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Telomerase activity in human placenta cells

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Objective. The purpose of the study was to determine and characterize the expression, spreading and localization of telomerase-positive cells in normal human placenta at term.

Patients and methods. Fifteen placentas from healthy parturient women with the normal course of pregnancy and healthy newborn baby were investigated histologically and immunohistochemically by means of horseradish immunoperoxidase-labeled mouse monoclonal antibody against human telomerase reverse transcriptase (NCL-hTERT) (catalytic unit). Immunohistochemical staining was performed automatically in a DAKO TechMateÔ staining robot following the DAKO protocol for the biotin-streptavidin technique. Microscopic assessment was carried out using an MTV-3 digital image camera with an Olympus BH2-RFCA microscope.

Results. In normal human placenta, telomerase-positive cells were found in some endothelial cells, cytotrophoblast cells, and to less extent in syncytiotrophoblast, decidual and stromal cells. Some blood cells in mother blood and also in fetus blood exhibited different degrees of telomerase immunoreactivity. No telomerase-positive cells were found in the umbilical artery wall tissues.

Conclusion. The normal human placenta at term contains numerous telomerase-positive cells. Such cells usually are characterized by a high proliferative activity, implying that cells of a normal human placenta at term could be used as a source for primary cell cultures.

Key words: human placenta, telomerase activity, immunohistochemical staining

INTRODUCTION

Telomeres are the distal ends of human chromosomes, composed of tandem repeats of sequence TTAGGG (1) and associated proteins.

The possible functions of telomeres include stabilization of chromosome ends and prevention of their degradation, end-to-end fusion, rearrangement, and chromosome loss (2). This is essential for maintaining the integrity and stability of linear eukaryotic genomes. Telomere length regulation and maintenance contribute to normal human cellular aging and human diseases. Human telomeres undergo progressive shortening with cell division through replication-dependent sequence loss at DNA termini. The shortening of telomeres results in chromosomal instability, leading to cellular senescence. A possible cause for shortening of human telomeres is the repression of telomerase, a specialized ribonucleoprotein polymerase containing an integral RNA with a short template element that

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directs the synthesis of telomeric repeats at chromosome ends. Telomerase reactivation is thought to be essential for stabilizing telomere length to attain cellular immortality. The synthesis of telomeres is mainly achieved by the cellular reverse transcriptase telomerase, an RNA-dependent DNA polymerase that adds telomeric DNA to telomeres.

Telomerase is a specialized reverse transcriptase enzyme that elongates telomeres and synthesizes telomeric DNA. It is repressed in most adult human somatic cells. This results in telomere shortening with each cell division, leading to a process contributing to cell senescence. Human somatic cells have a finite proliferative capacity both *in vitro* and in vivo and enter a viable growth-arrested state. Activation of telomerase is important for cells to proliferate indefinitely (3). Strong telomerase activity was detected in human cancer cells (4) and weak telomerase activity in some normal somatic cells such as hematopoietic cells, epidermal keratinocytes, and cervical epithelial cells (5–7).

The ribonucleoprotein–telomerase complex, present in most cancer cell lines and in certain germline and stem cells, is a specialized reverse trancriptase that synthesizes telomeric repeats (8). Two essential components are present in human telomerase: an integral human telomerase RNA component (hTERC) on chromosome 3q 26.3, which serves as template for the synthesis of telomeric repeats, and protein subunit, human telomerase reverse transcriptase (hTERT) on 5p 15.33, which provides catalytic activity (9). hTERC is present in all cells, whereas the expression of hTERT is confined to cells that express telomerase activity (10). Reconstitution experiments, both *in vitro* and in vivo, strongly suggest that hTERT is the major determinant of human telomerase activity (11). Earlier we have found $CD34^+$ cells in human placenta (12). These cells could be considered as stem cells in adults. The aim of this study was to analyze the presence of telomerase-positive cells in normal human placenta.

PATIENTS AND METHODS

The study was conducted with human placentas. Permission to use human material was obtained from the local Ethical Committee prior to the study. Fresh human placentas were obtained immediately following spontaneous vaginal deliveries or Caesarean sections from the Opole Mother and Child Health Center, Opole, Poland.

Placenta specimens from 15 healthy parturient women with a normal course of pregnancy and healthy newborn baby were fixed in 10% neutral formalin, embedded in paraffin and processed for routine histological and immunohistochemical analysis.

Immunohistochemistry. The primary antibodies used were anti-human telomerase NCL-hTERT (catalytic unit) reverse transcriptase mouse monoclonal IgG2a, kappa (1:30, Novocastra Laboratories Ltd, Newcastle upon Tyne, UK).

Paraffin sections 5 μm thick were mounted on DAKO capillary slides (TechMate™, DAKO, Glostrup, Denmark), deparaffinized in xylene and rehydrated in graded ethanol series and 10 mM phosphate-buffered 0.9% saline, pH 7.4 (PBS). For antigen retrieval, the slides were placed into Antigen Retrieval Buffer™ (DAKO) and microwaved in 10 mM citrate buffer, pH 6.0, in a microwave processing labstation for histology, microMED T/T Mega Histoprocessing Labstation (Milestone Inc., Atlanta, USA), for 10 minutes at 98 °C according to the manufacturer's program, then cooled at room temperature for 30 minutes, washed in PBS and immunostained at 22 °C using the following protocol: 1) the primary antibody, diluted with DAKO ChemMate™ antibody diluent, for 1 hour; 2) secondary antibody containing biotinylated goat anti-mouse IgG antibodies (DAKO) for 30 minutes; 3) peroxidase block 3 times for 5 minutes; 4) peroxidase-conjugated streptavidin for 30 minutes; 5) HRP Substrate Buffer and 6) substrate solution containing 3,3'-diaminobenzidine tetrahydrochloride (ChemMate™ detection kit) for 15 minutes. Between each step, the sections were washed with DAKO ChemMate™ washing buffers three times and dried with absorbent pads.

Replacement of primary antibody with mouse or goat IgG diluted to the same concentration as the primary antibody were used as negative staining controls. After immunostaining, the sections were counterstained with haematoxylin or left without counterstaining, washed, dehydrated in ethanol series, cleared in xylene and mounted in a synthetic mounting medium (Mountex, Histolab Products AB, Gothenburg, Sweden).

Semi-quantitative microscopic assessment of immunohistochemical staining was performed under 400× magnification (high power field). For general histological evaluation the slides were stained with haematoxylin and eosin.

RESULTS

Staining of the samples with haematoxylin and eosin revealed a normal histological structure of the placentas under study. The expression of telomerase reactivity was found in some endothelial and much more frequently in cytotrophoblast cells (Fig. 1). In some interstitial and syncytiotrophoblast cells the reactivity of telomerase was also found, nevertheless, it was usually rather faint. Umbilical cord artery cells showed no telomerase reactivity (Fig. 2). In decidual cells, the reactivity of telomerase was either absent or varied from weak to mild (Fig. 3). Some blood cells both in mother and fetus blood exhibited different degree of telomerase immunoreactivity (Fig. 4).

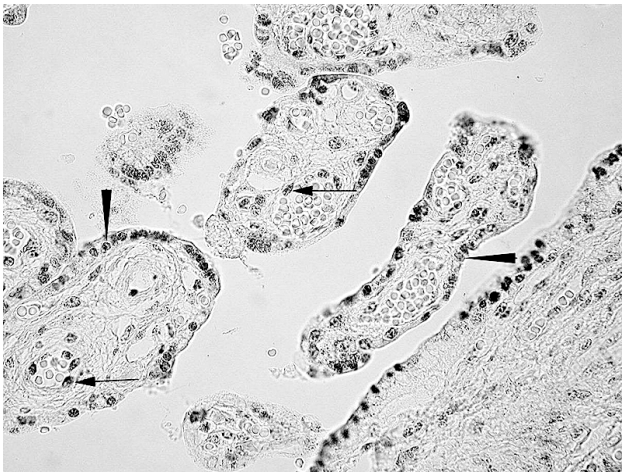

Fig. 1. Expression of telomerase immunoreactivity in the human placenta. In endothelial cells (arrows) and in cytotrophoblast cells (arrowheads). Original magnification × 200

Fig. 2. Lack of telomerase immunoreactivity in human umbilical cord artery. Arrowheads indicate the intima. Original magnification × 200

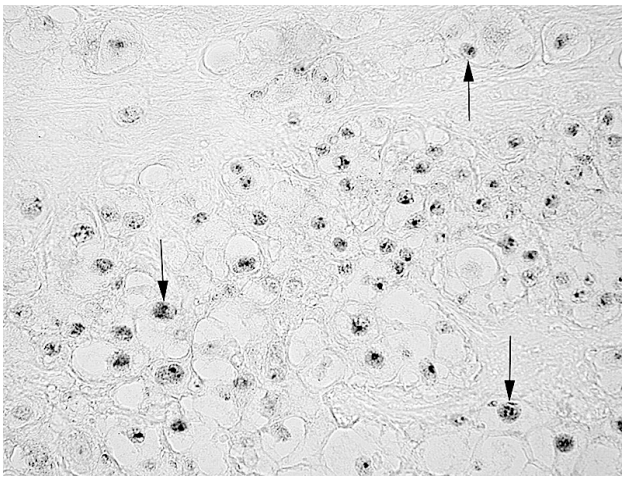

Fig. 3. Decidual telomerase-positive cells. Arrows indicate some cells with high immunoreactivity. Original magnification × 200

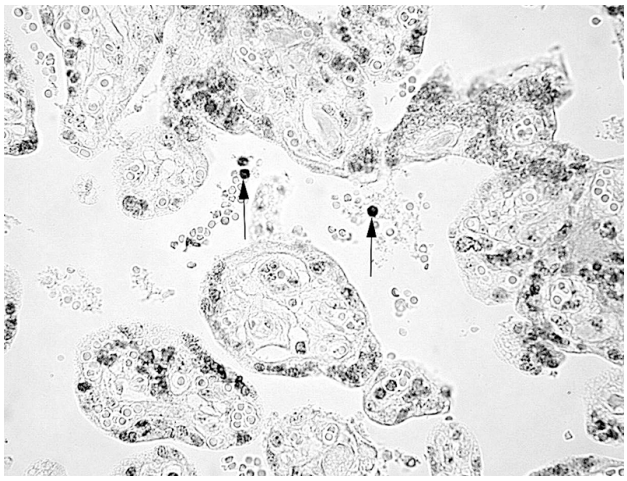

Fig. 4. Telomerase-positive cells in the blood. The arrows indicate some telomerase-positive cells in mothers blood. Original magnification × 200

DISCUSSION

It is known that normal somatic tissues are generally telomerase-negative, except for stem cells in renewing tissues. Transcriptional regulation of hTERT is believed to be the major mechanism of telomerase regulation in human cells. The highly ordered telomeric DNA-protein complex allows cells not only to distinguish telomeres from damaged DNAs and to protect them from degradation and fusion, but also to sense and control telomere homeostasis by regulating telomerase accessibility. The unique cellular reverse transcriptase is essential for maintaining telomere stability and is required for the proliferation of cells and/or cellular immortality in cancer or stem cells. During pregnancy, human trophoblast continues to proliferate and acts as a proliferating stem cell source for the development of chorion and formation of placenta (13). Chorionic development begins soon after implantation of the blastocyst. During invasion of the trophoblast into myometrium, the cytotrophoblast continues to proliferate. Significant telomerase activity was observed in early chorion at 5 to 9 weeks of gestation (13). Then the activity decreased, and only faint or no telomerase was found in term placentas (13). In our study, we observed a considerable activity of telomerase in many cells of human placentas, but not in all. This is consistent with other reports (14–18). The mechanisms of telomerase expression in the chorion are unclear. Probably the telomerase activity observed in the chorion may be related to the stem cell population of the trophoblast. Although the chorion is clearly distinguished from the embryo at early stages of gestation, stem cell population may be the common source of telomerase activity in both embryo and placenta tissues. Over the course of gestation, the chorion differentiates into mature terminal villi with a reduced proliferative capacity. Proliferation activity also gradually falls in the growing fetus. In this study, we found that the rate of telomerase-positive cells and the level of telomerase activity remained considerab-

ly high in human term placentas. In our previous work, we found presence of 34-positive cells in term human placentas (12). Since it is known that in human placenta telomerase expression correlates with proliferation capacity (13, 14), and that telomerase activity is expressed in stem cells in renewing tissues (19–21), it is possible to presume that at least part of CD34+ cells in human placenta express telomerase activity and maintain the capacity to proliferate. Telomerase activity may contribute to the maintenance of telomere length of stem cells with a high proliferative activity, which may in turn guarantee continuous and permanent growth. Stem cell biology is a very promising field with a lot of potential to generate new therapies (22). Further investigations are needed to elucidate if some human placenta cells could be considered as a potential candidate of a source of human adult stem cells.

CONCLUSION

The rate of telomerase-positive cells and the level of telomerase activity are rather high in human term placentas. Presumably, telomerase-positive cells still preserve their proliferation capacity.

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TELOMERAZËS AKTYVUMAS ÞMOGAUS PLACENTOJE

S a n t r a u k a

Imunohistochemiðkai iðtyrëme telomerazës teigiamø làsteliø pasiskirstymà þmogaus placentoje, kai buvo normalus nëðtumas ir gimë normalus kûdikis. Normalioje placentoje telomerazës ekspresijà nustatëme visø choriono gaureliø pavieniuose kapiliarø endoteliocituose bei citotrofoblasto làstelëse. Silpnesne telomerazës ekspresija pasiþymëjo ir pavienës decidualinës bei gaureliø stromos làstelës. Pavieniø telomerazës làsteliø aptikta ir motinos bei vaisiaus kraujyje. Telomerazës neigiamø làsteliø buvo virkðtelës arterijoje.

Tyrimo rezultatai rodo, kad subrendusioje placentoje iðlieka nemaþa telomerazës teigiamø làsteliø populiacija, tikriausiai dar pajëgi proliferuoti.

Raktaþodþiai: þmogaus placenta, telomerazë, imunohistochemija