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Telomeres and Telomerase Activity in the Human Placenta

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Abstract

Placenta is a transient organ ensuring the intrauterine development of the individual. To meet fetal requirements, rapid and continuous cell proliferation enlarges the areas of tissues maintaining maternofetal transport. The cell division in placenta is accompanied with shortening of telomeres leading to cell senescence. Telomerase activity, on the other hand, ensures replication of telomeres and allows the organ to serve till the end of pregnancy. This balanced process may be negatively influenced by unfavorable circumstances. Here, we summarize available data on telomere length as well as telomerase activity in placentas from normal and complicated pregnancies; attention is also paid to the comparison of methods used in relevant studies.

Keywords: pathology, placenta, pregnancy, telomerase, telomere

1. Introduction

The core of cell proliferation is the division of cells and replication of chromosomes. Among other factors, it is also regulated by the length of telomeres since short telomeres will either recruit telomerase, or, in the absence of telomerase, induce senescence, apoptosis, or genome instability, or activate a DNA damage response (e.g., telomere recombination). The main function of telomeres is the protection of chromosomal integrity during DNA replication; moreover, they themselves are protected by a shelterin protein complex. Telomeres stabilize the ends of linear chromosomes and prevent the ends from being recognized as a double strand break. In human cells, telomeres contain hexameric tandem repeats, 5'TTAGGG 3', of DNA sequence. To maintain the proliferative ability of cells, the elongation of telomeres is executed by adding telomeric DNA repeats to the 3' chromosomal ends by telomerase. In the absence of telomerase, the telomeres shorten in every cell division. Telomerase as an RNA-dependent DNA polymerase repairs the sequences of telomeres after each cell division; but in humans, this enzyme is active in stem cells, germ cells [1–3], and cancer cells only.

During development of an individual, mature oocytes and cleavage stage embryos display low or absent telomerase activity, whereas in the blastocyst stage, its activity is high again. As in cells during early cleavage, the telomeres become remarkably longer, and an alternate lengthening of telomeres may play a role in their elongation. Processes of telomeric DNA recombination between homologous sister chromatids take place in the cleavage stage, and the length of telomeres is then maintained from the blastocyst stage onward by telomerase [4]. Telomeric DNA recombination between telomeres on separate chromosomes such as gene

conversion and the elongation of telomeres by DNA polymerase activity by mechanisms like break-induced replication can lengthen telomeres independently of telomerase activity [5].

The mechanism of DNA replication results in progressive shortening of the ends of linear DNA molecule. That shortening limits the life span of individual cells and it is referred to as replication senescence [6]. Recently, it is well known that telomere attrition is observed during normal cellular aging, but telomere dysfunction may also contribute to the onset and progression of age-related diseases like atherosclerosis and myocardial infarction [7]. Telomere attrition is regarded as one of the so-called hallmarks of aging as proposed in [8].

Many tissues and organs contain cycling undifferentiated stem cells that provide cells for their renewal. The frequency of their mitotic division is different among tissues; examples of the most active are epidermal cells and cells of the bone marrow. Those cells are characterized by high levels of telomerase expression and disorders of telomere homeostasis cause, or at least take part in, the pathogenesis of serious inherited diseases, for example, dyskeratosis congenita [9], aplastic anemia, other bone marrow syndromes [10–12] and/or idiopathic pulmonary fibrosis [13]. Moreover, increased incidence of diabetes mellitus was identified in patients suffering from those pathologies [14]. Inherited bone marrow failure syndromes threaten also the prenatal development due to fetal malformations and intrauterine growth retardation accompanied with an abnormally small placenta [15].

The correct function of telomerase-telomere complex depends on both genetic predispositions and external factors (age, reactive oxygen species, and exogenous genotoxic factors). Mutations in the telomerase holoenzyme in either of the two genes, *TERT* encoding the reverse transcriptase, or *TERC* encoding the RNA template for the synthesis of telomeres by telomerase, can cause remarkable telomere attrition even in hemizygous individuals [16] and may take part in hereditary conditioned telomere disorders [17–20].

2. Placenta in pregnancy

The prenatal development of an individual is conditioned by placenta, the transient organ that functions exclusively for the time of pregnancy. In order to meet fetal requirements, the placenta holds the functions of still undeveloped fetal organs, for example, lung and kidney. It is the site of transport of oxygen, ions, nutrients, and maternal immunoglobulins from mother to fetus, and carbon dioxide and wastes from fetus to mother. The placenta also maintains pregnancy by production of steroid and protein hormones and other factors. Due to its position between maternal and fetal bloodstreams, the placenta acts as a barrier against infectious agents and regulates the maternal immune tolerance, gas exchange, and fetal nutrition. On the other hand, its structure and function are negatively impacted by maternal and fetal metabolic disturbances in pathological pregnancies.

Placental tissues originate in extraembryonic structures, that is in trophoblast, the outer layer of the blastocyst, which invades maternal tissues and gives rise to the cytotrophoblast and syncytiotrophoblast, and in extraembryonic mesoderm that is requisite for the formation of placental vasculature and supporting connective tissue.

The progress of fetal growth and maturation of fetal organs is essentially accompanied by the growth of placental size. It is performed by continuous cell proliferation till the term of gestation [21] and balanced with differentiation and apoptosis in all tissue compartments. The weight of term placenta is 500–600 g, the estimated surface area of syncytiotrophoblast available for maternofetal transport is 11–13 m², and the inner fetal capillary surface area is about 12 m² [22].

Human placenta is formed by chorionic plate and its repeatedly branched projections, chorionic villi, that are immersed in maternal blood circulating in the intervillous space. The growth of villi goes hand in hand with fetal growth. They develop into various types during pregnancy, and their classification depends on size and structural features. Basically, each villus is covered by the layer of trophoblast consisting of continuous cytoplasmic mass with numerous nuclei, that is, syncytiotrophoblast, and cuboidal cells of cytotrophoblast appearing as a continuous layer underneath syncytiotrophoblast in early gestation, but sparsely spread in term placenta. As nuclei in syncytiotrophoblast do not divide, cytotrophoblast cells play the role of stem cells of trophoblast. They undergo mitotic division, and their fusion with syncytiotrophoblast enlarges its mass. As shown by detection of cell cycle markers (e.g., Ki67, PCNA), they display proliferative potential over the duration of pregnancy [23–25].

The other source of placental tissues is extraembryonic mesoderm. For the placental development, this cell population gives rise to the mesenchyme adjoining villous trophoblast. Mesenchymal derivatives, that is, cells of connective tissue, endothelial cells, pericytes, and smooth muscle cells then form villous stroma and vascular bed. It is obvious that they follow the growth of trophoblast, and their proliferation and subsequent differentiation contribute to the formation of new functionally efficient villi, and thus to the enlargement of the organ and its functional capacity. It is significant particularly in the third trimester when the rapid development of terminal villi accompanies the rapid enlargement of fetal size [22]. Previous studies have demonstrated the proliferative potential of cells in villous vascular bed and stroma in early pregnancy as well as at term [24, 25]. The proliferative potential of cells in terminal villi of normal human term placenta is also demonstrated in **Figure 1**.

In order to provide nutrition for the increasing metabolic requirements of growing fetus, the placenta displays continuous cell proliferation during its entire existence. Despite the fact that fetal demands attain their maximal level at term, it is expected that cells in placenta at term decrease their proliferative capacity due to telomere shortening and undergo senescence similar to somatic cells in other organs. It is also supposed that those changes in telomere length might take part in the initiation of parturition [26]. Telomere shortening during pregnancy was found also in the placenta of mice [27]. As shown in another study in mouse, the proportion of representative short telomeres, that is, 3- and 5-kb telomere fragments,

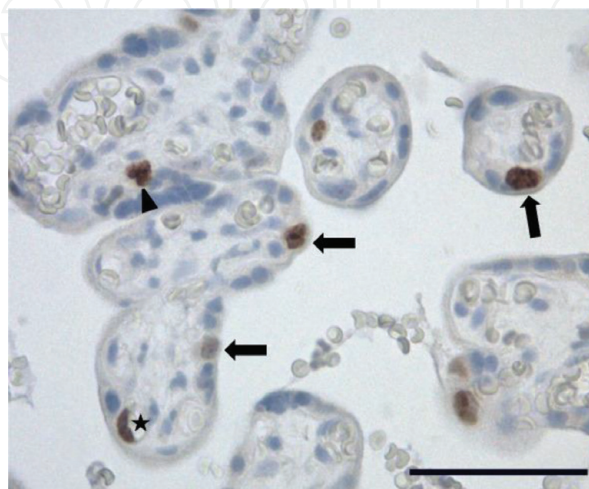


Figure 1.
 Proliferative activity of cytotrophoblast (arrows), stromal cell (arrowhead), and capillary endothelium (asterisk) in terminal villi of normal term placenta demonstrated by Ki-67 immunohistochemistry.
 Bar = 50 μ m.

significantly increases toward the end of pregnancy. The authors conclude that the quantity of representative short telomeres increases prior to parturition and takes part in the mediation of cellular aging in the placenta, finally leading to parturition [26]. Those findings are consistent with other studies regarding the impact of short telomere rather than average telomere length [28].

Due to its location in maternal uterus, the placenta is influenced by any abnormal metabolic conditions in both mother and fetus. The negative influence demonstrates itself in pathological structural features as well as in impaired function. In such organ displaying mitotic activity, altered telomere homeostasis may take part in those adverse changes. Here, we summarize data on telomere length and telomerase activity in placentas from normal pregnancies and pregnancies complicated by metabolic disturbances threatening pregnancy outcome, as are maternal diabetes mellitus, preeclampsia, and intrauterine growth restriction.

3. Analysis of telomere homeostasis—methodological approaches

In view of the facts mentioned above, telomere length emerged as a promising marker generally in medicine, but the utility of such measurements highly depends on valid methodologies [7]. The average telomere length is highly variable among different cell types and among different individuals. There are also gender differences, which could be detected at birth. Additionally, the average telomere length declines with age [16].

Telomeres may be repaired either by the enzyme telomerase using its RNA template or by recombination. The methodologies examining telomere homeostasis in different tissues are therefore based not only on the determination of the length of telomeric DNA sequences, but they examine also the status and expression of the reverse transcriptase (*hTERT*) and the RNA template (*hTERC*) genes and recombination events on selected telomeres. In the following paragraphs and in **Table 1**, we summarize the main features and limitations of methodologies employed in placenta research, alternatively the methods applicable on human samples.

3.1 Methods examining telomeric DNA sequences

Terminal restriction fragment (TRF) analysis was originally developed to determine the lengths of telomeres in 1988 by Moyzis et al. [54]. Genomic DNA is isolated and then digested with a mixture of restriction enzymes that are selected to avoid the cutting of telomeric and subtelomeric sequences. The DNA fragments obtained after such a digestion are separated in agarose gel and hybridized with the probe containing telomeric sequences [55]. The need for high amounts of highly integral DNA samples represents one of the main limitations of this methodology [7].

Polymerase chain reaction (PCR)-based methodologies were elaborated to overcome limits of the previous method. Cawthon [56] reported the technique based on quantitative PCR (qPCR). The sequences of primer pairs proposed by Cawthon avoid the primer dimer formation which frequently occurs when repetitive sequences are amplified. Cawthon's qPCR technique and its subsequent modifications [56–58] belong to the most frequently used methods. Telomere length is quantified relatively—the ratio T/S between the quantity of repetitive telomeric sequences (T) and the quantity of sequences representing a single copy gene (S) is calculated. These ratios correlated very well with the absolute telomere lengths measured by TRF method in Cawthon's original study [56]. An attempt to standardize this methodology over large spectrum of laboratories was made [59].

Method	References
Telomere length analysis based on isolated DNA	
TRF	[29–31]
STELA	[32–34]
Q-PCR, T/S ratio	[29, 35–38]
Telomere length analysis based on examination of interphase nuclei	
Interphase Q-FISH	[36, 39–45]
Determination of gene copy numbers in situ	
hTERT-FISH	[36]
hTERC-FISH	[36, 39, 41, 46]
hTERT expression	
Immunohistochemistry	[43, 45, 47]
RT-PCR for hTERT	[29, 41, 48–51]
hTERC expression	
RT-PCR for hTERC	[29]
Telomerase activity determination	
TRAP	[33, 38, 48, 52, 53]
TRAP <i>in situ</i>	[52, 53]
Genome-wide technologies	
Illumina methylation array	[37]

Table 1.
Experimental methods used in placenta research.

O’Callaghan and Fenech [60] described the modification of qPCR-based technique allowing the determination of an absolute telomere length due to the use of artificially synthesized DNA standard containing the defined number of telomeric repeats.

PCR-based techniques are quite popular among researchers because they require lower DNA quantity and usual laboratory equipment. Due to their relatively low costs, they may be employed in high-throughput epidemiologic studies. One short-coming of PCR-based methodologies is that the resulting values inform only about the average telomere length in the specimens.

Single telomere length analysis (STELA) targets telomeric DNA from a single chromosome using primers specific to subtelomeric sequences [61]. All individual chromosomal subtelomeric regions do not contain suitable specific sequences; therefore, the method is able to examine only a limited set of chromosomes [7]. Using this methodological approach, Garcia-Martin et al. [32] found considerable intra-sample variability in examined placentas.

Quantitative fluorescence in situ hybridization (Q-FISH)-based techniques are focused more on the work with cells than with isolated DNA. A fluorescent probe, mostly peptide nucleic acid (PNA) probe, complementary to telomeric repetitive sequences is hybridized to denatured DNA of metaphase chromosomes or interphase nuclei. The cells may be fresh, frozen, or formaldehyde-fixed and paraffin-embedded. The application of *metaphase Q-FISH*, which was developed in 1996 by Lansdorp and colleagues [62], results in the estimation of the length of all 92 telomeres in human cells. It allows the detection of telomere free ends. The most serious limitation of this approach is the requirement of metaphases from mitotically active cells. The method is labor intensive [7, 62].

Interphase Q-FISH, first described in 1998 by de Pauw and colleagues [63], is applicable on nondividing cells. It compares the fluorescent signals obtained after hybridization with a telomere-specific probe and with a probe targeting a single copy gene. Its results inform, similar to the results of qPCR methodologies, about the average length of the telomeres in examined cell, the method is not able to recognize each individual telomere as metaphase Q-FISH.

A method for telomere capture evaluation based on interphase Q-FISH has been established by Amiel et al. [64]. When telomeres shorten to the critical length, repair pathways are activated. In the process of telomere capture, a critically short telomere obtains a new telomeric sequence from another chromosomal end. In the original method, the number of fluorescent signals for a single copy gene, SNRP, which is localized on chromosome 13, was compared with the number of signals for 15qter region of this chromosome [64] to follow not only random aneuploidy but also telomere capture or translocation of telomere. The methodology was also used in placenta research [39, 48].

The *pq-ratio assay* described in 2001 by Perrem et al. [40] belongs to methods that directly examine telomere recombination. This assay measures the variation in telomere lengths at the p and q arms of a chromosome. The telomere ratio for most chromosomes is expected to be $q/p \sim 1$, because the telomeres at both ends of a given chromosome shorten at a similar rate. If recombination is used to maintain the ends, then it could alter the length of at least one telomere by a random amount of telomeric repeats. This results in variable values for the pq-ratio. The pq-ratio assay is very sensitive, and the data may become biased as the telomeres shorten. Small changes on a short telomere may be overrepresented and telomeres with no signal will not be represented at all [40, 65, 66].

Chromosome orientation-FISH (CO-FISH) was first described in 1993 [67, 68] as a method for strand-specific FISH. The method is dependent on cultivation of analyzed cells because it requires incorporation of bromodeoxyuridine (BrdU) into newly synthesized strands. This step allows subsequent enzymatic removal of BrdU containing strands after their damage caused by UV light in the presence of the dye Hoechst 33258. The remaining strands then serve as single-stranded targets for FISH. This approach allows differentiation between the telomeres produced via leading- or via lagging-strand DNA synthesis, and it enables the study of sister chromatid exchanges (SCE) and inversions in telomeric regions [69, 70].

The methodology of *directional genomic hybridization (dGH)* represents the cytogenomic extension of strand-specific hybridization. *Telo-dGH* recognizes terminal exchange events—terminal inversions and generally different forms of genetic recombination occurring near the telomeres, namely sister chromatid exchange (SCE) [71].

Flow-FISH represents the modification of interphase Q-FISH, which was introduced in 1998 by Hultdin et al. [72]. The cells in suspension are hybridized with fluorescent probes and then examined using flow cytometry. This approach is technically very demanding because the unfixed cells are often fragile and clustering. The technique is very sensitive to preservation of cells. FISH probes may also have affinity to cytoplasmic structures [7]. Higher numbers of cells are needed (typically $> 1 \times 10^5$), the assay determines mean telomere lengths and it does not account for aneuploidy or SFE (signal free ends) but the methodology was successfully used in numerous studies [73–75].

TELI-FISH is a combined FISH/immunofluorescence method which was developed in 2002 to assess human telomere lengths from standard formalin-fixed paraffin-embedded tissues. Combination with immunostaining allows the simultaneous identification of specific cell types. The assay requires very few cells (10–15).

Validation showed excellent agreement with the commonly used TRF method based on Southern blotting [76].

Microdissection followed by qPCR allows selection of cells for analysis using classical histological technique and it is suitable for the studies based on archival material [35]. Microdissected cells may be examined by qPCR methodology, and therefore, its main disadvantage is that it provides information only about the relative average length of telomeres in analyzed samples. The methodology does not result in the determination of the lengths of the longest or the shortest telomeres contained in the sample. Comparison of T/S ratios related to a reference sample allows evaluation of differences between the groups of samples (for instance between healthy controls and patients).

Whole genome sequencing (WGS) captures sequence information from the entire genome, including the telomeres, and is increasingly being applied in research and in the clinic. In 2014, Ding et al. [77] demonstrated a novel method, TelSeq, which allows measurement of average telomere length by using whole genome or exome sequencing data. It was the first study that evaluated in detail the relationship between the frequency of telomere repeats and telomere length. With the potential to be a relatively high-throughput method, this may overtake qPCR as the method of choice in future studies. Their study was the first computational method that had been validated against an established experimental method (Southern blot measurements of the mean length of terminal restriction fragments).

3.2 Methods examining genes coding for telomerase and its RNA components, their expression, and telomerase activity

The telomere length is closely associated with telomerase (human telomerase reverse transcriptase—hTERT) activity (TA) and the availability of its RNA component (hTERC or TERRA—telomeric repeat-containing RNA) in tissues.

The copy number of both genes *hTERT* and *hTERC* in nuclei in archive tissues or cultivated cells is examined by *FISH methodology*. This approach was applied also in placenta research [36, 41, 46, 48]. *RNA-FISH* is based on the use of fluorescently labeled probes and allows the cellular localization of TERC [78].

The presence of telomerase itself may be detected in tissues by means of *immunohistochemistry*—a methodology which is generally well established in laboratories of pathologists [76]. This methodology was also widely used in placenta research—see reference in **Table 1**.

In **Table 1**, the studies that examined the expression of *hTERT* using *reverse transcription and subsequent qPCR* to quantify the amount of *hTERT* transcripts are also summarized.

The telomerase activity may be measured by a wide panel of methodologies based on addition of telomerase substrate and detection of amplified telomerase products by *telomere repeat amplification protocols*—*TRAPs*—developed by Kim et al. [79]. Recently existing numerous methodological modifications of this approach were reviewed by Mensa et al. [78].

hTERC (TERRA) is a long noncoding RNA, which can be transcribed from nearly all telomeres in mammalian cells because its transcription starts from their subtelomeric regions. Therefore, the quantity of such transcripts can be measured by *RT-qPCR* starting from RNA isolated from analyzed cells or tissues followed by reverse transcription and PCR with chromosome-specific primers. Molecular mechanisms associated with the role of TERC in telomere reparation are intensively studied [80, 81].

Northern blotting or *RNA dot blotting* needs mostly radiolabeled probes and cannot detect the minor changes in quantity of TERC [78].

Detection of G4 quadruplexes using antibodies is based on the fact that during hTERC transcription, the RNA:DNA hybrids at the chromosome ends are formed, and they can fold into G-quadruplexes [78]. The G-quadruplex structure formed by telomere DNA plays also an important role in the regulation of the telomerase reaction [82].

3.3 Methods examining epigenetic changes and chromatin structure

Not only the presence of DNA sequences of genes *hTERT* and *hTERC* but also their functional state determined epigenetically plays a crucial role in the regulation of their expression.

Wilson et al. [37] used *array technology* (Illumina Infinium Human Methylation 450 BeadChip) to study methylation alterations in genes *hTERT*, *DNMT1*, and *DNMT3A* in human placentas. *MALDI-TOF technology* was employed to determine the level of DNA methylation of *hTERC* in placenta using Sequenom EpiTyper platform [29]. Lower levels of gene methylation were found in normal placentas compared with other somatic cells.

Quantitative Telomeric Chromatin Isolation Protocol (QTIP) was introduced in 2013 by Grolimund et al. [83]. It allows the comprehensive determination of telomere protein composition and the quantitative comparison of telomere protein compositions between cells with different telomeric states. Chromatin is cross-linked, immunopurified, and analyzed by mass spectrometry. The methodology may be also adapted for examination of other chromatin regions within the genome [83].

4. Placenta in normal pregnancy

The assessment of telomere length in normal pregnancy gives important data regarding dynamics of placental cellular proliferation. The study on third trimester placentas using the qPCR has shown decreased telomere length between gestational weeks 28 and 42 (13.98–10.56 kbp) [38]. The application of qPCR and Southern blot-based terminal restriction fragment (TRF) assay confirmed considerably longer telomeres in first trimester villi than in term placentas, telomeres of which were found to be longer than those in cord blood mononuclear cells [29, 84].

Some authors took into consideration that the position of villous tissue in the placenta may influence the telomere length, and therefore collected and processed samples of the whole placenta from more locations [29, 37, 84]; nevertheless, no site-specific differences of telomere length were determined except [84] showing that the telomerase activity was detected in term placenta restricted to biopsy sites near umbilical cord only. Moreover, the Southern blot-based TRF assay discovered longer telomere length in placental samples than in cord blood cells [84]. The study by qPCR proved that telomeres in placentas of female fetuses are longer than in placentas of male fetuses at the same gestational week. This finding suggests an influence of hormonal milieu during intrauterine development [37]. On the other hand, the other study performed by single telomere length analysis (STELA) revealed neither influence of fetal sex nor influence of the mode of delivery [32].

The above-mentioned findings in chorionic villi and normal placenta suggest that telomere length is maintained by active telomerase during pregnancy. Nevertheless, the papers dealing with this topic present equivocal results. Using TRAP assay, Wright et al. [33] found no detectable telomerase activity in placenta. To the contrary, telomerase activity studied by TRAP assay and *in situ* TRAP assay was found in both chorionic

villi at 5–14 weeks of gestation and normal placentas at 23–42 weeks of gestation [49]. Expression of telomerase protein was detected by immunohistochemistry in cytotrophoblast of chorionic villi [47, 85]. Decreasing relative telomerase activity was shown in comparison of chorionic villi in first, second and third trimester. In normal villi from 6 to 40 gestational weeks, the presence of telomere RNA component (TERC) was demonstrated, whereas telomerase reverse transcriptase (TERT) was not found in samples from second and third trimesters [86]. Immunohistochemical reaction revealed telomerase expression in trophoblast and stroma of villi in 10th week, but not in term placenta [87]. Using RT-PCR method, the hTERT-RNA expression was found in normal chorionic villi from 6th to 10th gestational week as well as in normal placentas from 12th to 41st gestational week, whereas hTERT protein expression was found in chorionic villi, but only in the fourth part of placental samples [88].

5. Placenta in pregnancy pathologies

It is evident that normal placental growth and development carried out by cell proliferation is conditioned by appropriate telomere length ensured by homeostasis of telomerase system. For optimal course of those processes, normal metabolic milieu is necessary in mother, placenta, and fetus. The most critical condition of intrauterine development is adequate oxygen supply. The early pregnancy phase, that is, first 10 weeks, runs under low oxygen levels. This relative hypoxia before the constitution of the fetoplacental and uteroplacental blood circulation induces various factors, one being the hypoxia-inducible factor 1, that upregulates hTERT expression (and telomerase activity), and its decrease with gestational age is in correlation with decrease of telomerase activity logically followed by telomere shortening [88]. As the placenta consumes about 40% of the oxygen supplied to fetoplacental unit [89], the hypoxic conditions have negative impact on all processes running there. And hypoxia also represents a key factor in genesis of pregnancy pathologies discussed in the following parts of this chapter.

5.1 Maternal diabetes mellitus

There are two main forms of maternal diabetes mellitus, the insulin-dependent form with onset before conception, and gestational diabetes diagnosed usually in second half of pregnancy that disappears after birth. In the insulin-dependent type diabetes, an autoimmune process destroys β -cells of the islets of Langerhans completely and the patient is then treated by insulin supplementation.

As shown in experiments with mice, the type 2 diabetes, and similarly gestational diabetes, may involve telomere shortening during pathogenesis. Shorter telomeres are associated with impaired β -cell regeneration, impaired glucose-stimulated insulin secretion by disorders of insulin release leading to impaired glucose tolerance as well as to increased β -cell senescence [14, 90].

Both forms of maternal diabetes are characterized by maternal hyperglycemia and thus higher amount of glucose transported to fetus. Metabolic complications derived from hyperglycemia threaten the mother; manifest themselves in placental structure and function; have negative influence on fetal well-being, perinatal morbidity and mortality; and long-lasting effect on the postnatal life of the individual. In pregnancies complicated by maternal diabetes mellitus, the alterations of placental structure and function as well as the pregnancy outcome depend on the quality of metabolic control. Placentas from poorly controlled diabetes are larger and heavier and microscopic picture shows disturbances of villous maturation [22]. The oxidative stress produced by imbalance of glucose and oxygen supply

in fetoplacental unit is compensated by enhanced placental angiogenesis demonstrated by higher villous capillary branching [91]. It is possible to suppose that both, the higher placental weight and enhanced angiogenesis, are a consequence of escalated cell proliferation leading to exhaustion of telomeres available for mitotic division.

Regarding the telomere length in placenta from pregnancies complicated by maternal diabetes, the available data are not quite consistent. In the qPCR-based study examining large cohort of placentas, maternal diabetes was found associated with longer telomere length [38]. In another study comparing telomere length in cytotrophoblast of placentas from poorly controlled maternal diabetes and normal pregnancies by FISH method, the result indicated shorter telomeres in the diabetic group [42]. In the same groups of patients, the immunohistochemically identified telomerase expression, the expression of mRNA for hTERT, and the expression of *TERC* gene copy number were lower in diabetic placentas [41]. On the other hand, no difference of mean telomere length was found in peripheral villi of normal placentas and placentas in well-controlled maternal diabetes examined by laser capture microdissection and qPCR [35], although the study on the same placental material discovered lower proliferative potential of cytotrophoblast and vascular endothelium of terminal villi in maternal diabetes [25]. Single telomere length analysis (STELA) was used for measurement of telomere length in normal placentas and placentas from gestational diabetes treated either by lifestyle intervention or by metformin or insulin therapy. The result showed that the therapy by metformin or insulin protected from telomere shortening in placentas of male fetuses [34].

5.2 Preeclampsia

Preeclampsia is a disease of pregnancy characterized by new-onset maternal hypertension and proteinuria. It may begin in 28–34 gestational weeks (early-onset preeclampsia) or after 34 weeks (late-onset preeclampsia). It is commonly accepted that the preeclampsia originates in deficient placentation, that is, decreased invasion of the maternal tissues by extravillous trophoblast. Under normal conditions, the endothelium and smooth muscle cells of uterine spiral arterioles are replaced with trophoblast and their diameter becomes wide allowing delivery of blood at low pressure to the intervillous space. The decreased trophoblastic invasion produces narrow uteroplacental arteries and the resulting malperfusion of the intervillous space causes oxidative stress of the fetoplacental unit manifested among others by reduced development of the villous tree and placental growth retardation. Increased placental proliferative activity found using detection of PCNA and Ki67 in preeclampsia may be a sign of increased cell turnover [92]. The associated systemic vascular inflammation in maternal organism may cause injury of multiple organs.

In placentas in preeclampsia, the analysis of villous cytotrophoblast based on the quantitative FISH method showed shorter telomeres, more end-to-end telomere aggregates, and abnormal *TERC* gene copy number as well as decreased hTERT expression detected by immunohistochemistry [39, 43, 44]. Common expression of hTERT protein and HIF-1 α in term preeclamptic placenta gives an evidence of response to hypoxia by telomerase upregulation [88]. Nevertheless, the measurement of average telomere length by qPCR did not show significant differences between control, early-onset preeclamptic, and later-onset preeclamptic placentas [37]. Another study has also shown no differences of telomere length between normal placentas and placentas in preeclampsia [30]. Enhanced levels of placental hTERT-mRNA in preeclampsia [50] if not associated with longer telomeres as a result of enhanced telomerase activity suggests that there is a conceivable disturbance in translation or post-translation processes of the enzyme protein.

6. Intrauterine growth restriction

In intrauterine growth restriction (IUGR), the growth and development of fetus is delayed by 3–4 weeks regarding the gestational age and the birth weight is low, under 10th percentile for gestational age. Fetal growth restriction is associated with restricted placental size caused by arrest mechanisms reducing cell proliferation [45]. There are two types of IUGR, symmetric and asymmetric. The fetus displaying symmetric IUGR has normal body proportion, the fat and muscle tissue are reduced. It is usually associated with genetic factors causing, for example, already mentioned bone marrow syndromes [9–12] or infections. The asymmetric IUGR is characterized by normal size of head and reduced chest and abdominal circumference due to reduced fat and muscle tissue. It is often associated with placental insufficiency arising, for example, in preeclampsia and may be related to oxidative stress.

Studies performed by quantitative FISH method and RT-PCR on placental cytotrophoblast in IUGR gave an evidence of shorter telomeres, lower telomerase activity, decreased hTERT mRNA, and decreased *TERC* gene copy number [45, 46, 48]. The relative telomere length and hTERT expression were found lower in cytotrophoblast of placentas in IUGR as well as in IUGR combined with preeclampsia [43].

Lower proliferative potential found in placenta in IUGR [52, 70, 76, 93] seems to be consistent with decreased telomerase activity in cytotrophoblast of IUGR placenta [52, 76]. In placenta associated with asymmetric IUGR, only weak, if any, telomerase activity, hTERT expression, and copy numbers of telomerase reverse transcriptase were found by qPCR and in situ TRAP assay [49, 51, 53]. Shorter telomeres associated with higher expression of cell senescence markers were found in placenta samples in IUGR [31] and shorter telomeres detected by quantitative FISH technique and reduced average telomere length detected by qPCR were shown in [36]. To the contrary, no difference was revealed in average telomere length assessed by qPCR between normal placenta and placenta in IUGR [37].

7. Conclusions

Appropriate fetal growth and development is conditioned by appropriate placental growth and development. It is accomplished by balanced cell proliferation, differentiation, and apoptosis. Cell proliferation is influenced by the length of telomeric sequences of chromosomes and their elongation due to telomerase activity. This review article summarizes available data on telomere length and telomerase activity of placenta in pregnancy-complicating situations, that is, maternal diabetes mellitus, preeclampsia, and intrauterine growth restriction (**Table 2**) as well as methods used for this research (**Table 1**).

In normal placentas, longer telomeres and higher telomerase activity were found in early pregnancy, they gradually decreased till the term. Although the available studies on placentas from pregnancies complicated by maternal diabetes are not numerous, their results corroborated by experimental studies suggest that diabetic metabolic conditions contribute to telomere shortening and that the appropriate metabolic control achieved by adequate treatment may function as a prevention of this adverse process. The results of research on telomere length and telomerase activity in preeclampsia are still equivocal and rather suggest debatable comparability of methods applied in those studies. The telomere length in placenta associated with IUGR was found lower and accompanied with decreased expression and activity of components of telomerase apparatus.

Diagnosis	Telomere length	Telomerase activity
Normal placenta	Telomere length is reduced in the course of pregnancy; placental telomere length is not site-specific [32, 33, 37, 38, 84]	hTERT expression and telomerase activity were found [47, 49, 84, 85]; telomerase activity decreases in the course of pregnancy [86–88]
Placenta associated with maternal diabetes mellitus	Maternal diabetes is associated with longer placental telomeres [38]; there is no difference in placental telomere length in well-controlled diabetes [34, 35]; telomeres are shorter in placentas from poorly controlled diabetes [42]	Lower expression of hTERT, hTERT-mRNA, lower <i>TERC</i> copy number in placentas from poorly controlled diabetes [41]
Placenta associated with preeclampsia	No differences of telomere length between normal placentas and placentas in preeclampsia [37, 84]; shorter telomeres, more end-to-end telomere aggregates, higher telomere aggregates count [43, 44]	Lower expression of hTERT [44], abnormal <i>TERC</i> copy gene number [39]; higher expression of hTERT mRNA [50]
Placenta associated with IUGR	Shorter telomeres [31, 36, 45]; no difference in telomere length [37]	Lower expression of hTERT, hTERT-mRNA, lower <i>TERC</i> copy number [46, 48, 51–53]

Table 2.
Summarized data on telomere length and telomerase activity in normal placenta and placenta in case of maternal diabetes mellitus, preeclampsia, and IUGR.

Maternal diabetes mellitus, preeclampsia as well as IUGR do not only complicate pregnancy but are also taken as causes of adverse outcomes for individuals later in postnatal period. Further investigation of the effect of factors influencing telomere length and telomerase activity may contribute to better understanding of those links. The placental DNA is identical to the DNA of fetal cells and the period of its existence equal to the prenatal period of the fetus spent in the same maternal environment. As telomeres are susceptible to external conditions of maternal milieu (i.e., oxidative stress, reactive oxygen species, exogenous genotoxic insults), those epigenetic influences may accelerate their shortening [94]. Prospective studies in experimental models and of course in long-time prospective studies in human should elucidate if there is a relation of final telomere length in placentas at term and newborn and if the measurement of placental telomere length could have a predictive potential for individual.

Recently, the immunomodulatory role of telomeric sequences was recognized [78, 95]. Telomeric sequences originated from trophoblasts may circulate in the pool of cell-free DNA in maternal plasma and contribute to timing of parturition [26] by stimulation of maternal immune response against placenta. The role of telomeric sequences contained in cell-free DNA in plasma of healthy persons in the regulation of immune system performance was also described [95]. Additionally, the telomeric sequences were found also in cytoplasm where they regulate inflammatory response via their interaction with TLR9 receptor [78].

In view of all these facts, the study of telomeres and their homeostasis in placenta seems to be crucial for the understanding of pathogenesis in the broad spectrum of pregnancy complications.

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Conflict of interest

There are no conflicts of interest.

Abbreviations

FISH	fluorescence in situ hybridization
IUGR	intrauterine growth restriction
HIF	hypoxia induced factor
hTERT	human telomere reverse transcriptase = catalytic component of telomerase activity
IUGR	intrauterine growth retardation
qPCR	quantitative polymerase chain reaction
RT-PCR	reverse transcriptase polymerase chain reaction
SFE	signal free ends
<i>TERC</i>	telomerase RNA component gene
<i>TERT</i>	telomerase catalytic component gene
TRAP	telomeric repeat amplification protocol
TRF	terminal restriction fragment

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