滋養層細胞疾病之尾部酵素活性

Telomerase Activity in Gestational Trophoblastic Diseases

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Abstract

Telomeres are the distal ends of human chromosomes composed of tandem repeats of the sequence TTAGGG. Possible functions of telomeres include prevention of chromosome degradation, end-to-end fusions, rearrangements, and chromosome loss. Human telomeres undergo progressive shortening with cell division which results in chromosomal instability. In our study we used telomerase activation protocol to measure telomerase activation in pregnancy, gestational trophoblastic neoplasm as well as a variety of malignant gynecological tumors. We found that telomerase activity in trophoblasts from gestational trophoblastic diseases is possibly higher than that in normal pregnancy or spontaneous abortion. Our results suggest that the telomerase activity in trophoblasts is critically regulated over the course of gestation. The findings of the present study also appear to support the emerging concept that normal somatic cells with stem cell-like characteristics can express telomerase activity.

Keyword: Gestational trophoblastic diseases, Gynecological Cancer, Telomerase

Introduction

Normal human somatic cells have a limited life span both in vitro and in vivo. It has been hypothesized that this limited capacity for proliferation is regulated by telomere length [1,2]. Telomeres are the 4 to 15 kb of simple DNA repeats (TTAGGG) located at the ends of chromosomes [3,4,5]. It has been shown that telomeres provide a protective cap at the chromosome ends, stabilizing the structure to prevent genetic instability [4,5,6]. Furthermore, telomeres can facilitate the replication of chromosomes and in some cases regulate the expression of genes at the chromosome termini [7]. Due to the inability of DNA polymerase to completely replicate the ends of double-stranded DNA, telomeres progressively shorten with each round of cell division [8,9,10]. It is hypothesized that cells recognize this stochastic loss of telomeres and, when the telomeres reach a critical length, enter a

program of cellular senescence [1,11,12]. This programmed senescence is believed to be a mechanism utilized by multicellular eukaryotes to prevent uncontrolled proliferation. Unicellular eukaryotes and human germline cells, which must maintain telomere length, have been shown to contain an enzyme activity called telomerase, which adds the six base repeats to the telomere ends [13,14,15]. Most normal somatic cells have undetectable levels of this enzyme [16]. Strong evidence suggests that immortalization requires a mechanism to restore telomere length, usually by activation of normally silent telomerase complexes [17]. Although telomerase can be detected in most immortal cells and cancers, little is known about how it is activated.

Gestational trophoblastic disease is more prevalent in our country than in the Western world. This disease category contains hydatidiform mole, invasive mole and choriocarcinoma. Its pathology can progress from a benign state to malignancy. In this study, we will measure telomerase activity in the tissue samples obtained from cases of gestational trophoblastic disease in order to determine whether hydatidiform mole has a higher level of telomerase activity than placental tissue that is from a normal pregnancy and is of same gestational age; to ascertain whether choriocarcinoma has high telomerase activity; and to find out whether hydatidiform mole with high telomerase activity is more likely to have malignant sequelae.

Material and Methods

Patients were recruited from National Taiwan University Hospital. During diagnostic D&C or during surgery, specimens of hydatidiform mole, invasive mole or choriocarcinoma were collected and put in phosphate-buffered saline and kept on ice until they are processed. A histopathological examination to confirm the diagnosis was conducted. All specimens for this study will be snap-frozen in liquid nitrogen and stored at -80oC until analysis. The tissue samples from other gynecological cancers were also collected. Telomerase activity was measured by TRAP assay.

Results

In the results of our study, ten of the eleven (90.9%) cases of hydatidiform mole and all three cases of choriocarcinoma were all positive telomerase activity. In the contrast, 35 specimens of

trophoblastic tissue from placentas at 5 to 9 weeks of gestation were examined for telomerase activity using the TRAP assay. Twenty-four (76%) were telomerase-positive. Trophoblasts from early spontaneous abortions also exhibited telomerase activity but at a low level. Five of 13 (38%) were found to exhibit telomerase activity. In contrast, none of the 13 (0%) late trophoblasts at 36 to 41 weeks gestation expressed telomerase activity. Significant telomerase activity was observed in trophoblasts in the early pregnancies.

In our study using telomerase activation protocol (TRAP) assay, we found that telomerase activation in a variety of malignant gynecological tumors (Table 1), suggesting that telomerase activation is a critical step in cancer development of these malignancies.

Our results showed that the positive rates of telomerase activity among early normal pregnancy (26/35), spontaneous abortion (5/13), hydatidiform mole (10/11), and choriocarcinoma (3/3) are significant different (p=0.0136). Nevertheless, in comparison between normal early pregnancy and gestational trophoblastic disease, the positive rate is slightly outside the significance level (P=0.0887) (Table 2).

Discussion

Telomeres are the distal ends of human chromosomes composed of tandem repeats of the sequence TTAGGG. Possible functions of telomeres include prevention of chromosome degradation, end-to-end fusions, rearrangements, and chromosome loss. Human telomeres undergo progressive shortening with cell division which results in chromosomal instability, leading to cellular senescence. A possible cause for shortening of human telomeres is due to the repression of telomerase, a specialized ribonucleoprotein polymerase containing an integral RNA with a short template element that directs the synthesis of telomeric repeats at chromosome ends. Telomerase reactivation is thus thought to be essential for stabilizing telomere length to attain cellular immortality. Telomerase activation is required for cellular immortalization and is found in most malignant tumors. Normal human somatic cells have a limited life span both in vitro and in vivo. It has been hypothesized that this limited capacity for proliferation is regulated by telomere length. Normal somatic cells are generally telomerase-negative, except for stem cells in renewing tissue.

The category of gestational trophoblastic disease contains

hydatidiform mole, invasive mole and choriocarcinoma. Its pathology can progress from a benign state to malignancy. Marked trophoblastic proliferation can be found in the disease entity. It is still unclear whether telomerase reactivation is present in the disease, which represents a disease from benign to malignancy.

In a normal pregnancy, the early chorion is composed of a mass of proliferating trophoblasts. Chorionic development begins soon after implantation of the blastocyst. During invasion of trophoblast into myometrium, cytotrophoblast continues to proliferate and acts as proliferating stem cells which guarantee growth of the trophoblast and subsequent development of the chorion and placenta. The role of trophoblasts as stem cells prompt us to examine the telomerase activity in the trophoblasts in chorion as well in the first year of our study.

In our study using telomerase activation protocol (TRAP) assay, we found that telomerase activation in a variety of malignant gynecological tumors, suggesting that telomerase activation is a critical step in cancer development of these malignancies.

From results of this study, we found that the telomerase activity in trophoblasts is critically regulated over the course of gestation. The findings of the present study also appear to support the emerging concept that normal somatic cells with stem cell-like characteristics can express telomerase activity.

These findings suggest that telomerase activity in trophoblasts from gestational trophoblastic diseases is possibly higher than that in normal pregnancy or spontaneous abortion. However, it is necessary to increase the case number of gestational trophoblastic diseases to clarify whether hydatidiform mole has higher telomerase activity than normal pregnancy placenta of the same gestational age. We all also need to determine whether choriocarcinoma has high telomerase activity and whether hydatidiform mole with high telomerase activity is more likely to have malignant sequelae in the following years.

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Table 1. Preliminary results of expression of telomerase activity in various gynecological tumors

	Positive	Total	Positive rate
Normal uterine cervix	0	12	0
CIN	8	26	31
Cervical cancer	7	10	70
Endometrium	1	6	17
Endometrial cancer	2	6	34
Normal ovary	0	12	0
Benign ovarian tumor	1	7	14
Ovarian cancer	7	8	88

Table 2. Preliminary results of expression of the telomerase activity in gestational trophoblastic diseases and pregnancies

	Positive	Negative	Total
Normal early pregnancy	26(74)	9(26)	33
Spontaneous abortion	5(38)	8(62)	13
Normal late pregnancy	0	13	13
Hydatidiform mole	10(91)	1(9)	11
Choriocarcinoma	3(100)	0	3