Comparison of Telomerase Activity in Ovary Carcinoma Tissues and Para-Carcinoma tissues

Zhijun Xia[1], Yinghan Chen[1], Shulan Zhang[1]

The Second Clinical College, China Medical University, Shenyang 110003 China.

Abstract **Objective** To study the expression state of Telomerase activity of ovary carcinoma and para-carcinoma. **Methods** Telomere repeat amplification protocol-sliver staining (TRAP-Sliver staining) was used to detect telomerase activity in 36 cases ovary carcinoma tissue samples, 32 cases para-carcinoma tissue samples and 30 cases benign ovary tissue samples. **Results** The detective rates of Telomerase were 91.67%, 3.13% and 6.67% and in ovary carcinoma, para-carcinoma tissua and cystadenoma respectively. The detective rates of Telomerase were significant higher in ovary carcinoma group than those in the other two groups ($p<0.01$). But there was no significant difference of Telomerase between para-carcinoma and cystadenoma tissues samples. **Conclusion** There was significant difference in the expression state of Telomerase activity between ovary carcinoma tissues and para-carcinoma, which can supply theoretical warranty for that operation on reserve ovary function of ovary carcinoma patients .

Key Words ovary carcinoma; para-carcinoma tissues; telomere

There is close correlation between the activation of telomerase and the formation and development of malignant tumors. Although activation of telomerase may not be the early event of carcinogenesis, telomerase is definitely activated late in the progression of many cancers. Expression of telomerase might not be the causal factor of carcinogenesis but play a crucial role in the maintenance of development of tumors[1]. The detective rates of telomerase were significantly higher in carcinomas than in para-carcinomas and benign tissues, indicating that telomerase activity is closely related to malignant tumors.

MATERIALS AND METHODS

Sources and Processing of Samples Ovarian tumor samples were obtained from women with previously untreated ovarian disease, including 36 cases with malignant tumors (32 samples of para-carcinomas that showed no evidence of ovarian tumor formation or malignant tissue according to pathological section were simultaneously collected from them) and 30 cases with benign tumors, who underwent surgery between July 1997 and June 2000 at No.2 hospital affiliated with China Medical University. Diagnostic verifications and tumor subtyping or grading were postoperatively performed by practicing surgical pathologists in ovarian histopathology. Surgical staging was done according to the current recommendation of the International Federation of Gynecology and Obstetrics. Of all the 36 patients with ovarian cancer, there were 19 serous cystadenocarcinoma (among them, stage Ⅱ 3 cases, stage Ⅲ 9 cases and stage Ⅳ 7 cases) 12 mucous cystadenocarcinoma (among them, stage Ⅲ 6 cases and stage Ⅳ 6 cases)and 5 mixed epithelial carcinoma(among them, stage Ⅲ 2 cases and stage Ⅳ 3 cases). Of all the 30 cases with benign tumors, there were 21 serous cystadenomas and 9 mucous cystadenomas. In addition, the normal ovarian tissues were also collected from 30 cases with uterine myoma diagnosed during laparotomy. The ages of the patients in the malignant tumors group, benign tumors group and normal ovaries group were ranged from 21 to 64 (median 51.6), 17 to 65 (median 49.2) and 22 to 56, respectively. Each sample was rinsed immediately with physiological saline and stored at -75℃ before analysis.

Assay of Telomerase expression Activity Telomerase assays were performed with TRAPEZETM kit (Oncor American) following the TRAP (i.e. telomeric repeat amplification protocol) method described by Kim et al. Briefly, 25ul reaction mixes , containing TRAP reaction solution and 2ul of tissue extract, were incubated at 30 ℃ for 30 minutes. The preparations were then amplified by using the following thermal cycling profile: 35 cycles of 94℃-30s, 60℃-30s and 72℃-30s. The PCR products were subjected to electrophoresis on 12.5% nondenaturing polyacrylamide gels at 100 voltages for 2 hours. Samples that contained telomerase were identified by silver-staining on the autoradiography.

The telomerase-negative controls without telomerase tissue extracts were assayed following the same procedure. The telomerase-positive controls were cell masses with telomerase activity provided by TRAPEZETM telomerase kit. The preparations of telomerase-positive

controls homogenized with 200ul of ice-cold analysis buffer detergent containing CHAPS were used to determine the telomerase activity by the same TRAP assay.

The assessment of results

Evidence of telomeric repeat ladder indicates presence of telomerase expression activity. All the samples were classified as negative (without DNA ladders), weakly positive (with less than three DNA ladders) and positive (with at least three DNA ladders)

In addition, since some cell/tissue extracts containing the inhibitor(s) of Taq enzyme could result in false negative, TRAP assays for the samples that were negative for telomerase activity were repeated in the presence of internal control oligopeptide K1 and TSK 1 to ascertain the presence or absence of telomerase inhibitor(s) in the samples.

Statistical Analysis Data were analyzed using T-test and χ^2 test . These biochemical data were presented as the group mean ±SE.

RESULTS

Thirty one of the 36 carcinoma specimens were classified as positive, 2 as weakly positive, and 3 as negative for telomerase activity. All the three telomerase negative carcinomas specimens were mucous cystadenocarcinomas. Weak telomerase activity was detected in 1 of the 32 para-carcinomas tissues; all the others were negative. Weak telomerase activity was detected in 2 of the 30 benign ovarian tumors, both of which were cystadenomas; the others were all negative for telomerase activity. All the normal controls were negative for telomerase activity. The rates of telomerase expression differed significantly between normal ovaries, benign ovarian tumors or ovarian para-carcinomas tissues and ovarian carcinomas tissues (p<0.05). On the contrary, the difference of telomerase expression between the para-carcinomas tissues and normal controls was not statistically significant, so was the difference between benign ovarian tumors and normal controls (p>0.05).

The results of the telomerase assay for all of the case are shown in Table 1. The telomerase expression in ovarian carcinoma, para-carcinoma tissues and benign ovarian tumors are shown in Figure 1.

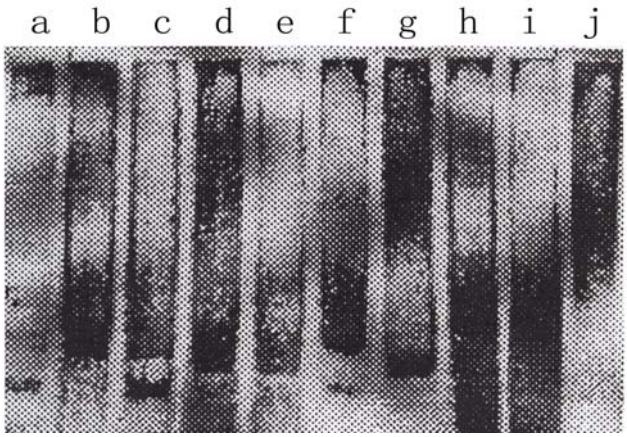

Fig-1. Telomerase activity in ovarian carcinomas, ovarian para-carcinomas, and benign ovarian tumors. Lanes a, j and g represent positive control, negative control and normal control, respectively. Lanes e and f represent para-carcinomas and the remaining lanes represent carcinomas.

DISSCUSSION

According to the relative findings of tumor molecular biology, telomeres are essential for the maintenance of stability of chromosomes and the length of telomeric DNA sequence is closely associated with the life of cell [2]. The telomeric DNA sequences become progressively shorter during DNA replication. It is thought that chromosomes are unable to undergo additional rounds of replication once their telomeres have shortened below a critical size. Telomeres shorten with cell division is a process thought to contribute to cell senescence and the proliferative crisis of transformed cells [3]. Therefore, telomere is often called as cell mitotic 'counter' or cell senescence 'internal biological clock' [4] .

Telomerase that is a ribonuleoprotein enzyme, containing a template RNA complementary to that of the

Table 1 The expression of Telomerase in normal ovary, benign ovarian tumor, ovarian para-carcinoma and ovarian carcinoma

Histology	number of patients	Telomerase activity			Detective rate
		positive	weakly positive	negative	
Carcinomas	36	31	2	3	91.67
Para-carcinomas	32	0	1	31	3.13
Benign tumors	30	0	2	28	6.67
Normal ovaries	30	0	0	30	0

telomeric repeat, can elongate telomeric DNA by adding telomeric repeats to the 3' end of telomeric DNA. Alternate template-directed synthesis of telomeric DNA and repositioning of telomerase on the chromosome results in an increase in telomere length. Thus telomere stabilization by telomerase can lead to unlimited cell proliferation and contribute to their 'immortality' [5]. Telomerase which is usually undetectable in normal somatic cells, is generally expressed in malignant tumor cells. Activation of this enzyme in cancer cells may contribute to their 'immortality' and is even important in tumor progression. The estimation of telomerase activity could provide new ways to develop the therapy of tumors and by examining the expression of the telomerase in tumors of low malignant potential (LMP), we might determine the trend of these tumors.

According to this report, the rates of telomerase expression significantly differed between ovarian para-carcinomas tissues or benign ovarian tumors and ovarian carcinomas tissues. The highly expression of telomerase in ovarian carcinomas suggests that telomerase activity could be associated with malignant cells immortality and contribute to their evasion of proliferative limitation of cells senescence. As demonstrated by some investigations, the strength of telomerase activity is not an independent factor of the prognosis of ovarian cancer[6]. But it may be possible to use the presence of telomerase activity as a tumor specific marker in order to complement more conventional approaches (e.g. CA125 and TGF) to monitor disease status following therapy. Although the merit of this approach, such as find micro recurrent foci and subtle foci, has not yet been tested experimentally, the high sensitivity of the TRAP assay and the apparent high specificity of telomerase for the neoplastic phenotype add to its potential attractiveness.

In this study, three mucous cystadenocarcinomas specimens were telomerase negative. This result may be caused by the interference of stagnant mucin with the detection of telomerase activity. In spite of the significant difference between the telomerase activity of ovarian carcinomas and para-carcinomas, further investigation needs to be made to test the correlation between telomerase activity and the progression of ovarian epithelial cancer.

REFERENCES

1.Carreiras F, Cruet S, Staedel C, et al. Human ovarian adenocarcinoma cells synthesize vitromectin and use it to organize their adhesion . Gynecol Oncol , 1999, 72：312~322.

2. Cruet S , Salamanca C , Mitchell GW , et al . Alphavbeta3 and vitromectin expression gy normal ovarian surface epithelial cells: role in cell adhesion and cell proliferation. Gynecol Oncol , 1999，75：254-260.

3. Hiyama E，Kodama T，Shinbara K，et al. Telomerase activity is detected in pancreatic cancer but not in benign tumors. Cancer Res , 1997，57（2）：326~331.

4. Brien TP, Kallakury BVS, Lowry CV, et al. Tolomerase activity in benign endometrium and endometrial carcinoma. Cancer Res, 1997，57 (6)：2760.

5. Kasran MB, Canmaan CE, Leonard CJ. p53 dyficiency rescues the adverse effectes of telomere loss and cooperates with telomere dysfunction to acceleration carcinogenesis. Cell , 1999，97：527-538.

6. Xia Zhijun , Zhang Shulan , Zhou Zhuo . Effect of transforming growth factorα on the clinical application of CA125 in patients with ovary carcinoma. J Chin Med Univ , 2000, 29(6): 465-466.