— Original Article —

Early Detection of Recurrence of Ovarian Cancer using Telomerase Assay in Peritoneal Washing Fluids

Department of Perinatology and Gynecology, Kagawa University School of Medicine
Masayuki Ohno, Kenji Kanenishi, Tatsuya Igarashi, Atsuko Shiota, Toshiyuki Hata

Abstract

OBJECTIVE: The aim of this study was to evaluate whether telomerase activity in peritoneal washing fluids can be used as novel means for early detection of the recurrence of ovarian cancer.

METHODS: Twenty patients with ovarian cancer (stage Ⅰc in 2, Ⅱa in 2, Ⅱc in 2, Ⅲa in 2, Ⅲb in 1, Ⅲc in 4, and Ⅳ in 7) were recruited for the study. These patients received maximal debulking as a primary treatment, and were followed up using telomerase activity measurement (TRAP assay) and cytological examination in peritoneal washing fluids.

RESULTS: In 8 patients with telomerase negative and negative peritoneal washing cytology, no recurrence was ascertained. Of the 6 peritoneal washing cytology negative cases, 3 were telomerase positive. When these 3 were reevaluated for peritoneal cytology, malignant ascites were identified in all 3 patients later. Of the 12 patients with telomerase positive, 9 died, whereas none died in 8 patients with telomerase negative ($p<0.005$). Patients with telomerase positive had significantly poorer outcomes than those with telomerase negative ($p<0.005$).

CONCLUSIONS: Our preliminary results reveal that the telomerase test in peritoneal washing fluids can be used as an adjuvant to cytopathological methods in early detection of the recurrence of ovarian cancer. Our results suggest that positive telomerase activity in peritoneal washing fluids may indicate the poor prognostic factor in patients with ovarian cancer.

KEY WORDS: *Ovarian cancer, recurrence, telomerase, peritoneal washing fluids, cytology*

Introduction

Telomerase is a ribonucleoprotein that synthesizes telomeric DNA onto chromosomal ends using an RNA component as a template. [1,2] Extension of telomeric repeats by telomerase prevents telomere shortening with cell divisions and contributes to chromosomal stability, possibly leading to immortalization of the cells. [3,4] Telomerase activity has been found in a variety of malignant tumors but only rarely in benign tumors or normal tissues. Specifically, telomerase activity is present in 95% of gynecologic malignancies and in 88% of epithelial ovarian carcinomas, but it is undetectable in most benign tissue. [5] Therefore, telomerase activation might be common in gynecologic malignant tumors, and be a valuable diagnostic parameter that could help to identify potentially progressive lesions. [6,7]

Malignant ascitic effusion is a common presentation of ovarian cancer, and reflects peritoneal dissemination. Transcelomic seeding of malignant cells antedates the development of ascites, so its detection has prognostic significance. [8] The diagnosis of malignant ascitic fluid and the differentiation among malignant, paramalignant and nonmalignant effusions by conventional diagnostic methods are sometimes difficult, and usually only

Table 1 Clinical characteristics and telomerase activity in each ovarian cancer

Case	Age	Stage	Histology	Telomerase activity	Follow up (months)	Outcome
1	36	2c	serous	(−)	112	NED
2	43	1c	endometrioid	(−)	100	NED
3	46	1c	endometrioid	(−)	99	NED
4	53	3b	serous	(+)	62	AWD
5	46	4	serous	(+)	35	DOD
6	50	4	clear cell	(+)	20	DOD
7	48	3a	mucinous	(−)	48	NED
8	52	2c	serous	(+)	34	DOD
9	63	4	serous	(+)	28	DOD
10	77	3c	serous	(+)	24	DOD
11	51	3a	clear cell	(+)	23	DOD
12	60	4	serous	(+)	22	DOD
13	42	2a	endometrioid	(−)	37	NED
14	56	3c	mucinous	(+)	4	DOD
15	58	2a	serous	(−)	26	NED
16	67	3c	serous	(−)	24	NED
17	72	4	serous	(+)	6	DOD
18	73	4	serous	(+)	20	AWD
19	74	4	serous	(+)	20	AWD
20	61	3c	undifferentiated	(−)	17	NED

NED = no evidence of disease; AWD = alive with disease; DOD = death of disease.

48-60% of malignant peritoneal fluid in patients with ovarian cancer could be diagnosed by cytological examination of peritoneal fluid. [9,10] Tseng et al. [11] revealed a high sensitivity and specificity of both telomerase testing and conventional cytology in peritoneal fluids. These authors also suggested that the telomerase test in peritoneal fluids can be used as an adjuvant to cytopathological methods in the diagnosis of malignant peritoneal ascites, particularly in cases of negative cytology. These findings encouraged us to evaluate the potential usefulness of telomerase test in washings or ascitic fluids from the peritoneal cavity as a means of detecting residual or recurrent cancer cells in patients treated for ovarian cancer. The aim of this study was to determine whether telomerase test is a useful indicator of recurrence risk and ultimate outcome.

Patients and Methods

A total of 20 patients (aged 36-77 years, mean 56.7 years) with histologically confirmed primary epithelial ovarian cancer were studied between September 1992 and August 2000 at Kagawa Medical University Hospital, Miki, Japan (Table 1). These patients received maximal debulking as a primary treatment, and chemotherapy was not given before surgery. The patients were staged according to criteria recommended by the International Federation of Obstetricians and Gynecologists (FIGO). [12] There were 2 stage I patients, 4 stage II patients, 7 stage III patients, and 7 stage IV patients. The staging system defined by FIGO assumes that an adequate staging operation has been performed. [13] Tumors were classified histologically according to World Health Organization (WHO) criteria[12] as serous (n=12), mucinous (n=2), endometrioid (n=3), clear cell

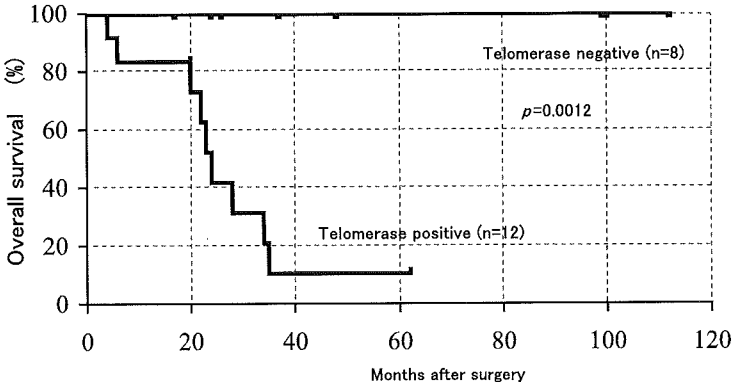

Figure 1 Kaplan-Meier survival curves for patients with ovarian cancer cording to telomerase activity.

Table 2 Patient outcome according to telomerase activity

Group	n	Telomerase activity			Outcome		
		At surgery	After treatment	Final test during follow up	NED	AWD	DOD
I	6	(-)	(-)	(-)	6	0	0
II	3	(-)	(-)	(+)	0	1	2
III	2	(+)	(+ or -)	(-)	2	0	0
IV	9	(+)	(+ or -)	(+)	0	2	7

NED = no evidence of disease; AWD = alive with disease; DOD = death of disease.

(n=2), and undifferentiated (n=1). All 20 patients received cisplatin- and taxan-based regimens after first surgery. The study was approved by the local ethical committee of Kagawa Medical University, and standardized informed consent was obtained from each patient.

Ascitic fluid was withdrawn from all 20 patients at the time of laparotomy just after opening the peritoneum. About 500 cc of normal sterile saline was used to wash the whole pelvis if no ascites was noted within the peritoneal cavity. Along with routine investigations[11], the ascitic fluid was submitted for cytological examination and telomerase assay. Data acquired from cytology and telomerase assay were collected in a double-blind fashion until analysis. Reservoir was placed just before closing the peritoneum, and used for the route for intraperitoneal chemotherapy and retrieval of ascitic fluids during follow-up period. Telomerase activity was determined using the TRAPeze telomerase detection kit (Oncor, Inc., Gaithersburg, MD), as described previously.[14] The difference between telomerase status in peritoneal ascites was determined using chi-square test. Survival curves were plotted using the method of Kaplan-Meier, and the log-rank test was used to determine the difference between life tables. A value of $p<0.05$ was considered statistically significant.

Results

Patient outcome according to telomerase activity was shown in Table 2. In 8 patients with telomerase negative and negative peritoneal washing cytology at fi-

nal test during follow up, no recurrence was ascertained. Of the 6 peritoneal washing cytology negative cases at final test during follow up, 3 were telomerase positive. When these 3 were reevaluated for peritoneal cytology, malignant ascites were identified in all 3 patients later. Of the 12 patients with telomerase positive at final test during follow up, 9 died, whereas none died in 8 patients with telomerase negative ($p<0.005$). Out of 11 alive patients, 8 showed no evidence of disease, and 3 were alive with disease (Table 1). Patients with telomerase positive had significantly poorer outcomes than those with telomerase negative ($p<0.005$) (Figure 1).

Discussion

Duggan et al. [15] reported that TRAP assay is more sensitive than cytologic examination in detecting cancer cells in the peritoneal cavity of patients with ovarian carcinoma. These authors suggested that the presence of telomerase activity in abdominal fluids and washings from patients treated for ovarian carcinoma may be a strong indicator of residual disease and improve the ability to detect early disease recurrences. In this study, 3 cases were histologically negative but telomerase positive during follow-up period (Cases 4, 5 and 8 in Table 1). When histology was repeated in these cases, all 3 showed positive histology later (3 months later in Case 4, 3 months later in Case 5, and 15 months later in Case 8). Several factors support the merit of this approach for the detection of ovarian carcinoma. The high sensitivity of the TRAP assay ensures the detection of trace amounts of tumor cells in the presence of large excesses of normal cells. [15] For example, TRAP assay is extremely sensitive, enough to detect telomerase activity in extracts equivalent to 100 immortal cancer cells, whereas the conventional peritoneal assessment is difficult if there are less than 1000 cells. [11] In this study, reservoir was placed just before closing the peritoneum, and used for the route for intraperitoneal chemotherapy and retrieval of ascitic fluids during follow-up period. Peritoneal washings could easily obtained from patients with ovarian carcinoma by use of lavage through long-term abdominal catheters. Therefore, the telomerase test in peritoneal washing fluids could be used as an adjuvant to cytopathological methods for early detection of residual disease of ovarian carcinoma in patient follow-up protocols.

Telomerase activity has been found in a variety of malignant tumors but only rarely in benign tumors or normal tissues. Telomerase activation might therefore be a valuable diagnostic parameter that could help to identify potentially progressive lesions. [7] Telomerase activity is associated with development and extension of epithelial ovarian cancer. [16] The decrease of telomerase activity levels parallel cell growth impairment, and the observed telomerase activity remaining after treatment with antineoplastic agents is most likely to reflect activity from the remaining viable cells. [17,18] Therefore, the disappearance of telomerase activity might be a reliable marker of tumor cell killing. Moreover, the diagnostic and therapeutic implications of telomerase activation need to be clarified in clinical trials. In this study, of the 12 patients with telomerase positive at final test during follow up, 9 died, whereas none died in 8 patients with telomerase negative. Patients with telomerase positive had significantly poorer outcomes than those with telomerase negative. These results suggest that the telomerase test might be a novel clinical prognostic indicator of human ovarian carcinoma. However, the data and its interpretation should be taken with some degree of caution because of the small number of subjects studied. Further study is needed to clarify the clinical usefulness of telomerase assay as a prognostic indicator in ovarian carcinoma.

References

1) Greider CW, Blackburn EHA. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature 1989;337:331-337.

2) Yu GL, Bradley JD, Attardi LD, Blackburn EH. In vitro alteration of telomerase sequences and senescence caused by mutated Tetrahymena telomerase RNAs. Nature 1990 ; 344 : 126-132.

3) Counter CM, Avilion AA, LeFeuver CE, Stewart NG, Greider CW, Harley CB, Bacchetti S. Telomere shortening associated with chromosome instability rested in immortal cells which express telomerase activity. EMBO J 1992 ; 11 : 1921-1929.

4) Counter CM, Hirte HW, Bacchetti S, Harley CB. Telomerase activity in human ovarian carcinoma. Proc Natl Acad Sci USA 1994 ; 91 : 2900-2904.

5) Zheng PS, Iwasaka T, Yamasaki F, Ouchida M, Yokoyama M, Nakao Y, Fukuda K, Matsuyama T, Sugimori H. Telomerase activity in gynecologic tumors. Gynecol Oncol 1997 ; 64 : 171-175.

6) Kyo S, Kanaya T, Ishikawa H, Ueno H, Inoue M. Telomerase activity in gynecological tumors. Clin Cancer Res 1996 ; 2 : 2023-2028.

7) Park TW, Riethdorf S, Riethdorf L, Loning T, Janicke F. Differential telomerase activity, expression of the telomerase catalytic sub-unit and telomerase-RNA in ovarian tumors. Int J Cancer 1999 ; 84 : 426-431.

8) Creastman WT, Rutlege F. The prognostic value of peritoneal cytology in gynecologic malignant disease. Am J Obstet Gynecol 1971 ; 110 : 773-778.

9) Yoshimura S, Scully RE, Taft PD, Herrington JB. Peritoneal fluid cytology in patients with ovarian cancer. Gynecol Oncol 1984 ; 17 : 161-167.

10) Ziselman EM, Harkavy SE, Hogan M, West W, Atkinson B. Peritoneal washing cytology : uses and diagnostic criteria in gynecologic neoplasms. Acta Cytol 1984 ; 28 : 105-110.

11) Tseng CJ, Jain S, Hou HC, Liu WW, Pao CC, Lin CT, Horng SG, Soong YK, Hsueh S. Applications of the telomerase assay in peritoneal washing fluids. Gynecol Oncol 2001 ; 81 : 420-423.

12) International Federation of Gynecology and Obstetrics : Changes in definition of clinical staging for carcinoma of the cervix and ovary. Am J Obstet Gynecol 1989 ; 156 : 263-264.

13) Cannistra SA. Cancer of the ovary. N Engl J Med 1993 ; 329 : 1550-1559.
Obstet Gynecol 1989 ; 156 : 263-264.

14) Dowdy SC, O'Kane DJ, Keeney GL, Boyd J, Podratz KL. Telomerase activity in sex cord-stromal tumors of the ovary. Gynecol Oncol 2001 ; 82 : 257-260.

15) Duggan BD, Wan M, Yu MC, Roman LD, Muderspach LI, Delgadillo E, Li WZ, Martin SE, Dubeau L. Detection of ovarian cancer cells : Comparison of a telomerase assay and cytologic examination. J Natl Cancer Inst 1998 ; 90 : 238-242.

16) Oishi T, Kigawa J, Minagawa Y, Shimada M, Takahashi M, Terakawa N. Alteration of telomerase activity associated with development and extension of epithelial ovarian cancer. Obstet Gynecol 1998 ; 91 : 568-571.

17) Faraoni I, Turriziani M, Bonmassar E, De Vecchis L, Graziani G. Development of a novel in vitro chemosensitivity assay : Telomerase as a possible marker of tumor cell survival. J Chemotherapy 1996; 8 : 394-398.

18) Faraoni I, Turriziani N, Masci G, De Vecchis L, Shay JW, Bonmassar E, Graziani G. Decline in telomerase activity as a measure of tumor cell killing by antineoplastic agents in vitro. Clin Cancer Res 1997; 3 : 579-585.