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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※　　婦科腫瘤血管生成之研究:臨床到基礎　　　　　　※
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計畫主持人：謝長堯

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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

執行單位：台大醫院婦產科

中　華　民　國　　89　年　　10　月　20　日

中文摘要

目的:評估血管內皮細胞生長因子是否可以預測子宮頸癌轉移至淋巴腺及是否可當作獨立預後因子。

材料與方法:130 位接受子宮根除手術及雙側淋巴腺根除的早期子宮頸癌(Ib-IIa 期)病患,腫瘤血管內皮細胞血管生長因子的濃度以酵素免疫分析法規 Enzyme immunoassay)測量比較影響預後之病理組織項目及細胞血管生長因子。

結果:有子宮頸癌復發之 23 位病人(16.3%)比未復發之病患有較高的血管內皮細胞生長因子濃度(1020 vs 112pg/ml protein, p<0.001)。以 400pg/mg 當 cutoff value,敏感度為 75%,特異度為 70%,陽性預測值為 41%,陰性預測值為 92%,只有過度表現的細胞血管內生長因子才可被當作獨立的影響復發之預後因子(hazard ratio 6.44, P<.001)。過度表現的細胞血管內皮細胞生長因子(hazard ratio 4.50, P=0.021)及陽性淋巴血管栓塞瘤(hazard ratio 4.11, P=.045)為獨立的影響存活的預後因子。

結論: 腫瘤組織血管內皮細胞生長因子可以被當作一種生物指標來預測早期子宮頸癌病患之骨盆腔淋巴腺的情況及可作為獨立的影響結果之預後因子。

英文摘要

Objective: To evaluate vascular endothelial growth factor as a marker for predicitng lymph node metastasis and an independent prognostic factor of early-stage cervical carcinoma.

Materials and Methods: One hundred thirty-five women with stage IB-IIA cervical carcinoma underwent radical abdominal hysterectomies and pelvic lymph node dissections. Intratumoral cytosol vascular endothelial growth factor concentrations were assayed with enzyme immunoassay. Histopathologic items and cytosol vascular endothelial growth factor – influencing clinical outcomes were compared.

Results: Twenty-two women (16.3%) who had disease recurrence had higher levels of cytosol vascular endothelial growth factor (1020 versus 112 pg/mg protein, *P*<.001) as compared with those without recurrence. Using a vascular endothelial growth factor cutoff value of 400 pg/mg protein resulted in best sensitivity of 75%, best specificity of 70%, positive predictive value of 41%, and negative predictive value of 92%. Only over expressed cytosol vascular endothelial growth factor (hazard ratio 6.44, *P*<.001) was an independent prognostic factor of disease-free survival. The over expressed cytosol vascular endothelial growth factor (hazard ratio 4.50, *P*=.021) and positive lymphovascular emboli (hazard ratio 4.11, *P*=.045) were independent prognostic factors of overall survival.

Conclusions: Cytosol vascular endothelial growth factor might be a biomarker for the status of pelvic lymph nodes in early-stage cervical carcinoma and an independent prognostic indicator of its outcome.

計畫緣由與目的

Although cervical cancer rates have declined significantly in Western countries during the past several decades, it remains one of the most common forms of cancer in women in developing countries.[1,2] Along with receding incidence of invasive cervical cancer is frequent diagnosis in its early stages.[3] Most women are seen first with stage I disease, implying favorable outcomes. Tumor size, depth of stromal invasion, lymphovascular emboli, parametrial invasion, and pelvic lymph node metastasis are prognostic factors of early-stage cervical cancer.[4-7]

Radical hysterectomy with pelvic lymphadenectomy is frequently used to treat stage IB and IIA cervical cancer.[8] Knowledge of pelvic lymph node metastasis is crucial for treatment planning. Five-year survival is 70–90% in women without lymph node involvement and decreases to 40– 60% with metastasis.[9,10] Hence women with positive pelvic lymph nodes are considered at risk of recurrence and are treated more aggressively.[11] Nodal status dose not fully account for clinical outcomes, in fact, 10–15 % of women without pelvic lymph node involvement have tumor recurrence, and approximately half with involvement are cured of disease after adjuvant radiotherapy.[12] More accurate prognostic parameters that correlate with outcome allow improved understanding of the biologic behavior of the tumor and could help define a subgroup of women at risk of

recurrence, which makes it possible to individualize treatment.

The growth of solid tumors and their metastatic spread are believed to be angiogenesis-dependent, which was confirmed by several studies.[13,14] Vascular endothelial growth factor, which induces growth of a capillary network arround a tumor and acts as a highly specific mitogen on endothelial cells, is an important factor in tumor angiogenesis.[15] Vascular endothelial growth factor was associated with disease-free and overall survivals in ovarian cancer.[16] We evaluated the correlation between vascular endothelial growth factor, microvessel density, and clinicopathologic parameters in cervical cancer previously;[17] however, the qunatitation of vascular endothelial growth factor and its correlation with disease-free and overall survivals are still unclear in cervical carcinoma.

The purposes of this study were 1) to determine if intratumoral cytosol vascular endothelial growth factor could be a marker for predicting pelvic lymph node metastasis, and 2) to evaluate whether intratumoral cytosol vascular endothelial growth factor important in outcomes of early stage cervical carcinoma.

結果

Patients' ages ranged from 29–71 years with a mean age of 50.6 years. Sixty-four (47.4%) were postmenopausal. One hundred twenty-two (90.4%) presented with stage IB and 13 (9.6%) with stage IIA cervical cancer. Pathologic examination showed 103 (76.3%) squamous cell carcinomas, 25 (18.5%) adenocarcinomas, five (3.7%) adenosquamous cell carcinomas and two (1.5%) small cell carcinomas. Pelvic lymph node metastases were found in 28 of 135 women (20.7%). Tumor size ranged from 0.3– 6.0 cm (mean 2.6). Fifty women (37.3%) received postoperative adjuvant radiotherapy. The cytosol vascular endothelial growth factor concentration ranged from 0– 3050 pg/mg (median 154.0). Disease-free intervals of recurrent women were from 2– 41 months (median 12.5) and nonrecurent women from 12– 70 months (median 23).

There were 22 women (16.3%) with disease recurrence later. Demographic characteristics between recurrent and nonrecurrent groups are shown in Table 1, with no significant differences between groups. Women with recurrence had higher median cytosol vascular endothelial growth factor protein levels (1020 versus 112 pg/mg protein, $P<.001$) compared with those without. As shown in Table 2, significantly higher median cytosol vascular endothelial growth factor concentrations were noted in tumors larger than 4 cm (775.0 versus 111.0 pg/mg protein, $P=.013$), with deep cervical stromal invasión (more than half thickness) (423.0 versus 86.5 pg/mg protein, $P<.001$), lymphovascular emboli (770.0 versus 111.5 pg/mg protein, $P=.003$), parametrial invasion (423.0 versus 114.5 pg/mg protein, $P=.01$), pelvic lymph node metastasis (807.5 versus 116.0 pg/mg protein, $P=.003$) compared with tumors less than 4 cm, with superficial stromal invasion, no lymphovascular emboli, no parametrial invasion, or no pelvic node metastasis. Other clinicopathologic characteristics such as stage and menopausal status showed no significant difference in the cytosol vascular endothelial growth factor concentrations.

The diagnostic efficacy of cytosol vascular endothelial growth factor for evaluating lymph node metastasis was analyzed. The receiver operating characteristics (ROC) curve illustrating the performance characteristics of vascular endothelial growth factor for predicting lymph node metastasis was shown. Using a vascular endothelial growth factor cutoff value of 400 pg/mg, resulted in the best sensitivity with 75%, the best specificity of 70%, positive predictive value of 41%, and negative predictive value of 92% (Table 3).

Table 4 shows the results of assessment of risk factors of recurrence for those women, and only overexpressed cytosol vascular endothelial growth factor (hazard ratio 6.44 (2.37, 13.54), $P<.001$) is the independent prognostic factor in disease-free survival . Table 5 shows the results of multivariable proportional hazards model analyses on risk factors that might influence the overall survival. The cytosol vascular endothelial growth factor (hazard ratio 4.50 (1.25, 16.22), $P=.021$) and lymphovascular emboli (hazard ratio 4.11 (1.05, 16.34), $P=.045$) are independent prognostic factors by multiple regression analysis.

討論

Tumor angiogenesis is a complex process that involves endothelial cell proliferation, digestion of the extracellular matrix surrounding capillaries, endothelial cell migration, and differentiation into functioning capillaries. Abundant evidence supports the concept that tumors can induce angiogenesis through many angiogenic molecules. Vascular endothelial growth factor is the most potent angiogenic molecule at present, not only inducing endothelial cell proliferation, but increasing vascular permeability. Our previous studies showed that vascular endothelial growth factor could reflect the disease severity and had good correlation with microvessel density in cervical cancer.[17,18] This study supports our previous reports and found that tumors with deep stromal invasion, parametrial invasion, lymphovascular emboli, and lymph node metastasis had higher cytosol vascular endothelial growth factor than those with superficial stromal invasion, and without parametrial invasion, lymphovascular emboli, or lymph node metastasis. That strongly suggested that vascular endothelial growth factor concentration indicates the local invasive activity and metastatic potential of cervical carcinoma.

Prognostic factors of early-stage cervical carcinoma include tumor size, depth of stromal invasion, parametrial invasion and lymph node metastasis, and the dominant prognostic factor affecting survival after radical hysterectomy is the lymph node status.[19] At present, adjuvant radiotherapy or systemic chemotherapy is administered after surgery to women at high-risk of relapse with indications of positive pelvic node, positive section margin, parametrial invasion, or bulky tumors.[3, 20] Adjuvant radiation can reduce the incidence of local recurrence, which is beneficial for patients with positive surgical margin or parametrial invasion. For women with lymph node metastasis, adjuvant radiotherapy may reduce pelvic recurrent rates but the survival benefit is still unclear. Because of complication rates are higher in patients treated with surgery and adjuvant radiotherapy, it is better to treat women with known lymph node metastasis by radiotherapy alone or concurrent chemoradiation. Preoperative evaluation of lymph node status is improtant because lymph node metatsasis or not will change the treatment modality for early stage cervical carcinoma patients. Noninvasive diagnostic tools such as sonography, CT, or magnetic resonance imaging are currently used to evaluate the lymph node status.We found that cytosol vascular endothelial growth factor could be a marker for evaluating possible lymph node metastasis and might be used for treatment palnning in early stage patients. We suggest that when cytosol vascular endothelial growth factor is higher than 400 pg/mg, pelvic lymph node metastasis should be suspected highly. Radiation therapy with or without concurrent chemotherapy seems to be better for women having higher than 400 pg/mg cytosol vascular endothelial growth factor protein levels.

Identification of factors that correlate with survival can give clinically relevant information to physicians and elucidate the basic biology of the disease. This study showed that vascular endothelial growth factor affects prognoses of women with cervical cancer in stage IB and IIA. As shown by Cox proportional hazards model, vascular endothelial growth factor showed the only prognostically relevant information independent of other established prognostic factors of stage IB and IIA cervical cancer. Status of lymph node metastasis, parametrial invasion, lymphovascular emboli, or tumor size were not the prognostic factors in this study. Our explanation is that those factors always had close correlations with each other, so they could not be independent factors in our survey.

There is abundant evidence that tumor angiogenesis influences prognosis of various cancers. Tumor angiogenesis, measured by microvessel density, has been reported as a prognostic factor in cervical carcinoma,[21] and seems to be a new factor of disease severity and prognosis.[20,22] Microvessel density influences the disease-free and overall survival of patients,[21] however, methodologic problems such as inter- and intraobserver variability, heterogenicity of tumors, and selection of the area of most intense neovascularization ("hot spot"), remain unsolved. Quantitation of angiogenic molecules from urine, serum, or tumor tissues provide alternative methods. In our survey, we tried to evaluate if vascular endothelial growth factor, an angiogenic factor, could be a prognostic factor for cervical cancer patients. Those with overexpressed vascular endothelial growth factor had poorer disease-free and overall survival in this study. Chiarotto et al reported that vascular endothelial growth factor could be up-regulated in the presence of hypoxia,[23] which might be why cervical cancer patients even receiving adjuvant radio- or chemotherapy still have higher recurrent incidences. We suggest that cervical cancer patients with over-expressed cytosol vascular endothelial growth factor, instead of other risk factors, even those receiving postoperative adjuvant radiation or chemotherapy, should be closely monitored to detect the early disease recurrence.

Chemotherapeutic cytotoxic agents are acting directly against tumor cells, while antiangiogenic molecules are targeting on the stromal component of the tumor. That might be why some clinical trials report high response rates to antiangiogenic therapy in many solid tumors, including those that commonly do not respond or respond poorly to conventional chemotherapy.[24] The value of adjuvant chemotherapy for preventing relapse of cervical cancer has not been proven yet, and our data showed that growth, spread, and outcome of cervical carcinoma are angiogenesis related, so we also stress the need for clinical trials to study the therapeutic effect of antiangiogenic substances on carcinoma of the uterine cervix.

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Table 1. Demographic Characteristics and Cytosol Vascular Endothelial Growth Factor According to Recurrence

	Nonrecurrence	Recurrence	*P*
Patient number	113	22	
Age	50.9±10.5	48.8±8.7	.08*
Gravidity	5.4±6.2	4.2±2.1	.55*
Parity	3.5±1.8	3.0±1.6	.31*
Menopasue			
Yes	55	9	.51†
No	58	13	(X^2=.67)
Cytosol vascular endothelial growth factor (pg/mg)	112	1020	<.001‡

* Student *t* test

† χ^2 test

‡ Mann-Whitney U test

Table 2. Median Vascular Endothelial Growth Factor Concentrations in Clinicopathologic Characteristics

Variables	Patient number	Vascular endothelial growth factor concentration (pg/mg) (25%, 75%)	*P*
Stage			
I	122	146.0 (0, 785.25)	.59
II	13	219.0 (64, 849)	
Menopause			
Yes	64	166 (57.5, 852.5)	.42
No	71	116 (0, 814)	
Tumor size			
≥ 4 cm	36	775.0 (144.25, 1046.5)	.013
< 4 cm	99	111.0 (0, 423)	
Depth of stromal invasion			
≥1/2	75	423.0 (50, 980)	<.001
<1/2	60	86.5 (0, 226.7)	
Lymphovascular emoli			
Yes	43	770.0 (38, 1219)	.003
No	92	111.5 (0, 419.7)	
Parametrial invasion			
Yes	39	423.0 (88, 1050)	.01
No	96	114.5 (0, 735)	
Pelvic lymph node metastasis			
Yes	28	807.5 (140, 1156.25)	.003
No	107	116.0(0, 460)	

Table 3. Vascular Endothelial Growth Factor Concentration for Predicting Lymph Node Metastases

Lymph node metastases	< 400 pg/mg protein	> 400 pg/mg protein	Patient Number
Yes	7	21	28
No	77	30	107
Patient number	84	51	135

Table 4. Multivarible Proportional Hazards Model on the Disease-free Survival

	Recurrent patients	Non-recurrent patients	Hazard ratio (95% CI)	*P*
Patient number	22	113		
Tumor size ≥ 4 cm				
Yes	6	30	0.59	.34
No	16	83	(0.21, 1.71)	
Deep stromal invasion				
(≥1/2 cervical thickness)				
Yes	15	60	0.91	.88
No	7	53	(0.28, 2.98)	
Lymphovascular emboli				
Yes	12	31	1.45	.52
No	10	82	(0.47, 4.46)	
Number of metastatic lymph nodes (≥3)				
Yes	4	6	0.77	.72
No	18	107	(0.17, 3.37)	
Parametrial invasion				
Yes	7	32	0.67	.49
No	15	81	(0.22, 2.09)	
Over-expressed cytosol VEGF (> 800 pg/mg				
protein)	14	19	6.44	<.00
Yes	8	94	(2.37, 13.54)	1
No				

CI= confidence interval

VEGF= vascular endothelial growth factor

Table 5. Multivarible Proportional Hazards Model on the Overall Survival

	Patients Dead	Patients Alive	Hazard ratio (95% CI)	*P*
Patient number	13	122		
Tumor size ≥ 4 cm				
Yes	5	31	1.12	.86
No	8	91	(0.32, 3.97)	
Deep stromal invasion (≥1/2 cervical thickness)				
Yes	10	65	1.00	1.00
No	3	57	(0.21, 4.83)	
Lymphovascular emboli				
Yes	9	34	4.11	.045
No	4	88	(1.05, 16.34)	
Number of metastatic lymph node (≥3)				
Yes	3	7	0.58	.51
No	10	115	(0.11, 2.93)	
Parametrial invasion				
Yes	6	33	1.08	.90
No	7	89	(0.30, 3.97)	
Over-expressed cytosol VEGF (> 800 pg/mg protein)				
Yes	8	25	4.50	.021
No	5	97	(1.25, 16.22)	

CI= confidence interval

VEGF= vascular endothelial growth factor