Human Telomerase Reverse Transcriptase (hTERT) Expression in Borderline Ovarian Tumors: An Immunohistochemical Study

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Objective: *To investigate the expression of human telomerase reverse transcriptase (hTERT) in epithelial borderline ovarian tumor (BOT) by immunohistochemistry with correlation to clinicopathologic variables.*
Material and Method: *Paraffin-embedded tissue sections of 62 borderline ovarian tumors (47 mucinous, 14 serous, and 1 clear cell) and 12 epthelial ovarian carcinomas were immunostained with antibodies to hTERT. The intensity and quantity of the immunostaining was determined and analyzed with clinicopathological characteristics.*
Results: *hTERT expression was detected in 48.4% of BOT and all cases of epithelial ovarian carcinoma. In immunoreactive BOT, 50% of cases were scored as high expression. Serous BOT had the highest rate of hTERT expression. There was no significant statistical difference of hTERT immunoreactivity between histologic types of BOT. No hTERT immunoreactivity was observed in the benign parts of the same slides of each immunoreactive case. hTERT immunoreactivity was positively correlated with FIGO stage (p = 0.04), but not with other variables. The mean follow-up time of BOT cases was 81.63 months and no recurrence or death was noted.*
Conclusion: *hTERT expression was found in half of BOT and all of epithelial ovarian carcinoma. High hTERT expression was associated with FIGO stage.*

Keywords: *Immunohistochemistry, Ovarian neoplasms, Telomerase, Telomerase reverse transcriptase, TERT protein*

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Epithelial ovarian carcinomas are the most common ovarian malignancies and account for 5% of all cancers among women[1]. Epithelial ovarian carcinomas most commonly occur in postmenopausal women and are associated with poor prognosis. They are highly aggressive tumors and about two-thirds of the patients are diagnosed in advanced stages at initial admission[2]. In contrast, borderline ovarian tumors (BOT) are usually diagnosed at an early stage and the median age of diagnosis is younger than that of invasive ovarian cancers[3-5]. In general, BOT have an excellent prognosis even in advanced stages with the survival rate of 77-100%[6,7]. In Western countries, the most common histologic type of BOT is serous subtype. Borderline mucinous tumors are less common than serous borderline tumors in most reports by 20% to over 100% but are equally prevalent in Japan[8,9].

The biological and molecular basis of the ovarian cancer is not completely understood. BOT is also considered as a precursor of ovarian carcinoma[10,11]. Many studies have been focused on telomerase. The ribonucleoprotein enzyme telomerase plays a crucial role in the maintenance of stable telomeres of chromosomes. Telomeres are the specialized DNA-protein structures, which are located at the distal ends of chromosomes and are comprised

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of up to hundreds of a 6-base-pair sequence (5'-TTAGGG-3')[12]. It is assumed that telomeres are important for DNA replication in protecting chromosome ends from DNA degradation, end-to end fusion, rearrangements, and chromosome loss[13]. In somatic cells, telomeres are progressive shorten with each cell cycle until a critical length is reached signaling cell-cycle exit. It is hypothesized that cell senescence can be overcome by reactivation of telomerase that restores telomeric sequences[14,15]. To date, telomerase activity has been found in immortalized cell lines, stem cells, germ cells, and the majority of malignant human tumors, but not in most human somatic cells[15-19]. Telomerase consists of three subunits: an RNA component that acts as a template for DNA replication; a telomerase associated protein of unknown function; and the human telomerase reverse transcriptase (hTERT), which is responsible for its catalytic activity[20].

The aim of the present study was to analyze the telomerase activity in epithelial borderline ovarian tumors by anti-hTERT antibody with regard to clinico-pathologic variables.

Material and Method

Case selection

Sixty-two cases of epithelial borderline ovarian tumor were retrieved from surgical pathology files of the Division of Gynecologic Pathology, the Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, between 1996 and 2002. Only patients who underwent primary surgery without previous chemotherapy which had available clinical follow-up date and paraffin-embedded tissue specimens were included in the present study. Twelve cases of epithelial ovarian carcinomas were also randomly selected to study. Hematoxylin-eosin-stained sections from each case were reviewed to confirm the histological diagnosis and assess pathological features and subtype. The most representative paraffin block for each case was selected for immunohistochemical analysis.

From a retrospective review of medical records, the patient's demographic and surgical data were collected. Clinical data were retrieved from the database files of the Division of Gynecologic Oncology and the institution computerized clinical information system. Surgical staging was determined with the criteria that are recommended by the International Federation of Gynecology and Obstertrics (FIGO).

Immunohistochemical study

The authors studied the catalytic unit of telomerase with mouse monoclonal anti-hTERT antibody (NCL-hTERT, clone 44F12, Novocastra Laboratories, Newcastle upon Tyne, UK; 1:50 dilution), with biotinylated anti-mouse secondary antibody (Dako, Cytomatia, CA; 1:100 dilution). Sections were deparaffinized and subjected to immunohistochemical staining, with standard streptavidin-biotin-peroxidase techniques, and diaminobenziding (DAB) as the chromogen. Sections of 4 to 5 micron thick underwent antigen retrieval by steam treatment in a citrate buffer. Endogenous peroxidase activity was removed by 3% hydrogen peroxide (10 minutes). Nonspecific binding sites were blocked with 3% normal horse serum in phosphate buffered saline solution (PBS) for 20 minutes at room temperature. After incubation with the primary antibodies, slides were incubated with streptavidin/peroxidase at 1:500 in PBS for 30 minutes at room temperature, then rinsed with PBS and incubated for 10 minutes in DAB solution and counterstained with Meyer's hematoxylin. Negative control was performed by substituting the primary antibody with nonimmune sera. Slides of tonsils were served as external positive controls. Appropriate positive and negative controls were run simultaneously. The immunohistochemically stained sections were evaluated without previous knowledge of the clinical outcome of each patient.

Evaluation of the hTERT expression

Only brown_staining in nucleus of tumor cells was considered as immunoreactive. The staining intensity and the percentage of tumor cells positively stained were analyzed. The intensity of staining was evaluated subjectively using a scale ranging from 0 (none), 1 (weak), 2 (medium), and 3 (strong). The proportion of positive cells was scored at low magnification (40x) by evaluating the entire tumor area. "Negative for hTERT expression" was defined as less than 10% of tumor cells staining, regardless of the intensity. "Low expression" was defined as intensity 1 and > 10% cell staining, or intensity 2 and 10-75% cell staining, or intensity 3 and 10-50% cells staining. "High expression" was defined as intensity 2 and > 75% of cells staining, or intensity 3 and > 50% of cells staining.

Statistical analyses

Statistical analyses were performed with the SPSS for Windows software (version 13; SPSS Inc,

Chicago, IL, USA). The correlation between hTERT expression and the other variables was assessed with the Chi-square and Fisher's exact tests. Statistical significance was defined as a probability value (p-value) < 0.05.

Results

Clinicopathologic variables

Of 62 cases of BOT included in the present study, 47 (75.8%) were mucinous BOT and 14 (22.6%) were serous BOT. In mucinous BOT group, 25 (53.2%)

Table 1. Clinicopathological characteristics of borderline ovarian tumors and epithelial ovarian carcinoma

Characteristics	Borderline tumor (n = 62)		Epithelial carcinoma (n = 12)	
Age (mean; range)		44.44 (17-85)		52.83 (26-77)
Parity				
Nulliparous		29 (46.8%)		4 (33.3%)
Multiparous		33 (53.2%)		8 (66.7%)
Menopause				
Premenopause		38 (61.3%)		4 (33.3%)
Postmenopause		24 (38.7%)		8 (66.7%)
Histologic type:	Serous	14 (22.6%)	Serous	4 (33.3%)
	Mucinous	47 (75.8%)	Mucinous	3 (25.0%)
	Clear cell	1 (1.6%)	Endometrioid	1 (8.4%)
			Clear cell	4 (33.3%)
FIGO stage				
I		62 (100%)		5 (41.6%)
II		0		1 (8.4%)
III, IV		0		6 (50.0%)

Table 2. Association between hTERT expression and clinicopathological characteristics in BOT

Characteristics	Total number of patients	hTERT expression			p-value
		Negative	Low	High	
Age					0.872
< 20	5	3 (60.0%)	1 (20.0%)	1 (20.0%)	
20-40	24	11(45.8%)	7 (29.2%)	6 (25.0%)	
40-60	23	12 (52.2%)	4 (17.4%)	7 (30.4%)	
> 60	10	6 (60.0%)	3 (30.0%)	1 (10.0%)	
Parity					0.448
Nulliparous	29	17 (58.6%)	7 (24.1%)	5 (17.3%)	
Multiparous	33	15 (45.5%)	8 (24.2%)	10 (30.3%)	
Menopause					0.717
Premenopause	38	21 (55.3%)	8 (21.1%)	9 (23.6%)	
Postmenopause	24	11 (45.8%)	7 (29.2%)	6 (25.0%)	
FIGO stage					0.044
1a	57	32 (56.1%)	13 (22.8%)	12 (21.1%)	
1b	5	0	2 (40.0%)	3 (60.0%)	
Histologic type					0.094
Serous	14	4 (28.6%)	4 (28.6%)	6 (42.8%)	
Mucinous	47	28 (59.6%)	10 (21.3%)	9 (19.1%)	
Endocervical-like	22	12 (54.5%)	4 (18.2%)	6 (27.3%)	
Intestinal-like	25	16 (64.0%)	6 (24.0%)	3 (12.0%)	
Clear cell	1	0	1 (100%)	0	

were intestinal-like type and 22 (46.8%) were endocervical-like type. All 62 cases of BOT were FIGO stage 1 (Table 1); 57 (91.9%) cases were stage 1a, while the remaining cases were stage 1b (Table 2). The mean age of the patients was 44.44 years (17-85 years). The mean follow-up time was 81.63 months with a range of 43 to 131 months. No recurrence or death was noted.

Cases with epithelial ovarian carcinoma comprised of four serous, three mucinous, four clear cell, and one endometrioid carcinoma. The mean age of the patients was 52.83 years (26-77) (Table 1). Four patients died due to recurrent or advanced disease.

The clinicopathological characteristics of both groups are demonstrated in Table 1. BOT tended to be found in the younger age group and premenopausal period. However, no significant statistical difference in general demographic features between borderline tumor and carcinoma was observed.

hTERT immunostaining

Positive expression of hTERT was seen in all 12 cases of carcinoma and 30 out of 64 BOT cases. All epithelial ovarian carcinoma revealed high expression of hTERT (Fig. 1).

Association between hTERT expression and clinicopathological characteristics in BOT is shown in Table 2. In positive immunoreactive BOT, 50% of cases demonstrated high expression (Fig. 2). According to histologic types, high hTERT expression was mostly detected in serous tumor although no statistical significance was demonstrated ($p = 0.094$). There was quite similar hTERT expression in both subtypes of mucinous tumor. Notably in the same slide of each immunoreactive case, all benign parts were negative for hTERT expression (Fig. 3). There was no significant correlation between hTERT expression and patient age, parity, menopausal status, and histologic type. A significant association was only noted between hTERT expression and the FIGO stage as positive expression was more detected in cases with 1b ($p = 0.04$).

Discussion

Epithelial ovarian neoplasms can be divided into three distinct clinicopathological groups: benign, malignant, and borderline (BOT) or low malignant potential tumors. They are very heterogenous in their histology and clinical behavior. The BOT show a significant less aggressive behavior than epithelial ovarian carcinoma. However, subset of these BOT cases can progress to malignancy. Although all steps

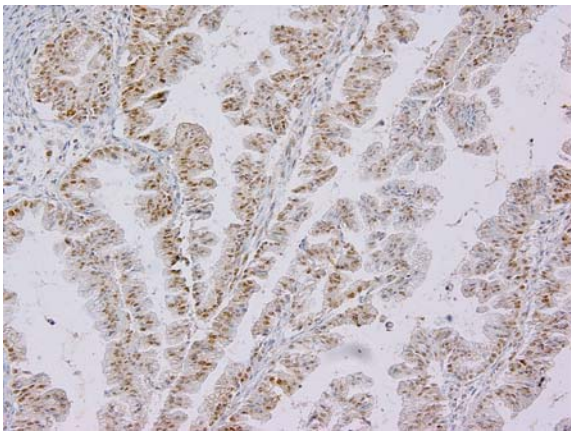

Fig. 1 High nuclear hTERT immunoreactivity in well-differentiated mucinous cystadenocarcinoma (x200)

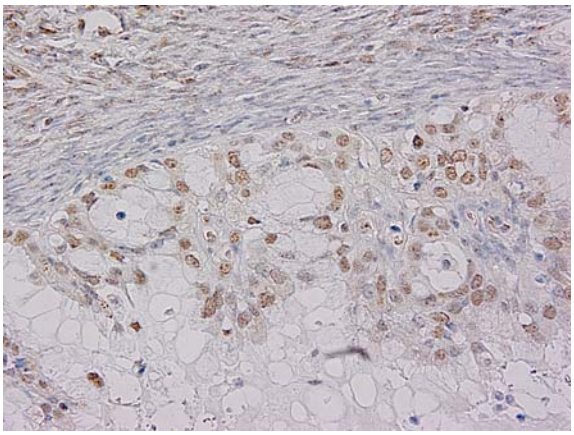

Fig. 2 Nuclear hTERT immunoreactivity in mucinous borderline ovarian tumor. Stromal fibroblasts also reveal scattered nuclear staining (x400)

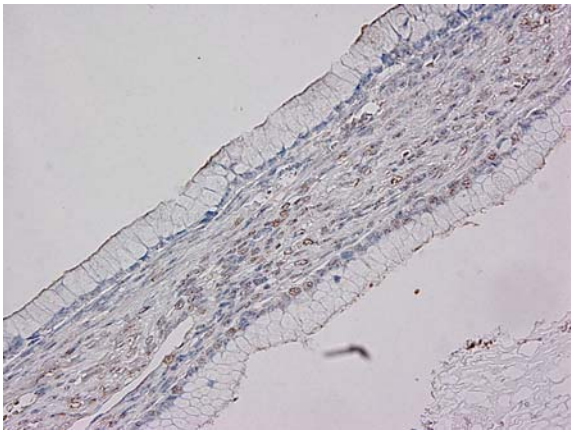

Fig. 3 The benign part in mucinous borderline ovarian show negative immunostaining for hTERT (x400)

of pathogenesis leading to carcinoma are yet unknown, accumulation of genetic alteration is considered to have an important role. In recognition of telomerase activity as an indicator of proliferative potential, high telomerase activity involves in unlimited cell divisions, which relate to clonal expansion and inducing the accumulation of gene mutations[18,21].

In the present study, positive hTERT immunoreactivity was seen in all cases of carcinoma and 48.4% of BOT cases. Although the authors studied only 12 cases of epithelial ovarian carcinoma, all tumors were positive with high expression for telomerase activity. This result was similar to those previously reported[22,23].

Majority of BOT in most Western countries is serous type, while mucinous BOT is much more common in our institute[24,25]. Similar incidence was also observed in the present study. 75.8% of BOT in the present study were mucinous type, which comprised of 53.2% intestinal-like type and 46.8% endocervical-like type. It is well documented that BOT has an indolent clinical course and delayed recurrence. The majority of patients can be cured by surgery alone in the early stage, although relapse and death from recurrent disease can occasionally occur[7]. As all cases of BOT in the present study were FIGO stage 1, unsurprisingly, no recurrence or death was observed despite the rather long period of follow-up.

In contrast to carcinoma, hTERT expression was seen in only 48.4% of BOT cases. The prevalence of hTERT expression in BOT varied greatly, depended on the different methods and positive-staining criteria. However, the overall result in our study was quite similar to those previously reported[26,27]. Serous tumor had a higher rate of hTERT expression when compared to mucinous tumor, but without statistical significance. A case of clear cell BOT also revealed positive hTERT immunoreactivity. Interestingly, all benign epithelial linings in the same slide of each immunoreactive case were negative for hTERT expression. These findings were correlated with other studies, which reported the low rate of hTERT expression in benign ovarian tumor[27,28].

In the present study, only FIGO stage was significantly associated with hTERT immunoreactivity. It seems like more extended disease was positively correlated with high hTERT expression. Therefore, this finding had limitation to interpret because all BOT cases in the present study were FIGO stage 1 and the majority of cases were stage 1a.

This present study has limitations including the limited number of cases, the only one institutional experience, and the semiquantitative staining interpretation. However, despite of these limitations, it adds to the authors' understanding the association between hTERT expression and borderline ovarian tumors. Up regulation of hTERT is important for telomerase activation during malignant tumor progression. Although hTERT itself is not sufficient for malignant transformation of cells, it can increase the cell's life span that allows the accumulation of additional genetic mutations[9]. As previously mentioned, high hTERT expression was observed in most carcinoma, approximately half of borderline tumors, and none in the benign tissue. The present findings partly supported the role of telomerase activity to pathogenesis of ovarian cancer. In addition to immunohistochemistry, further larger quantitative studies may provide more clear information about the role of telomerase activity in borderline ovarian tumors, including the pathogenesis of ovarian cancer.

In conclusion, the present study has demonstrated hTERT immunoreactivity in 48.4% of BOT and 100% of epithelial ovarian carcinoma. High hTERT expression was associated with FIGO stage. These findings suggested a role of telomerase activity in the biology of ovarian tumors. Although telomerase may not be useful as a supplement means of cancer diagnosis, further studies are needed to elucidate its role in tumorigenesis.

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References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al Cancer statistics, 2006. CA Cancer J Clin 2006; 56: 106-30.
2. Cannistra SA. Cancer of the ovary. N Engl J Med 2004; 351: 2519-29.
3. Barakat RR. Borderline tumors of the ovary. Obstet Gynecol Clin North Am 1994; 21: 93-105.
4. Hopkins MP, Kumar NB, Morley GW. An assessment of pathologic features and treatment modalities in ovarian tumors of low malignant potential. Obstet Gynecol 1987; 70: 923-9.
5. Eltabbakh GH, Natarajan N, Piver MS, Mettlin CJ. Epidemiologic differences between women with borderline ovarian tumors and women with epithelial ovarian cancer. Gynecol Oncol 1999; 74: 103-7.

6. Zanetta G, Rota S, Chiari S, Bonazzi C, Bratina G, Mangioni C. Behavior of borderline tumors with particular interest to persistence, recurrence, and progression to invasive carcinoma: a prospective study. J Clin Oncol 2001; 19: 2658-64.
7. Trimble CL, Kosary C, Trimble EL. Long-term survival and patterns of care in women with ovarian tumors of low malignant potential. Gynecol Oncol 2002; 86: 34-7.
8. Scully RE, Young RH, Clement PB. Tumors of the ovary, maldeveloped gonads, fallopian tube, and broad ligament. In: Rosai J, editor. Atlas of tumor pathology. 3rd series, fascicle 23. Washington, DC: Armed Forces Institute of Pathology; 1998: 51-168.
9. Hart WR. Borderline epithelial tumors of the ovary. Mod Pathol 2005;18 (Suppl 2): S33-50.
10. Shih I, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol 2004; 164: 1511-8.
11. Bell DA. Origins and molecular pathology of ovarian cancer. Mod Pathol 2005; 18 (Suppl 2): S19-32.
12. Blackburn EH. Telomeres. Trends Biochem Sci 1991; 16: 378-81.
13. Blackburn EH. Telomerases. Annu Rev Biochem 1992; 61: 113-29.
14. Blackburn EH. The telomere and telomerase: how do they interact? Mt Sinai J Med 1999; 66: 292-300.
15. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al Specific association of human telomerase activity with immortal cells and cancer. Science 1994; 266: 2011-5.
16. Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW. Telomerase activity in human germline and embryonic tissues and cells. Dev Genet 1996; 18: 173-9.
17. Vaziri H, Dragowska W, Allsopp RC, Thomas TE, Harley CB, Lansdorp PM. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. Proc Natl Acad Sci U S A 1994; 91: 9857-60.
18. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer 1997; 33: 787-91.
19. Hiyama E, Hiyama K, Yokoyama T, Shay JW. Immunohistochemical detection of telomerase (hTERT) protein in human cancer tissues and a subset of cells in normal tissues. Neoplasia 2001; 3: 17-26.
20. Nakamura TM, Morin GB, Chapman KB, Weinrich SL, Andrews WH, Lingner J, et al. Telomerase catalytic subunit homologs from fission yeast and human. Science 1997; 277: 955-9.
21. 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: 568-71.
22. Wan M, Li WZ, Duggan BD, Felix JC, Zhao Y, Dubeau L. Telomerase activity in benign and malignant epithelial ovarian tumors. J Natl Cancer Inst 1997; 89: 437-41.
23. Sood AK, Coffin J, Jabbari S, Buller RE, Hendrix MJ, Klingelhutz A. p53 null mutations are associated with a telomerase negative phenotype in ovarian carcinoma. Cancer Biol Ther 2002; 1: 511-7.
24. Trivijitsilp P, Triratanachat S, Niruthisard S, Tantayaporn K. The frequency of primary ovarian neoplasms at King Chulalongkorn Memorial Hospital during 1990-1997. Chula Med J 1999; 43: 213-24.
25. Niruthisard S. Common epithelial cancers of the ovary at Chulalongkorn Hospital (1985-1989). Chula Med J 1991; 35: 735-43.
26. Brustmann H. Immunohistochemical detection of human telomerase reverse transcriptase (hTERT) and c-kit in serous ovarian carcinoma: a clinicopathologic study. Gynecol Oncol 2005; 98: 396-402.
27. Datar RH, Naritoku WY, Li P, Tsao-Wei D, Groshen S, Taylor CR, et al. Analysis of telomerase activity in ovarian cystadenomas, low-malignant-potential tumors, and invasive carcinomas. Gynecol Oncol 1999; 74: 338-45.
28. Murakami J, Nagai N, Ohama K, Tahara H, Ide T. Telomerase activity in ovarian tumors. Cancer 1997; 80: 1085-92.
29. Liu J, Yang G, Thompson-Lanza JA, Glassman A, Hayes K, Patterson A, et al. A genetically defined model for human ovarian cancer. Cancer Res 2004; 64: 1655-63.

การศึกษา human telomerase reverse transcriptase (hTERT) ในเนื้องอกรังไข่ชนิด borderline

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วัตถุประสงค์: *เพื่อสำรวจการแสดงออกของ human telomerase reverse transcriptase (hTERT) ในเนื้องอกรังไข่ชนิด borderline โดยวิธีการย้อม Immunohistochemistry*

วัสดุและวิธีการ: *ศึกษาการแสดงออกของ hTERT โดยวิธีการย้อม immunohistochemistry ในชิ้นเนื้อจากบล็อกพาราฟิน ซึ่งประกอบด้วย เนื้องอกรังไข่ชนิด borderline 62 ราย (mucinous 14 ราย, clear cell 1 ราย) และมะเร็งรังไข่ชนิดเยื่อบุผิว 12 ราย โดยประเมินจากความเข้มและปริมาณของการติดสีย้อม จากนั้นนำมาประเมินความสัมพันธ์ทางพยาธิวิทยาคลินิก*

ผลการศึกษา: *พบการแสดงออกของ hTERT ใน 48.4% ของเนื้องอกรังไข่ชนิด borderline และทุกรายของมะเร็งรังไข่ชนิดเยื่อบุผิว โดย 50% ของกลุ่มเนื้องอกรังไข่ชนิด borderline ที่ติดสีย้อม hTERT จัดอยู่ในกลุ่มที่มีการแสดงออกสูงซึ่งพบการแสดงออกสูงสุดในชนิด serous อย่างไรก็ตาม ไม่พบว่ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติในการแสดงออกของ hTERT ในชนิดต่าง ๆ ของเนื้องอกรังไข่ชนิด borderline ในการประเมินส่วนที่เป็น benign ในสไลด์แผ่นเดียวกันในกลุ่มเนื้องอกรังไข่ชนิด borderline ที่ติดสีย้อม hTERT พบว่าไม่มีการแสดงออกของ hTERT ในบริเวณที่เป็น benign ด้านความสัมพันธ์ทางพยาธิวิทยาคลินิก พบว่าการแสดงออกของ hTERT สัมพันธ์กับ FIGO stage เพียงอย่างเดียว ($p = 0.004$) ระยะเวลาเฉลี่ยในการตรวจติดตามผู้ป่วยเนื้องอกรังไข่ชนิด borderline ในการศึกษานี้นาน 81.63 เดือน ซึ่งไม่พบการกลับเป็นซ้ำ หรือ เสียชีวิตในระยะเวลาดังกล่าว*

สรุป: *การแสดงออกของ hTERT พบใน 48.4% ของเนื้องอกรังไข่ชนิด borderline และทุกรายของมะเร็งรังไข่ชนิดเยื่อบุผิว โดยการแสดงออกของ hTERT มีความสัมพันธ์กับ FIGO stage*