TELOMERASE RNA AND TELOMERASE ACTIVITY IN TROPHOBLASTIC TUMORS*

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Abstract: To gain better understanding of telomerase's possible role in the carcinogenesis of gestational trophoblastic tumors, the authors conducted RT-PCR amplification-based analysis and carried out telomeric repeat amplification to determine the levels of the human telomerase RNA (hTR) and that of telomerase enzymatic activity itself in 43 normal human placental tissues, 35 gestational trophoblastic tumor tissues and three choriocarcinoma cell lines. hTR was expressed in malignant gestational trophoblastic tumor tissues as well as choriocarcinoma cell lines. The results showed that hTR of early placenta villi and a part of hydatidiform mole were positive. But relatively low levels of the hTR could be found in placental tissues. Telomerase enzymatic activity was strongly positive in 32 of the 35 (91.4%) gestational trophoblastic tumor tissues and all the three choriocarcinoma cell lines. The enzymatic activity of telomerase itself was detectable at relatively low lelves in 14 of the 21 (66.7%) early placental villi, only three of the 22(13.6%) term placenta were weakly positive. These results suggest that telomerase activity may be correlated with the development of trophoblastic tumors, and so, may be a useful diagnostic marker for detecting the existence of malignant trophoblastic cells.

Key words: telomerase; RNA; trophoblast; tumor; placenta
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INTRODUCTION

Multiple genomic mutations and DNA damage can cause malignant transformation of human cells. Telomeres are specialized structures containing repeats of the sequence TTAGGG at the ends of eukaryotic chromosomes. Telomeres are thought to have an important role in chromosome protection and replication (Rhyu, 1995). Telomerase is a ribonucleoprotein that synthesizes telomeric DNA onto chromosomal ends (Greider et al., 1985). Telomerase activity is found in many human tumor tissues and most immortal cell lines but is not detected in many human somatic tissues except those of the germline cells (Lange, 1994, Kim et al., 1994). Thus, human telomerase may be an enzyme with a crucial role in regulating the proliferation of immortal tumor cells. The fact that telomerase is seemingly expressed in majority of malignant cells should lead to new diagnostic and therapeutic applications. Recently, the inhibition of telomerase activity by antisense human telomerase RNA (hTR) and peptide nucleic acids had been reported (Kim et al., 1994, Norton, et al., 1996).

The human trophoblast found in early placenta has been considered to be "pseudo-malignant" tissue, because it undergoes extensive proliferation during invasion into the maternal decidua which was compared with the invasion and proliferation of choriocarcinoma. But whether expression of telomerase is a prerequisite for the development of human placenta and gestational trophoblastic tumors (GTT) is unknown. In order to assess the possible association of the expression of telomerase RNA and telomerase itself with the development of normal human placenta and GTT, reverse transcription (RT) and nested polymerase chain reaction (PCR) were used in an assay of telomerase RNA and telomerase activity in human first trimester, term placenta, gestational trophoblastic tumor tissues and choriocarcinoma cell lines.

MATERIALS AND METHODS

Cell lines and tissue specimens Human

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JAR, BeWo and JEG3 choriocarcinoma cell lines were obtained from the American Type Culture Collection (Rockville, MD). JAR and JEG3 cells were maintained in RPMI-1640 (GIBCO-BRL, Gaithersburg, MD) medium with 10% fetal bovine serum. BeWo cells were maintained in F12K medium (also of GIBCO-BRL) with 15% FBS. All cells were cultured at 37 ℃ with 5% CO_2. Forty-three normal placental tissues specimens (21 early pregnant villi samples between 8 and 12 weeks gestation and 22 term placental tissues) were the subjects of this study. Thirty-five gestational trophoblastic tumor tissue samples were obtained from patients diagnosed and treatmented at our hospital. All tissues were confirmed by histopathological examination.

Determination of telomerase RNA by RT-PCR Total cellular RNA was extracted from cells and tissues with Tri-Reagent (Molecular Research Center Inc., Cincinnati OH). The hTR sequence information had been reported (Feng et al. 1995). The sequence of the primers were 5'-GGGAGGGGTGGTGGCCATTT-3' (antisense strand) and 5'-GTTTGCTCTAGAG AATGAACGG (sen-se strand) and the final amplification product was 150 base pairs. The sequences of the internal primers of β-actin were 5'-ACACTGTGCCCATCTACGAGGGG-3' and 5'-ATGATGGAGTTGAAGGTAGTTTC GTGGAT-3'. The reverse transcription and PCR reactions were carried out in a standard reaction as described before.

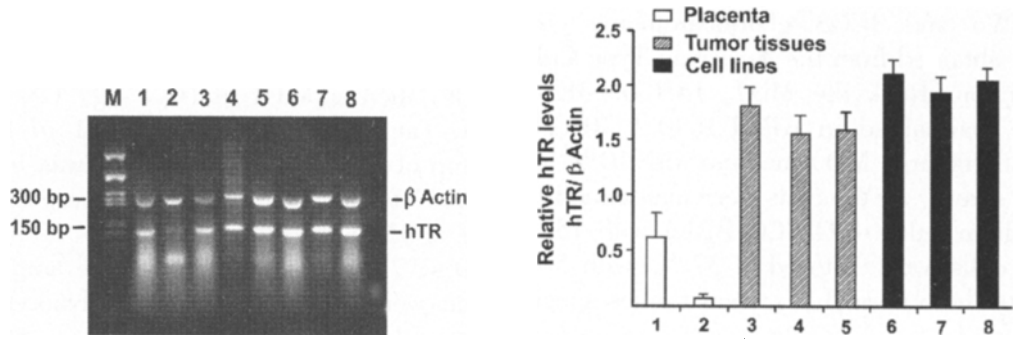
Telomerase TRAP assay Cells or portions of surgical tissues were removed and homogenized with 20 μL of cold lysis buffer. Telomerase activity was assayed essentially by the procedures of the telomere repeat amplification protocol (TRAP) assay (Kim et al. 1994). Freozen samples were suspended in ice-cold wash buffer (10 mmol/L Tris-HCL (pH 7.5), 1mmol/L $MgCl_2$, 1 mmol/L EGTA (pH 8.0), 0.1 mmol/L PMSF, 5 mmol/L β-mercaptoethanol, 0.5% CHAPSO, and 10% glycerol) incubated on ice for 30 min, and centrifuged at 12, 000% g for 30 min at 4 ℃, and the supernatant was stored at − 80 ℃. The TRAP assay was performed as follows. Two μg protein extract were added in 20 mmol/L Tris-HCl (pH8.3); 1.5 mmol/L $MgCl_2$ 63 mmol/L KCl, 0.005% Tween20, 1 mmol/L EGTA, and 50 μmol/L dNTPS, 1 μg T4g32 protein , 0.1 mg/ml BSA and 0.1 μg TS primer at room temperature 30 min. The mixture was incubated at 90 ℃ for 90s, then added 1 μl (0.1 μg) CX primer, 0.1U Taq DNA polymerase and 1 μl BSA and a drop of PCR oil. The mixture was incubated at 90 ℃ for 90s. These samples were then subjected to 35 PCR cycles at 94 ℃ for 30 s, 50 ℃ for 30 s, 72 ℃ for 60 s. After the amplified products were separated in 15% polyacrylamide gel, the gels were stained with silver according to manufacturer's recommendations. The positivity of telomerase was established by inspecting the stained gel under ultraviolet lights for the presence of the presence of the characteristic 6 base pair increment ladder. Each sample was assayed initially with 2.0 μg protein extract to identify the relative intensities of the TRAP products. All extracts were assayed with (+) or without (−) pretreatment with Rnase.

Statistical Analysis Statistical analysis was conducted using a χ^2 test to evaluate the significance of the differences. $P < 0.05$ was considered to be statistically significant.

RESULTS

Expression of hTR in Placenta and Trophoblastic Tumors

The obtained sensitivity of hTR by the amplification and detection methods used in this study was such that the hTR was clearly visible when approximately 100 JAR cells were used for the RT followed with nested PCR (data not shown). These figures were determined by amplifying serial dilutions of known numbers of JAR cells (data not shown). The hTR was expressed on both JAR, BeWo and JEG3 choriocarcinoma cell lines (Fig. 1a, lanes 6 − 8) in a part of hydatidiform mole, invasive mole and primary choriocarcinoma tissues (Fig. 1a, lanes 3 − 5). Although at much lower levels as compared with those of choriocarcinoma cell lines and tissues, the hTR was detected in the first trimester villi (Fig. 1a, lane 1) and in term placental villi (Fig. 1a, lane 2). The relative hTR levels (hTR/β-actin) showed that expression of hTR in early pregnancy villi were higher than that of the term plancental villi ($p < 0.05$). The expression of hTR in trophoblastic tumors and choriocarci-



(a) (b)

Fig.1 Expression of hTR in human placenta and trophoblastic tumors (a). Lanes 1 and 2 are early pregnant villi and term placental villi, respectively. Lane 3 – 5 are trophoblastic tumor tissues (Hydatidiform mole, invasive mole and choriocarcinoma). Lanes 6 – 8 are JAR, BeWo and JEG3 choriocarcinoma cell lines. Relative hTR levels (hTR/(-actin) in human placenta and trophoblastic tumors(b). 1: early pregnant villi; 2: term placental villi; 3 – 5: Hydatidiform mole, invasive mole and choriocarcinoma; 6 – 8: JAR, BeWo and JEG3 choriocarcinoma cell lines.

noma cells lines were higher than that of early pregnancy villi ($p < 0.01$) and were higher than that of term plancental villi ($p < 0.001$) (Fig. 1b and Table.1)

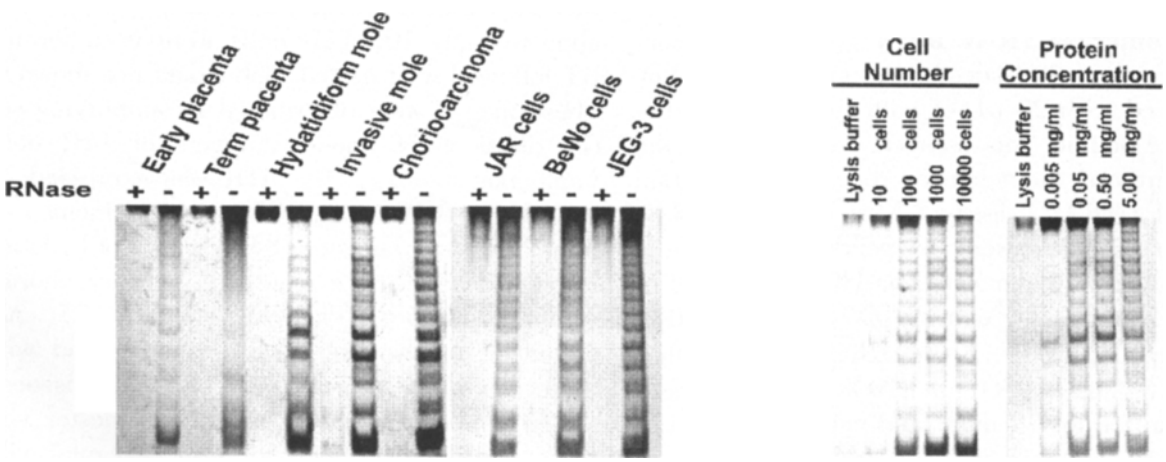
Table 1 Expression of hTR in human placenta and trophoblastic tumors

Types	Numbers	hTR/β-actin (X ± SD)
Early pregnancy villi	21	0.6 ± 0.24*
Term placental villi	22	0.08 ± 0.05
Trophoblastic tumors	35	1.75 ± 0.15**
Choriocarcinoma Cell lines	3	2.08 ± 0.12**

* Compared with term placental villi $P < 0.05$;
** Compared with early pregnancy villi $P < 0.01$;
Compared with term placental villi $P < 0.001$

Telomerase Activity in Placenta and Trophoblastic Tumors

Telomerase activities present in JAR, BeWo and JEG3 choriocarcinoma cell lines, respectively. Telomerase activities in hydatidiform mole, invasive mole and primary choriocarcinoma tissues were quite strong. Telomerase activity was detectabloe but at a much lower level in first trimester villi compared with either trophoblastic tumor tissues or Choriocarcinoma cell lines, whereas it was essentially absent in term placental tissues RNase pretreated extracts do not show telomerase activity (Fig.2a and Table 2).



(a) (b)

Fig.2 Telomerase activity in human placenta and trophoblastic tumors (a). RNase + : treated with RNase; RNase-: not treated with Rnase. The sensitivity of telomerase assay (b) was carried out using extracts containing 5 μg of protein from choriocarcinoma tissues, and were subjected dilutions 5 μg, 0.5 μg, 0.05 μg, and 0.005 μg. A 1000% dilution of the extract containing a final concentration of 0.005 μg of protein still showed a positive telomerase signal. Lysis buffer and a dilution series of an extract from JAR cell line were used as controls.

Table 2 Telomerase activity in human placenta and trophoblastic tumors

Types	Numbers	Telomerase activity*		
		(−)	(+)	(++)
Early pregnancy villi	21	7	4	10
Term placental villi	22	19	3	0
Hydatidiform mole	12	2	3	7
Invasive mole	14	1	4	9
Choriocarcinoma tissues	9	0	1	8
Cell lines	3	0	0	3

* (−), negative; (+), weakly positive; (++), strongly positive.

DISCUSSION

Telomeres, the ends of human chromosomes, seem to function in chromosome stabilization during replication by protecting the chromosomal ends against exonucleases and ligases. Consisting of several thousand copies of a repeat nucleotide sequence 5″-TTAGGG-3″. With each cycle of DNA replication, one end of the linear chromosome develops a short 8 – 12 bp (Rhyu et al., 1995). It has been proposed that telomere shortening may function as a "mitotic clock". Telomerase is a rionucleoprotein that synthesized telomeric DNA. Telomerase activity has been reported in several human tumor types. hTR is cloned as a template RNA of telomerase and is expressed in pre-crisis cell lines and non-neoplastic tissues, as well as in immortalized cell lines or tumor specimens and the expression level is not correlated with the level of telomerase activity (Feng et al., 1995).

In the present study, the relative level of hTR in human normal placental tissues as well as choriocarcinoma cell lines and tissues were determined by a factor of about 100 over simple PCR . It has been suggested that germline tissues and tumor cell lines expressed higher levels of hTR than the normal somatic cells and tissues (Feng et al., 1995), More recent studies had indicated that the regulation of hTR synthesis was not strictly correlated with that of the telomerase enzyme itself, and that expression of hTR in normal somatic cells that were telomerase negative had been reported (Feng et al., 1995, Avilion et al., 1996). Our results that hTR could be found in normal early pregnancy villi and term placental tissues accorded with previous observations of others.

It was known that trophoblastic cells from fetal villi can aggregate and invade maternal tissues during early pregnancy, and many features of this invasion of trophoblastic cells were not different from the invasion of tumor cells (Roberts et al., 1994, Song et al., 1988). Over-expression of certain oncogenes had been reported in malignant trophoblastic cells as well as in normal trophoblastic cells found in normal human placental tissues. This over-expression of certain oncogenes was highest in first trimester placental tissues and gradually decreased as gestational age increased (Park et al., 1992, Haidacher et al., 1995). Our data showed that telomerase activity was much higher in human early pregnant placental tissues, and became either very weak or undetectable when the placental tissues approached term. This differential expression of telomerase agreed with the notion that the telomerase activity is correlated with the proliferative activity of the trophoblastic cells and placental tissues. Telomerase activity were not detected in RNase pretreated all extracts of the cell lines and tumor tissues, because telomerase is an RNA-dependent DNA polymerase. Therefore, it have been suggested that inhibitor of telomerase by an antisense oligonucleotide that targent hTR might constitute a new class of anticancer drug for most types of cancer (Raymond et al.,1996, Iversen et al., 1995, Kanazawa et al., 1996)

It was postulated that activation of telomerase expression is necessary for overcomeing cellular senescence and that cancer cells must express telomerase to maintain their immortality (Kim et al., 1994). Of many different cancer types examined, an average of 85% exhibited telomerase activity, raising the possibility that telomerase can be used as an universal tumor marker for the identifying tumor cells. The finding that telomerase activity was very strong in choriocarcinoma tissues and cell lines may be useful for corroborative support for cytological and pathological examination for identifying malignant trophoblastic cells.

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References

Avilion, A. A., Piatyszek, M. A., Gupta, J., et al., 1996. Human telomerase RNA and telomerase activity in immortal cell lines and tumortissues. *Cancer Res.*, **56**: 645 - 650.

Feng, J. Funk, W. D., Wang, S. S., et al., 1995. The RNA component of human telomerase. *Science*, **269**: 1236 - 1241.

Roberts D. J., Mutter G., 1994. Advances in the molecular biology of gestational trophoblastic disease. *J Reproductive Medicine*, **39**: 201 - 208.

Song, H. Z., and Wu, P. C., 1988. Studies in Trophoblastic Diseases in China. Pergamon Press, Oxford, U. K. P. 3 - 27.

Greider, C. W., and Blackburn, E. H., 1985. Identification of a specific telomere terminal transferase activity in tetrahymena extracts. *Cell*, **43**: 405 - 413.

Haidacher, S., Blaschitz, A., Desoye, G. et al., 1995. Immunohistochemical evidence of P53 protein in human placenta and choriocarcinoma cell line. *Molecular Human Reprod*, **10**: 983 - 988.

Iversen. P. L., Pirruccello. S. J. Meta J. E. et al., 1995. Antisense oligonucleotides which demonstrate tumor specific action also inhibit telomerase in vitro. *Proc Am Assoc Cancer Res*. **36**:412.

Kanazawa, Y., Ohkawa, K., Ueda, K., et al., 1996. Hammerhead ribozyme-mediated inhibition of telomerase activity in extract of human hepatocellular carcinoma cells. *Biochemical and Biophysical Research Communications*, **225**: 570 - 576.

Kim, N. W., Piatyszek, M. A., Prowse, K. R., et al., 1994. Specific association of human telomerase activity with immortal cells and cancer. *Science*, **266**: 2011 - 2015.

Lange, T., 1994. Activation of telomerase in a human tumor. *Proc. Natl. Acad. Sci. USA*, **91**: 2882 - 2885.

Norton, J. C., and Piatyszek, M. A., 1996. Inhibition of human telomerase activity by peptide nucleic acids. *Nature Biotechnol*, **14**: 615 - 619.

Park, J. S., Namkoong, S. E., Lee, H. Y., et al., 1992 Expression and amplification of cellular oncogenes in human developing placenta and neoplastic trophoblastic tissue. *Asia-Oceania J. Obstet. Gynaecol.*, **18**: 57 - 64.

Raymond, E., Sun, D., Chen, S. F., et al., 1996 Agents that target telomerase and telomeres. *Current Opinion in Biotechnology*, **7**: 583 - 591.

Rhyu, M. S., 1995. Telomeres, telomerase, and immmortality. *J. Natl. Cancer Inst*. **87**: 884 - 894.