關性。統計分析各個變項，包括病毒陽性率與臨床特徵。比較淋巴結陽性和淋巴結陰性之間，其病毒陽性率沒有顯著差異(P>0.05)。 當不考慮病毒性因子時，沒有一變項與存活率具有相關性。然而，腫瘤週期、腫瘤大小、淋巴結狀態和雌激素接受器等變項與無復發存活率之間具有相關性存在(P<0.05)。就病毒因子而言，病毒的感染數量與存活率和無復發存活率具有相關性。當V0或 V(0, 1)組與其他多病毒感染組做比較時，二組其存活率、無復發存活率皆具有顯著差異(P<0.005；P<0.001)。研究結果發現HSV-1、HHV-8、EBV、CMV、HPV與存活率有關，然而，只有HHV-8和CMV與無復發存活率有關(P<0.05；P <0.01)。病毒性因子與人類乳癌具有顯著的相關性，不僅是在致癌性過程，也呈現在存活率、無復發存活率上。

關鍵詞：存活率、無復發存活率、病毒、淋巴結、聚合酶鏈反應、南方雜交法

Our previous study based on the results of PCR and Southern hybridization for the detection of HPV, CMV, EBV, HSV-1, HSV-2 and HHV-8 DNA in non-familial breast cancer patients suggest that the viruses associated with breast cancer are HHV-8>EBV (P<0.01). Therefore, efforts were made to further investigate the association between breast cancer with node status and viral infections. 62 breast cancer patients and their mammary specimens were enrolled in this retrospective study. The presence of these six potential oncogenic viruses was analyzed to establish the relationship between nodal status and treatment outcome. Statistical analysis were

used for the assessment of variables, including viral positivity and clinical feature. Viral positivity was not significantly different comparing node-positive and node-negative patients ($P>0.05$). When the viral factors were not entered for statistical analyses, no variable was significantly related to overall survival. However, tumor stage, tumor size, nodal status and estrogen receptor were significantly related to relapse-free survival ($P<0.05$). For viral factors, the number of infecting viruses is related to the overall and relapse-free survivals. Only when V0 or V(0, 1) was grouped for comparison with other multiply virus-infected subgroups, were the overall and relapse-free survivals significantly different ($P<0.005$ or <0.001). The results suggest that HSV-1, HHV-8, EBV, CMV and HPV were relate to overall survival, however, only HHV-8 and CMV were related to relapse-free survival ($P<0.05$ or <0.01). Virus factor is significantly related to human breast cancer, not only in terms of the oncogenetic process, but also in overall and relapse-free survivals.

Key words: overall survival; relapse-free survival; virus, lymph-node; polymerase chain reaction; Southern hybridization.

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ORIGINAL PAPER

Relationship between viral factors, axillary lymph node status and survival in breast cancer

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Abstract
Purpose Our previous study based on the results of polymerase chain reaction and Southern hybridization for the detection of Human papilloma virus (HPV), Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Herpes simplex virus (HSV)-1, HSV-2, and Human herpesvirus (HHV)-8 DNA in non-familial breast cancer patients suggest that the viruses associated with breast cancer are HHV-8 > EBV ($P < 0.01$). Therefore, efforts were made to further investigate the association between breast cancer with nodal status and viral infections.
Methods Sixty-two breast cancer patients and their mammary specimens were enrolled in this retrospective study. The presence of these six potential oncogenic viruses was analyzed to establish the relationship between nodal status and treatment outcome. Statistical analyses were used for the assessment of variables, including viral positivity and clinical feature.
Results Viral positivity was not significantly different comparing node-positive and node-negative patients ($P > 0.05$). When the viral factors were not entered for statistical analyses, no variable was significantly related to overall survival. However, tumor stage, tumor size, nodal status , and estrogen receptor were significantly related to relapse-free survival ($P < 0.05$). For viral factors, the number of infecting viruses is related to the overall and relapse-free survivals. Only when V0 or V(0, 1) was grouped for comparison with other multiply virus-infected subgroups, were the overall and relapse-free survivals significantly different ($P < 0.005$ or $P < 0.001$). The results suggest that HSV-1, HHV-8, EBV, CMV, and HPV were related to overall survival, however, only HHV-8 and CMV were related to relapse-free survival ($P < 0.05$ or $P < 0.01$).
Conclusion Virus factor is significantly related to human breast cancer, not only in terms of the oncogenetic process, but also in overall and relapse-free survivals.

Keywords Overall survival · Relapse-free survival · Virus · Lymph node · Polymerase chain reaction · Southern hybridization

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Abbreviations

CMV	Cytomegalovirus
EBV	Epstein-Barr virus
HHV	Human herpesvirus
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
HSV	Herpes simplex virus
NAT	Neoadjuvant therapy
NNAT	Non-neoadjuvant therapy
PCR	Polymerase chain reaction
UICC	Union Internationale Contre Ie Cancer

Introduction

Axillary nodal status, as assessed by traditional histological methods, is a proven independent prognostic factor for breast cancer. Sentinel lymph node biopsy is a surgical pathological staging procedure that not only allows the selective removal of the most likely sites for lymphogenic metastases, but also enables upstaging of breast carcinoma by identifying nodal involvement undetected by standard staging methods (Cserni 2004). Although the impact of axillary treatment on survival remains controversial, in recent times axillary lymph node positivity has been considered an indicator for elevated systemic-diffusion disease risk rather than as the possible source of systemic metastases (Catzeddu et al. 2004).

The concept of sentinel lymph node biopsy in breast cancer surgery is based on the fact that tumors drain via the lymphatic system in a logical way (Trifiro et al. 2004). Specialists have used hematoxylin and eosin stain and/or immunohistochemical techniques to examine metastatic axillary lymph nodes (Giuliano et al. 1995; Breslin et al. 2000; Julian et al. 2001). However, predicting metastases from the status of axillary lymph nodes is difficult (Noguchi et al. 1996).

The effectiveness of lymph nodes for entrapment of inanimate particles, bacteria, viruses, and red blood cells has been extensively evaluated; however, there are relatively few reports detailing investigation of the viral factors in breast cancer and how they affect lymph node metastasis. Viruses are one of the risk factors closely related to human cancer (Dimmock and Primrose 1994). Many widespread chronic diseases, previously thought to be due to metabolic imbalances or genetics modifications, are increasingly linked to infections. It is now estimated that viruses contribute to 20% of all human cancers (Dimmock and Primrose 1994).

Previous studies provide direct evidence that viruses exist in patients with breast cancer and suggest that viruses are one of the risk factors for breast cancer (Pogo and Holland 1997; Brower 2004). However, some of these works remain in dispute (Gopalkrishna et al. 1996; Chu et al. 1998; McCall et al. 2001). Richardson (1997) suggested that late exposure to human cytomegalovirus (CMV) is a risk factor for breast cancer. Labrecque et al. found that Epstein-Barr virus (EBV) was more frequently detected in malignant tissue by polymerase chain reaction (PCR) compared to non-cancerous tissue (Labecque et al. 1995). A recent study suggests that the presence of either Human papilloma virus (HPV)-16 or HPV-18 in the breast may be related to the development of a malignant phenotype (Damin et al. 2004). By contrast, Gopalkrishna et al. (1996) reported the absence of HPV DNA in breast cancer. It has also been suggested that Human herpesvirus (HHV)-8 is associated with breast cancer (Newton et al. 2003). Our previous study focused on the fact that the results of PCR and Southern hybridization for the detection of HPV, CMV, EBV, Herpes simplex virus (HSV)-1, HSV-2, and HHV-8 DNA in non-familial breast cancer patients suggest that the viruses associated with breast cancer are HHV-8 > EBV ($P < 0.01$) (Tsai et al. 2005). However, none of these studies discussed the relationship between viral factors and axillary lymph nodes and breast cancer survival. Instead, these workers emphasize the molecular etiology of breast cancer without investigating the relationship to clinicopathological features.

In this patient study, therefore, we have investigated the presence of these six potential oncogenic viruses, attempting to establish the relationship between viral factor, nodal status, and treatment outcome. Investigation of viral factors in breast cancer and their association with clinicopathological features might enable earlier diagnosis, ultimately improving prevention and treatment.

Patients and methods

Specimen collection and characterization

This study was performed with approval by Chung Shan Medical University Human Investigations Review Board (No. CS05044). As in the previous investigation (Tsai et al. 2005), samples were obtained from 69 females with primary non-familial breast cancer tumors and 60 specimens from non-cancerous or other individuals with thyroid tumors or fibroadenoma (non-breast cancer controls). All of the samples were examined by pathologists at Chung Shan Medical University Hospital, Taichung, Taiwan and collected at

this medical center from November 1999 to August 2003. The cancer stage and tumor size were defined according to the Union Internationale Contre Ie Cancer (UICC) system, and also analyzed by PCR and Southern hybridization for the presence of six viruses, HPV, CMV, EBV, HSV-1, HSV-2, and HHV-8. Two specimens from patients with a familial history of breast cancer and five breast cancer specimens with negative results for β-globin, which was used as internal control, were excluded from this study. Of 62 β-globulin-positive breast cancer cases, all were infiltrating carcinomas. None of 62 breast cancer patients had undergone presurgical radiotherapy, however, six had undergone neoadjuvant therapy (NAT). Of 62 breast cancer patients studied, the six treated with neoadjuvant chemotherapy were assigned to the NAT group, while the other 56 were assigned to the non-neoadjuvant therapy (NNAT) group. The median number of axillary lymph nodes examined in the NNAT group was 20 (range 11–38), significantly higher than the NAT analog (median = 10.4, range 2–19; $P = 0.023$).

Immediately after surgery, one set of the tumor samples and controls were placed in liquid nitrogen and stored at −80°C until analysis. The other set of tissues underwent routine histopathological diagnosis, including immunohistochemical staining for estrogen and progesterone receptors (Clones 1D5 and PgR636, DakoCytomation, Copenhagen, Denmark).

DNA extraction, polymerase chain reaction assay and southern hybridization

The DNA extraction, PCR assay, and Southern hybridization were established in our lab using the protocols described before (Tsai et al. 2004, 2005; Yang et al. 2004a, b).

Axillary lymph node examination

Lymphadenectomy specimens were dissected fresh following the Watanabe protocol for isolation of lymph nodes (Watanabe et al. 1992), with no clearing techniques used.

Statistical analysis

Relapse-free survival was calculated using the period from first day of surgery until the day of relapse or last follow-up visit. Overall survival was calculated from the first day of surgery until death or last follow-up visit. Life-table estimation was performed according to the method of Kaplan and Meier. The chi-square test was used to compare clinicopathological parameters between virus detection and nodal status. Wilcoxson sum rank test was used to compare axillary lymph node numbers with or without NAT. Univariate comparison of survival curves was assessed by log-rank test. Multivariate comparison of clinical feature was assessed by Cox proportional-hazards model. Kendall's τ-b-test was used to examine the correlation between number of viruses present and node positive/node negative samples. $P < 0.05$, $P < 0.01$, $P < 0.005$, or $P < 0.001$ was considered statistically significant.

Results

A total of 62 non-familial, female breast cancer patients and their surgically obtained mammary specimens were studied. The clinicopathological characteristics of the breast cancer patients are summarized in Table 1. All cancer tissues ($n = 62$) were screened for the presence of six different viruses using PCR and Southern blotting analysis, as previously reported (Tsai et al. 2005). Among the 62 breast cancer samples, eight (12.9%) were positive for HSV-1, 28 (45.2%) for EBV, 47 (75.8%) for CMV, eight (12.9%) for HPV, and 28 (43.8%) for HHV-8. In the non-breast cancer control groups, 8/12 (66.7%) were CMV-positive normal samples, 4/16 (25.0%) CMV-positive thyroid tumor samples, 20/32 (62.5%) were HSV-1-positive fibroadenoma samples, 16/32 (50.0%) were EBV-positive, 20/32 (62.5%) were CMV-positive, 2/16 (6.3%) were HPV-positive, and 28 (87.5%) were HHV-8-positive fibroadenoma samples. HSV-2 was not detected in either the breast cancer or the non-breast cancer groups. Among the viral gene-positive breast cancer samples, 12 (23.1%) were positive for one virus, 16 (30.8%) were positive for two viruses, 21 (40.4%) were positive for three viruses, and three (5.8%) were positive for four viruses. Among the 40 viral DNA-positive specimens of the non-breast cancer controls, only one type of virus was found in the normal and thyroid tumor specimen groups, and all fibroadenoma specimens had more than one viral type present. The presence of two- and three-viruses was found in eight (28.6%) specimens each, respectively, and four virus types were found in 12 (42.9%) specimens. Of 52 viral DNA-positive breast cancer specimens, 12 specimens (23.1%) were positive for one virus, 16 (30.8%) for two, 21 (40.4%) for three, and three (5.8%) were positive for four viruses. When viral positivity and clinical features were entered into the chi-square analysis, no significant correlation was demonstrated between viral positivity and age (50 year), tumor stage, tumor size, estrogen receptor or progesterone receptor status for our patient sample

Table 1 Clinicopathological characteristics of breast cancer patients

	Total patients no. (%)	Virus positivity no. (%)	*P*-value
Age (year)			
< 50	19 (30.6)	16 (84.2)	0.962
≥ 50	43 (69.4)	36 (83.7)	
Tumor stage			
I	18 (29.0)	17 (94.4)	0.333
II	42 (66.8)	34 (80.9)	
III	2 (3.2)	2 (100)	
Tumor size			
T1	13 (21.0)	12 (92.3)	0.580
T2	41 (66.1)	33 (80.4)	
T3	8 (12.9)	7 (87.5)	
Nodal status			
−	32 (51.6)	29 (90.6)	0.251
+	30 (48.4)	23 (76.7)	
ER status			
−	32 (51.6)	27 (84.3)	0.912
+	30 (48.4)	25 (83.3)	
PR status			
−	49 (79.3)	41 (85.3)	0.935
+	13 (20.7)	11 (84.6)	

($P > 0.05$). Although histological involvement of axillary lymph nodes is the standard risk factor used for prognostic evaluation, we found that viral positivity was not significantly different comparing node-positive and node-negative patients ($P > 0.05$).

When the clinical features but not viral positivity were entered into a multivariate Cox proportional-hazards model, none of the variables was significantly related to overall survival; however, tumor stage, tumor size, nodal status, and estrogen receptor were significantly related to relapse-free survival ($P < 0.05$; Table 2). We used Kendall's τ-*b*-test to examine the correlation between number of virus infections and survivals in breast cancer patients. The results suggest that the number of infecting viruses is related to the overall and relapse-free survivals of breast cancer patients (correlation coefficient = −0.275; $P = 0.021$). In order to further investigate the relationship between viral factors and overall and relapse-free survivals in our sample of breast cancer patients, univariate log-rank analysis was used. Both overall and relapse-free survival rates were significantly different ($P = 0.001$ and $P < 0.001$, respectively; Table 3) comparing V0–V4 subgroups (zero–four virus infections, respectively). Moreover, as shown in Table 3, significant differences were also demonstrated in comparisons of the respective survival rates for the virus infection subgroups: V(0,1), V2, V3, and V4; V(0, 1), V2, V(3, 4), V(0, 1), V(2, 3), V4, and, V(0, 1), and V(2, 3, 4) ($P < 0.005$ or $P < 0.001$) (n, $n + 1$..., indicates virus number). Except for the V0 vs. V1 vs. V2 vs. V3 vs. V4 variable, only when V(0,1) was grouped for comparison with other multiply virus-infected subgroups, however, were the overall and relapse-free survivals significantly different. These results suggest that the number of virus infections is related to the overall and relapse-free survivals in our sample of breast cancer patients, moreover, the overall and relapse-free survivals of multiply (more than two) virus-infected breast cancer patients group is significantly different from the no virus- or one virus-infected group.

Different virus-positive groups were further studied to clarify the role of individual viruses in breast cancer.

Table 3 Results of log-rank analysis for overall and relapse-free survival

Variable	*P*-value	
	Overall survival	Relapse-free survival
V0 vs. V1 vs. V2 vs. V3 vs. V4	0.001*	< 0.001*
V0 vs. V(1, 2) vs. V(3, 4)	0.359	0.189
V0 vs. V(1, 2, 3) vs. V4	0.645	0.598
V0 vs. V(1, 2, 3, 4)	0.515	0.979
V(0,1) vs. V2 vs. V3 vs. V4	0.013*	< 0.001*
V(0, 1) vs. V2 vs. V(3, 4)	0.005*	< 0.001*
V(0, 1) vs. V(2, 3) vs. V4	0.005*	< 0.001*
V(0, 1) vs. V(2, 3, 4)	0.001*	< 0.001*
V(0, 1, 2) vs. V3 vs. V4	0.543	0.187
V(0, 1, 2) vs. V(3, 4)	0.288	0.078
V(0, 1, 2, 3) vs. V4	0.539	0.312

*Statistically significant

Table 2 Results of multivariate Cox proportional-hazards analysis

Variable	Hazard ratio for death		Hazard ratio for relapse	
	95% CI	*P*-value	95% CI	*P*-value
Age (≥ 50 vs. < 50 year)	1.836 (0.268–21.807)	0.432	0.816 (0.430–4.437)	0.587
Tumor stage (I vs. II vs. III)	1.343 (0.429–8.787)	0.390	3.264 (1.535–10.448)	0.005*
Tumor size (T1 vs. T2 vs. T3)	1.567 (0.736–16.424)	0.116	3.547 (1.856–12.794)	0.001*
Nodal status (− vs. +)	1.238 (0.607–49.695)	0.130	5.229 (1.344–17.738)	0.016*
ER status (− vs. +)	1.364 (0.161–6.338)	0.991	4.143 (1.256–13.899)	0.020*
PR status (− vs. +)	1.234 (0.122–10.107)	0.927	1.235 (0.929–8.577)	0.067

*Statistically significant

The results of Kaplan–Meier estimation of overall and relapse-free survivals for the node-negative and node-positive virus-positive groups (N-V + and N+V+, respectively) are depicted in Fig. 1a, b. Both overall and relapse-free survivals were significantly different ($P < 0.05$ or $P < 0.01$). The results of the Kaplan–Meier estimation of the overall and relapse-free survivals for individual virus-infected cases deducted from N-V+ and N+V+ groups are presented in Fig. 1c–l. Thus, the statistical significance of individual virus infections can be revealed. For example, comparing the HSV-1-positive case-deducted N-V+ group with the HSV-1-positive case-deducted N+V+ group, the overall survival was not significantly different ($P > 0.05$), however, the

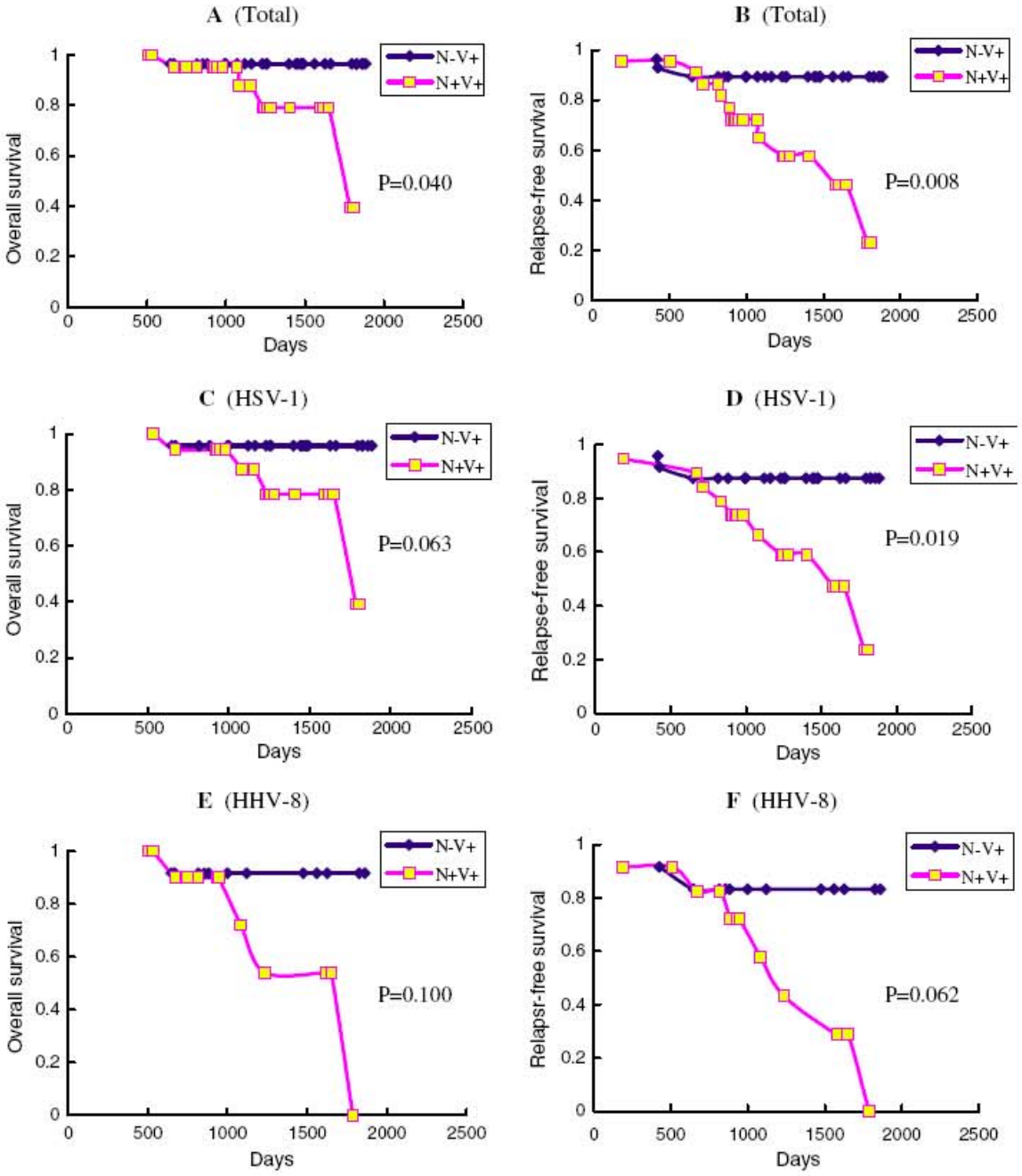

Fig. 1 Kaplan–Meier estimates of overall (panels **a**, **c**, **e**, **g**, **i**, and **k**) and relapse-free survival (panels **b**, **d**, **f**, **h**, **j**, and **l**) for different groups. **a** and **b** N-V+ ($n = 29$) and N+V+ ($n = 23$) groups. **c** and **d** HSV-1-positive case deducted N-V+ ($n = 24$) and N+V+ ($n = 20$) groups. **e** and **f** HHV-8-positive case deducted N-V+ ($n = 12$) and N+V+ ($n = 12$) groups. **g** and **h** EBV-positive case deducted N-V+ ($n = 12$) and N + V + ($n = 12$) groups. **i** and **j** CMV-positive case deducted N-V+ ($n = 2$) and N+V+ ($n = 3$) groups. **k** and **l** HPV-positive case deducted N-V+ ($n = 25$) and N+V+ ($n = 19$) groups

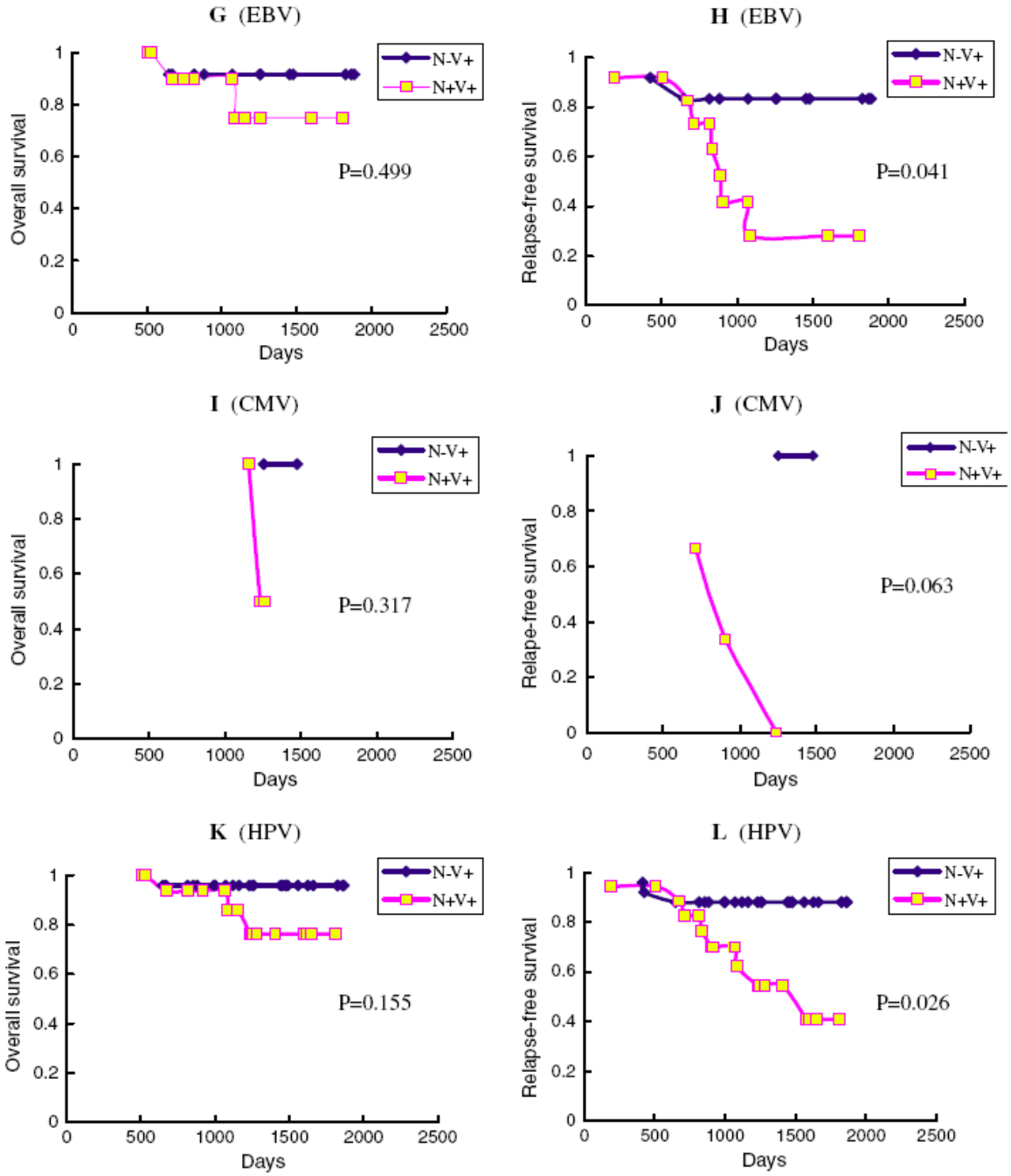

Fig. 1 continued

relapse-free survival remained significant ($P < 0.05$) (Fig. 1c, d, respectively). A statistically significant relationship between HSV-1 and overall, but not relapse-free, survival in our sample population was revealed, although the change of P values from 0.040 to 0.063 was minor. In these analyses, all five of the detected viruses (i.e. HSV-1, HHV-8, EBV, CMV, and HPV) were related to overall survival, however, only HHV-8 and CMV were related to relapse-free survival.

Discussion

Axillary lymph node involvement has been recognized as the most important prognostic factor in breast cancer, despite the fact that many other parameters have been evaluated in recent years (O'Hanlon et al. 2002; Reed et al. 2004). Recently, Reed et al. (2004) demonstrated that metastases larger than 2 mm predicted poorer relapse-free survival rate and that subcapsular

location of metastases was a strong predictor of overall survival in their sample. Further, multivariate analysis revealed that histological grade, tumor size, progesterone receptor status, and presence of occult metastasis in the lymph nodes had a prognostic impact on survival over a 25-year follow-up. Pedersen et al. (2004) demonstrated a significant effect on overall survival only for tumor size, positive lymph node status, histology grade, and receptor status from multivariate analysis. Kett et al. (2002) have shown that survival probability is determined both by status of axillary lymph drainage and number of metastatic axillary lymph nodes. Voordeckers et al. (2004) have indicated that the percentage of positive lymph nodes, as determined from axillary lymph node dissection, appears to be an important prognostic factor for survival, and that the ratio of improved nodes to absolute number of involved axillary lymph nodes is useful for prognostic assessment. In the present study, we have further suggested that, except for viral factors, no variable is significantly related to overall survival; however, tumor stage, tumor size, nodal status, and estrogen receptor were significantly related to relapse-free survival ($P < 0.05$) (Table 2).

A finding of negative lymph nodes is indicative only of removal of a given tumor before lymphatic dissemination and destruction of the tumor-containing node by immune nodes. Tumor-suppressor cell activity has been demonstrated in the draining lymph nodes from breast cancer tissue (Huang et al. 2000). Suppression of paracortical interdigitating dendritic cells and antigen-presenting processes in the sentinel node is marked by comparison to the non-sentinel analogs (Cochran et al. 2001; Cengiz et al. 2004). Therefore, the prospect for the control of metastases, we suggest that, in addition to immune factors, viral factors also contribute to the development of breast cancer.

In our previous report, we suggested that, although HHV-8 is more frequently associated with breast cancer than EBV ($P < 0.01$), HSV-1 and HHV-8 are more frequently associated with fibroadenomas than EBV ($P < 0.01$) (Tsai et al. 2005). It is also interesting to note that in our samples the number of infecting viruses associated with benign fibroadenoma was higher than for malignant breast cancer. Moreover, HSV-1 is associated with benign (fibroadenoma) but not malignant tumors (breast cancer) of breast tissue. Thus, the role of HSV-1 in mammary-tumor development warrants investigation. Moreover, it is interesting that except for the V0 vs. V1 vs. V2 vs. V3 vs. V4 variable, the overall and relapse-free survivals were significantly different only when V(0,1) was grouped for comparison with other multiply virus-infected subgroups (Table 3). The findings of our current and earlier studies (24) with respect to the significance of viral factors for overall and relapse-free survival and the viruses associated with breast cancer are summarized in Table 4. Based on these results, it seems reasonable to suggest that HSV-1, HHV-8, EBV, CMV, and HPV are related to overall survival, however, only HHV-8 and CMV appear related to relapse-free survival. It is thus reasonable to speculate that more virus infection by more virus types, i.e., multiple virus infection, would have greater effects on the immune system and overall and relapse-free survivals. In our previous report, we suggested that the HHV-8 virus had the strongest association with breast cancer tissues (Tsai et al. 2005). The present study reinforces this finding, with statistically significant relationships demonstrated between HHV-8 and both overall and relapse-free survivals. Thus, we suggested the importance of HHV-8 with respect to the oncogenic processes and survival of breast cancer cannot be ignored. For CMV, it was suggested that it is related to the rate of relapse-free survival (Fig. 1) but not oncogenesis in breast cancer (Tsai et al. 2005). More recently, Cengiz et al. (2004) have shown statistically significant increases in the number of metastatic lymph nodes, vascular invasion, and tumor size for breast cancer patients with thyroid disease and greater frequency of thyroid pathology compared to normal controls. Interestingly, we have previously suggested that CMV is the only virus associated with thyroid tumors (Tsai et al. 2005). If proven, this raises the possibility that it could account for the increased numbers of metastatic lymph nodes and the relatively low relapse-free survival in these patients. In spite of the associated oncogenic potential of EBV with breast cancer (Tsai et al. 2005), a relationship with relapse-free survival has not been proposed in this study. Widschwendter et al. (2004) have suggested that HPV may be transported from the original site of infection (cervical tissues) to

Table 4 The significance of viral factor to overall and relapse-free survival and virus associated with breast cancer

	Overall survival	Relapse-free survival	Associated oncogenic potential virus
HSV-1	+	–	–
HSV-2	N.A.	N.A.	–
HHV-8	+	+	+*
EBV	+	–	+*
HCMV	+	+	–
HPV	+	–	–

+ significant, – not significant, *N.A.* not available

*The viruses associated with breast cancer are HHV-8 > EBV ($P < 0.01$)

the breast via the blood stream, and that this may be the reason for the detection of HPV DNA in breast cancer samples where there is a cervical cancer history. This is consistent with our previous findings that HPV is significantly related to cervical and oral cancers but not to breast malignancy (Tsai et al. 2004, 2005; Yang et al. 2004a, b). Moreover, it was demonstrated in this study that HPV is significantly related to overall but not relapse-free survival in breast cancer patients.

For the association of virus with breast cancer, our results were based on parts of the DNA oncogenic viruses and a relatively small sample size. Further extensive study with large-scale samples and more virus types assayed is necessary. However, in the present study, multiple virus infection is significantly related to the overall and relapse-free survivals in our sample of breast cancer patients. This is consistent with our previous finding that the presence of multiple viruses predominates in breast cancer (Tsai et al. 2005). If this is true, immune-suppressive chemotherapy may further influence the virus infectious status. In Japan, Miura (1972) reported that the median number of nodes examined for non-adjuvant treatment breast cancer patients was 23.6. Giuliano et al. (1995) demonstrated that the median number of examined nodes was 19 (range 8–40). Although our sample size was relatively small, the median node numbers of 20 (range 11–38) and 10.4 (range 2–19) for the NNAT and NAT groups, respectively, are comparable with analogous findings from these reports, with the latter significantly lower than the former ($P < 0.05$). In our study, in spite of the small number of patients, the median number of dissected nodes was lower for the NAT group compared to the NNAT analog, suggesting a neoadjuvant chemotherapeutic effect.

To summarize, although viral positivity was not significantly different comparing node-positive and node-negative patients ($P > 0.05$), the number of virus infections is related to the overall and relapse-free survivals in our sample of breast cancer patients. Moreover, the overall and relapse-free survivals of multiply (more than two) virus-infected breast cancer patients group are significantly different from the no virus- or one virus-infected group. Therefore, we suggest that multiple viral factors may relate to human breast cancer not only in terms of the oncogenic processes revealed in our previous tissue-specimen study, but also in terms of overall and relapse-free survival, and ultimately reflect treatment efficacy.

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計畫成果自評：

本計畫第一部份將進一步進行其存活率預測與病毒感染因子的關聯性的追蹤分析，這部份已發表論文（Tsai JH, Hsu CS, Tsai CH, Su JM, Liu YT, Cheng MH, Wei JCC, Chen FL, **Yang CC**. Relationship between viral factors, axillary lymph-node status and survival in breast cancer. J Cancer Res Clin Oncol 2006, in press. **(**SCI, IF=2.503)），即將於 2007 年 1 月份出版。本計畫第二部份也已收集乳癌、子宮頸癌、口腔癌患者治療前後血清，進行 Metaloproteinase 2, IL-6 及 IL-11 檢測與比較分析，仍在進行。

附件二

可供推廣之研發成果資料表

■ 可申請專利　□ 可技術移轉　　日期：95 年 10 月 23 日

國科會補助計畫	計畫名稱：台灣地區乳癌患者存活率與病毒感染因子的關聯性及血清標記之研究 計畫主持人：楊繼江 計畫編號：NSC 94-2314-B-040-027 學門領域：BR 婦產科及泌尿科
技術/創作名稱	針對乳癌、甲狀腺瘤及纖維腺瘤相關病毒之用途、及含抗彼之醫藥組成物
發明人/創作人	楊繼江
技術說明	中文：一種針對乳癌、甲狀腺瘤及纖維腺瘤相關病毒之用途、及含抗彼之醫藥組成物，特別是指一種利用多種病毒因子檢測來得知罹患乳癌、甲狀腺瘤或纖維腺瘤的方法，更進一步提供乳癌、甲狀腺瘤及纖維腺瘤高危險群的預測、預防乳癌、甲狀腺瘤及纖維腺瘤的方法、乳癌、甲狀腺瘤及纖維腺瘤預後之預測，以及乳癌、甲狀腺瘤及纖維腺瘤之疫苗。
	英文：A kit of assaying for diagnosis, a composition for inhibition, and a vaccine for prevention the multiple viruses related to breast cancer, thyroid tumor or fibroadenoma. The present invention relates to the samples of breast cancer infected with human herpesvirus (HHV-8) and Epstein-Barr virus (EBV) showing statistical significance and the samples with non-cancerous and thyroid tumor infected with human cytomegalovirus (CMV) alone showing statistical significance. The present invention suggests that multiple viral factors are relate to human breast cancer not only in terms of the oncogenic processes revealed in our previous tissue-specimen study, but also in terms of overall and relapse-free survival, ultimately reflect treatment efficacy.
可利用之產業及可開發之產品	1. 乳癌、甲狀腺瘤及纖維腺瘤之檢測 2. 乳癌高危險群的預測 3. 乳癌、甲狀腺瘤及纖維腺瘤相關病毒之用途、及含抗彼之醫藥組成物，更進一步提供乳癌、甲狀腺瘤及纖維腺瘤之疫苗，以促進乳癌之治療。
技術特點	1. 本發明所提供之乳癌、甲狀腺瘤及纖維腺瘤之檢測，係以多種乳癌、甲狀腺瘤及纖維腺瘤相關病毒，檢測受測者有無罹患乳癌、甲狀腺瘤或纖維腺瘤，以做到乳癌早期診斷。 2. 本發明所提供之乳癌高危險群的預測，係檢測多種乳癌、甲狀腺瘤及纖維腺瘤相關病毒，係以多重病毒結合

	的效應與乳癌的關聯，評估受測者罹患乳癌之機率，以作為乳癌、甲狀腺瘤及纖維腺瘤風險的預測，達到預防乳癌、甲狀腺瘤及纖維腺瘤的目的。 3. 本發明之乳癌、甲狀腺瘤及纖維腺瘤相關病毒之用途、及含抗彼之醫藥組成物，更進一步提供乳癌、甲狀腺瘤及纖維腺瘤之疫苗，以促進乳癌之治療。
推廣及運用的價值	1. 一種針對乳癌、甲狀腺瘤及纖維腺瘤相關病毒之用途、及含抗彼之醫藥組成物，係在受測者檢體中確認單純疱疹病毒第一型(HSV-1)、單純疱疹病毒第二型(HSV-2)、愛波斯坦-巴爾病毒(EBV)、巨細胞病毒(CMV)、人類乳頭狀瘤病毒(HPV)及人類疱疹病毒第八型(HHV-8)的存在與否，作為乳癌、甲狀腺瘤及纖維腺瘤之檢測方法。 2. 本發明更進一步提供乳癌高危險群的預測、預防乳癌的方法、乳癌預後之預測，以及乳癌、甲狀腺瘤及纖維腺瘤之疫苗。

※ 1. 每項研發成果請填寫一式二份，一份隨成果報告送繳本會，一份送　貴單位研發成果推廣單位（如技術移轉中心）。

※ **2. 本項研發成果若尚未申請專利，請勿揭露可申請專利之主要內容。**

※ 3. 本表若不敷使用，請自行影印使用。