부인종양 제17권 제2호 2006
Vol. 17, No. 2, June, 2006

Overexpression of hTERT and c-erbB-2 are correlated in ovarian epithelial cancer

Sokbom Kang[1], Noh-Hyun Park[2], Yoo-Kyung Lee[2], Jae Weon Kim[2],
Yong-Sang Song[2], Soon-Beom Kang[2], Hyo-Pyo Lee[2]
Research Institute and Hospital[1], National Cancer Center, Goyang,
Department of Obstetrics and Gynecology[2], College of Medicine, Seoul National University, Seoul, Korea

Objective : It is still unclear how the abnormal hTERT expression is involved in the process of ovarian carcinogenesis. A recent report demonstrated that the introduction of c-erbB-2 could efficiently induce tumorigenicity of cells with the transfection of SV40 large T antigen and hTERT. It is designed to find correlation between overexpression of hTERT and c-erbB-2 in ovarian carcinogenesis.

Methods : Using immunohistochemistry, we tested whether overexpression of hTERT and c-erbB-2 were associated in ovarian cancer. Immunohistochemical staining of hTERT and c-erbB-2 was done in 63 cases of ovarian cancer. Overexpression of hTERT and c-erbB-2 were correlated to clinicopathological variables.

Results : Overexpression of hTERT was found in 7 (11.1%) of cases, whereas overexpression of c-erbB2 was founded in 3 (4.8%) of cases. It was found that overexpression of hTERT and c-erbB-2 were significantly correlated (p=0.03). Neither overexpression of hTERT nor that of c-erbB-2 was associated with any of clinicopathological variables, such as stage, grade, and histology.

Conclusion : Although the significant correlation between hTERT and c-erbB-2 was found, the low frequency of overexpression of hTERT and c-erbB-2 suggests that cooperation of hTERT and c-erbB-2 may be minor mechanism of ovarian carcinogenesis.

Key Words **:** Ovarian cancer, Immunohistochemistry, hTERT peptide, c-erbB-2 protein

INTRODUCTION

Ovarian cancer is the leading cause of death in women with gynecological malignant tumors.[1] Despite the recent developments in aggressive surgery and chemotherapy, the prognosis remains poor because ovarian cancers are often not diagnosed until advanced stages and the cancer cells frequently acquire drug resistance during repeated chemotherapies. Thus, there is an urgent need to develop new therapies. Epithelial ovarian cancer is believed to develop from the ovarian surface epithelium,[2] through the accumulation of aberrations of oncogenes and/or tumour suppressor genes. Many gene abnormalities have been detected in ovarian cancers, for example, the overexpression of c-erbB-2, mutation of K-ras, p53, BRCA1 or BRCA2, and the expression of telomerase activity.[3] Nonetheless, it is still unclear how the gene abnormalities are involved in the process of ovarian carcinogenesis.

A recent report demonstrated that the introduction of c-erbB-2 in the cells could efficiently immortalize by the transfection of SV40 large T antigen (LT) and hTERT and showed tumorigenicity. However, this idea of cooperation of c-erbB-2 and hTERT genes in ovarian carcinogenesis has not been supported by in-vivo evidence. Therefore, in the present study, we tried to investigate in-vivo evidence of the idea that hTERT and c-erbB-2 might play an important role in ovarian carcinogenesis.

논문접수일 : 2006년 4월 17일 채택일 : 2006년 5월 18일
교신저자 : 박노현, 110-744 서울시 종로구 연건동 28
서울대학교 의과대학 산부인과학교실
전화 : 02) 2072-3166 · 전송 : 02) 762-3599
Email : ksb1231@snu.ac.kr
This work was supported by a grant from the Seoul National University Hospital Research Fund (#04-2002-012).

MATERIAL AND METHODS

Sixty-three patients who had received initial surgical treatment for epithelial ovarian cancer at the Seoul National University Hospital, Seoul, Korea, from 1996 to 2001 were included in this study. Inclusion criteria were histopathological diagnosis of primary epithelial ovarian cancer, availability of clinical follow-up data, and availability of paraffin-embedded tissue specimens. Clinical data were obtained by retrospective review of the medical records. The median age of the patients was 52 years (range, 18-75 years). Detailed characteristics of cases were summarized in Table 1.

Immunodetection of c-erbB-2 and hTERT was performed on archival tissues from 63 cases of epithelial ovarian cancer. Sections of formalin-fixed and paraffin-embedded tissue were deparaffinized in xylene, then rehydrated using decreasing concentrations of alcohol ending in phosphate buffered saline (PBS). Endogenous peroxidase activity was quenched in 3% hydrogen peroxidase for 15 minutes. Sections were then subjected to steam heat antigen retrieval in citrate buffer, pH 6.0, for 30 minutes, washed in PBS solution, and incubated with Dako protein block (Dako, Carpinteria, CA) for 15 minutes. Sections were incubated for 60 minutes at room temperature with primary antibody in 0.1% bovine serum albumin (BSA) in PBS. Dilutions for the primary antibodies were as follows: c-erbB-2 (A0485; Dako, Carpinteria, CA) 1 : 100 and hTERT (H-231; Santa Cruz Biotechnology, Santa Cruz, CA) 1 : 40. The bound antibody was detected using the Dako Envision Plus rabbit peroxidase kit with diaminobenzidine as the chromogen. Finally, sections were counterstained in hematoxylin, dehydrated, and coverslipped. A section of each patient's tumor, not stained with primary antibody, was used as a negative control. The reactions were considered to be positive or negative according to whether or not TERT protein was detected in the nuclei or cytoplasm of the cancer cells. Complete membrane staining, Hercep Test (HercepTest, DAKO, Glostrup, Denmark) score 3+, was regared as overexpression of c-erbB-2.

Associations between clinicopathological parameters and hTERT and c-erbB-2 overexpression were analyzed by the Fischer's exact test and the x^2 test. All statistical analyses were performed using SPSS for Windows Version 10 (SPSS Inc., Chicago, IL).

Table 1. Clinicopathological characteristics of 63 ovarian cancer subjects

	No. (%)
Age	
Median (min-max)	52 (18-75)
Menopause	
Pre-menopause	36 (57.1)
Post-menopause	27 (42.9)
Stage	
Early (I-II)	27 (42.9)
Advanced (III-IV)	36 (57.1)
Histology	
Serous	35 (55.6)
Non-serous	28 (44.4)
Grade	
Grade I, II	46 (73.0)
Grade III	17 (27.0)
Ascites	
No	31 (49.2)
Yes	32 (50.8)
Residual disease	
Less than 2 cm	61 (96.8)
2 cm or over	2 (3.2)
hTERT	
Negative	56 (88.9)
Positive	7 (11.1)
c-erbB-2	
Negative	60 (95.2)
Positive	3 (4.8)

RESULTS

Clinicopathologic characteristics of 63 ovarian cancer patients were summarized in Table 1. Diffuse hTERT immunostaining was detected in the cytoplasm of ovarian cancer cells (Fig. 1A). Of the 63 ovarian cancers, 7 cases showed overexpression of hTERT (11.1%). Immunostaining of c-erbB-2 was detected in the membraneous part of ovarian cancer cells (Fig. 1B). Of the 63 ovarian cancers, 3 cases showed overexpression of c-erbB-2 (4.8%). Over-

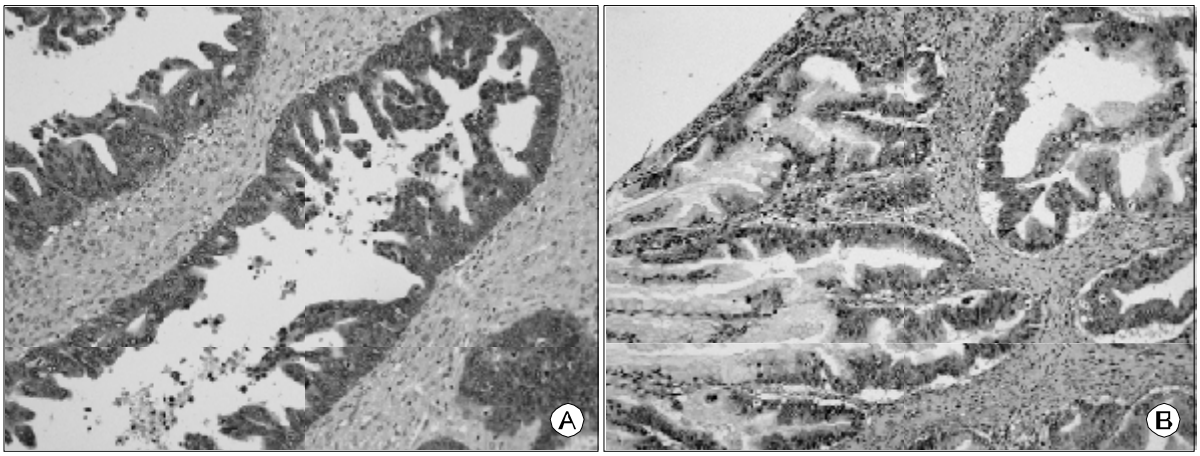

Fig. 1. Immunohistochemistry of epithelial ovarian cancers with hTERT and c-erbB-2 antibody. (A) Predominantly cytoplasmic c-erbB-2 signals in epithelial ovarian cancer. (B) Predominantly cytoplasmic hTERT signals in epithelial ovarian cancer. Original magnifications: ×200.

Table 2. Correlation between hTERT and c-erbB-2 over-expression*

	c-erbB-2 negative n (%)	c-erbB-2 positive n (%)
hTERT negative	55 (91.7)	1 (33.3)
hTERT positive	5 (8.3)	2 (66.7)

*p=0.03, by 2-tailed Fisher's exact test

expression of hTERT was correlated positively with expression of c-erbB-2 in ovarian cancer (Table 2, p=0.03).

Then, overexpression of hTERT and c-erbB-2 were correlated with various clinicopathologic variables (Table 3). Overexpression of hTERT was inversely correlated with advancement of stage (p=0.037). Overexpression of c-erbB2 was also inversely correlated with stage, but without significance (p=0.074). We also found that c-erbB-2 overexpression was more frequently, without statistical significance, found in ovarian cancer with non-serous histology.

DISCUSSION

In the present study, we found that hTERT expression was correlated with expression of c-erbB-2. Our results support a recent report suggesting that the introduction of c-erbB-2 in the cells could efficiently immortalize by the transfection of hTERT and showed tumorigenicity.[4]

In ovarian cancer, it was known that hTERT mRNA expression was correlated with telomerase activity.[5,6] It was also suggest that ovarian cancer cells express higher levels of hTERT mRNA than normal ovarian tissues.[5,7] Furthermore, some authors reported that hTERT expression, measured by real-time RT-PCR, is a possible independent marker of response to platinum-based therapy in advanced stage ovarian cancer patients.[8] Therefore, telomerase activity, represented by hTERT mRNA, may plays an important role in progression of ovarian cancer. However, this possibility is challenged by another recent report, suggesting that hTERT expression was not correlated with prognosis.[7] In our data, hTERT immunoactivity was not associated with clinicopathological variables. The clinical role of hTERT expression is yet to be determined.

Kusaraki et al. introduced c-erbB-2 into ovarian surface epithelial cells already immortalized by the transfection of LT and hTERT.[4] They found that the additional introduction of c-erbB-2 into cells further enhanced the growth activity. Also, they found that the immortalized ovarian surface epithelial cells expressing LT or both LT and hTERT did not form tumors in nude mice, while those cells additionally

Table 3. Comparison of clinical variables according to hTERT and c-erbB-2 overexpression

	hTERT overexpression			c-erbB-2 overexpression		
	Negative (n=56)	Positive (n=7)	p*	Negative (n=60)	Positive (n=3)	p
Age						
Less than 55	33 (58.9)	5 (71.4)	0.693	36 (60.0)	2 (66.7)	1.000
55 or over	23 (41.1)	2 (28.6)		24 (40.0)	1 (33.3)	
Menopause						
Pre-menopause	32 (57.1)	4 (57.1)	1.000	35 (58.3)	1 (33.3)	0.572
Post-menopause	24 (42.9)	3 (42.9)		25 (41.7)	2 (66.7)	
Stage						
Early (I-II)	21 (37.5)	6 (85.7)	0.036	24 (40.0)	3 (100.0)	0.074
Advanced (III-IV)	35 (62.5)	1 (14.3)		36 (60.0)	0	
Histology						
Serous	33 (58.9)	2 (28.6)	0.226	35 (58.3)	0	0.082
Non-serous	23 (41.1)	5 (71.4)		25 (41.7)	3 (100.0)	
Grade						
Grade I, II	41 (73.2)	5 (71.4)	0.615	43 (71.7)	3 (100.0)	0.557
Grade III	15 (26.8)	2 (26.8)		17 (28.3)	0	
Ascites						
No	26 (46.4)	5 (71.4)	0.257	29 (48.3)	2 (66.7)	0.613
Yes	30 (53.6)	2 (28.6)		31 (51.7)	1 (33.3)	
Residual disease						
Less than 2 cm	54 (96.4)	7 (100.0)	1.000	58 (96.7)	3 (100.0)	1.000
2 cm or over	2 (3.6)	0		2 (3.3)	0	

*All p value was calculated by Fisher's exact test

transfected with c-erbB-2 formed tumours. In the present study, we observed the positive correlation between c-erbB2 and hTERT expression. Therefore, it can be suggested that increased telomerase activity and c-erbB-2 may plays a role co-operatively in ovarian carcinogenesis. However, although the significant correlation between hTERT and c-erbB2 was found, the low frequency of overexpression of hTERT and c-erbB-2 suggests that cooperation of hTERT and c-erbB-2 may be minor mechanism of ovarian carcinogenesis.

The present study has some limitations. Expression of hTERT and c-erbB-2 was assessed by immunohistochemical methods and the frequency of expression of hTERT and c-erbB-2 was lower than the previous reports. Over-expression of c-erbB-2 was reported in 25-30% of the cases by immunohistochemical staining, ranged variously from 9 to 65% in different series.[9,10] Among them, the largest GOG study (n=837) showed only 4% of 3+ c-erbB-2 over-expression.[11] It was similar with our result, 4.8% of c-erbB-2 overexpression. Moreover most of studies regarded Hercep Test score 2+ and 3+ as c-erbB-2 overexpression. However our result considered only 3+ as c-erbB-2 overexpression. In addition, this positive correlation between c-erbB-2 and hTERT requires adequate validation in in-vitro model. Also, the relatively small size of sample may weaken the reliability of our data.

In conclusion, expression of hTERT was positively correlated with c-erbB2 expression in 63 ovarian cancer samples. Increased telomerase activity and c-erbB2 expression may be the one of the possible pathway of ovarian carcinogenesis. However, cooperation of hTERT and c-erbB-2 might be only the minor mechanism of ovarian carcinogenesis.

REFERENCES

1. Herbst AL. The epidemiology of ovarian carcinoma and the current status of tumor markers to detect disease. Am J Obstet Gynecol 1994; 170: 1099-105; discussion 1105-7.
2. Scully RE. Ovarian tumors. A review. Am J Pathol 1977; 87: 686-720.
3. Matias-Guiu X, Prat J. Molecular pathology of ovarian carcinomas. Virchows Arch 1998; 433: 103-11.
4. Kusakari T, Kariya M, Mandai M, Tsuruta Y, Hamid AA, Fukuhara K, et al. C-erbB-2 or mutant Ha-ras induced malignant transformation of immortalized human ovarian surface epithelial cells in vitro. Br J Cancer 2003; 89: 2293-8.
5. Kyo S, Kanaya T, Takakura M, Tanaka M, Yamashita A, Inoue H, et al. Expression of human telomerase subunits in ovarian malignant, borderline and benign tumors. Int J Cancer 1999; 80: 804-9.
6. Landen CN, Klingelhutz A, Coffin JE, Sorosky JI, Sood AK. Genomic instability is associated with lack of telomerase activation in ovarian cancer. Cancer Biol Ther 2004; 3: 1250-3.
7. Widschwendter A, Muller HM, Hubalek MM, Wiedemair A, Fiegl H, Goebel G, et al. Methylation status and expression of human telomerase reverse transcriptase in ovarian and cervical cancer. Gynecol Oncol 2004; 93: 407-16.
8. Buttitta F, Pellegrini C, Marchetti A, Gadducci A, Cosio S, Felicioni L, et al. Human telomerase reverse transcriptase mRNA expression assessed by real-time reverse transcription polymerase chain reaction predicts chemosensitivity in patients with ovarian carcinoma. J Clin Oncol 2003; 21: 1320-5.
9. Hellstrom I, Goodman G, Pullman J, Yang Y, Hellstrom KE. Overexpression of HER-2 in ovarian carcinomas. Cancer Res 2001; 61: 2420-3.
10. Mano MS, Awada A, Di Leo A, Durbecq V, Paesmans M, Cardoso F, et al. Rates of topoisomerase II-alpha and HER-2 gene amplification and expression in epithelial ovarian carcinoma. Gynecol Oncol 2004; 92: 887-95.
11. Bookman MA, Darcy KM, Clarke-Pearson D, Boothby RA, Horowitz IR. Evaluation of monoclonal humanized anti-HER2 antibody, trastuzumab, in patients with recurrent or refractory ovarian or primary peritoneal carcinoma with overexpression of HER2: A phase II trial of the Gynecologic Oncology Group. J Clin Oncol 2003; 21: 283-90.

hTERT 및 c-erbB-2 과발현과 상피성난소암의 관계

국립암센터 자궁암센터[1], 서울대학교 의과대학 산부인과학교실[2]

강석범[1] · 박노현[2] · 이유경[2] · 김재원[2] · 송용상[2] · 강순범[2] · 이효표[2]

목적 : 난소암 발생과정에서 hTERT 비정상적 발현은 아직 정확히 밝혀져 있지 않다. 최근 보고에 따르면 c-erbB-2가 SV40 large T 항원 및 hTERT에 의한 형질 전환을 통해 세포의 종양발생을 유도하는 데 기여한다고 알려지고 있다. 이에 면역조직화학 염색을 통해 c-erbB-2 및 hTERT의 과발현이 난소암과 상관성이 존재하는지 보고자 하였다.

연구 방법 : 본 연구에서는 난소암 63건에서 hTERT와 c-erbB-2에 대해 면역조직화학 염색을 시행하여 hTERT와 c-erbB-2의 과발현이 임상병리적 요인들과 상관관계가 있는지 살펴보았다.

결과 : hTERT의 과발현은 7건(11.1%)에서 관찰되어진 반면, c-erbB-2의 과발현은 3건(4.8%)에서만 나타났다. hTERT와 c-erbB-2의 과발현은 의미있는 상관관계를 보였다(p=0.03). 그러나 hTERT, c-erbB-2 모두 병기, 조직형 및 등급(grade)과 같은 임상병리적 요인들과는 의미있는 상관관계를 보이지 않았다.

결론 : 연구결과를 통해 hTERT와 c-erbB-2는 상관관계가 있음을 알 수 있다. 그러나 과발현의 빈도 자체가 낮으므로 이들간의 상관관계에도 불구하고 hTERT와 c-erbB-2의 상호작용은 난소암 발암과정에서 부수적인 역할을 할 것임을 시사한다.

중심단어 : 난소암, 면역조직화학 염색, hTERT peptide, c-erbB-2 protein