Placenta 55 (2017) 1–4

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Short communication

Relative amount of telomeric sequences in terminal villi does not differ between normal term placentas and placentas from patients with well controlled type 1 diabetes mellitus

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ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form
5 April 2017
Accepted 20 April 2017

Keywords:
Diabetes
Chorionic villi
Pregnancy
qPCR
Telomere sequence
Telomere/single copy gene ratio

ABSTRACT

We applied qPCR in order to compare relative telomere length in terminal villi microdissected from term control placentas and placentas of patients suffering from type 1 diabetes. Significant differences were found in the relative T/S ratios neither between placental groups nor between the diabetic placentas affected and non-affected with chorangiosis. We hypothesize that there is no relationship between decreased placental proliferative ability in maternal diabetes type 1 and telomere shortening.

1. Introduction

In third trimester, the continual placental growth is characterized by rapid development of terminal villi. The ability of placenta to enlarge areas of villous tissues involved in transport, i.e. syncytiotrophoblast and capillaries, continues till the end of pregnancy. However, the proliferative potential of cytotrophoblast and cells of capillary wall in terminal villi of placentas in diabetes mellitus was found significantly lower [1]. As maternal diabetes is associated with enhanced placental terminal villous volume and capillary bed [2–4], it seems that the proliferative potential could be in some extend exhausted due to enhanced cell proliferation.

In proliferating cells, telomeres are shortened with each cell division. In placenta, shortening of telomeres apparently represents a mechanism of placental senescence contributing to the timing of parturition [5]. Wilson et al. [6] observed longer telomeres in placentas of female fetuses compared to placentas of male fetuses. In placentas from pregnancies complicated by IUGR, both normal telomere length [6] and telomere shortening [7–9] were found. Both shorter [10] and intact telomere length [11] were also shown in placentas in preeclampsia. In maternal diabetes, shorter [12] telomeres were shown.

Based on our previous findings [1], here we tested the hypothesis that the lower proliferative potential in placental terminal villi is associated with shortening of telomeres. Samples of the same placentas used in the above mentioned study were used and methods of laser capture microdissection and qPCR were applied.

2. Material and methods

2.1. Placental samples

Specimens were collected from eight control placentas and sixteen placentas of mothers suffering from type 1 diabetes mellitus (T1DM). Mothers gave their written informed consent and the Ethics Committee of the General University Hospital in Prague

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http://dx.doi.org/10.1016/j.placenta.2017.04.016
0143-4004/

approved the collection of placentas. All mothers declared that they were nonsmokers. No patient with IUGR, abnormal results of Doppler velocimetry and chronic hypertension was included (Tables 1 and 2). Placental samples were collected by the systematic uniform random sampling [4]. Specimens were fixed in 4% formaldehyde solution and embedded in the paraffin wax.

For microscopic analysis of villous chorangiosis, one section of each block (approx. 21 blocks per placenta) taken from T1DM placentas was stained with hematoxylin and eosin. The criteria for chorangiosis were the occurrence of at least 10 capillary cross-sections in at least 10 villi found in at least one third of sections observed at 10x objective magnification. The analysis determined villous chorangiosis in 9 of 16 T1DM placentas.

2.2. Microdissection of terminal villi

For sampling of microdissected chorionic villi, three haphazardly chosen blocks per placenta were cut into serial 10 μm thick sections which were applied on the Frame Slides (PET membrane, 1.4 μm, Leica) and stained with hematoxylin and eosin. Fifteen fields per membrane (= forty five sections per placenta) containing terminal villi (approx. 200 μm^2 each depending on the morphology of placental sample) were prepared using microdissection (Laser Capture Microdissection, LMD 6500, Leica) and used for DNA isolation.

2.3. DNA isolation

To the microdissected tissue in the top of Eppendorf tube, 80 μl of Lysis buffer was added. Proteinase K solution (20 mg/ml in 20 m MTris-HCl, 1 mM $CaCl_2$, 50% glycerol, pH = 7.3, Qiagen) was diluted 1: 3 in 30 mM Tris-HCl, pH = 8 to prepare the Lysis buffer. Samples were incubated for 2 h at 56 °C. Proteinase K was than inactivated by heating (10 min, 94 °C). Processed samples were five times diluted in water. For qPCR reactions, 5 μl of this solution were used.

2.4. Determination of relative T/S ratio using qPCR

We used the methodology introduced by Cawthon [13], further developed by Gil and Coetzer [14]. In the field of placental research, this method was recently successfully applied by Wilson et al. [6]. The qPCR method based on detection of SYBR Green fluorescence was performed using QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems, USA). Final volume of reactions was 25 μl, the half of this volume consisted of Power SYBR® Green PCR Master Mix (Life Technologies, USA). Primers for the single copy gene 364B [13,14] amplification were used in 300 nM concentration, primers for telomere sequences (tel) [13] in 900 nM concentration.

Samples were run in triplicates. Temperature settings were divided in three main stages - hold stage (activation of the enzyme) included 2 steps—50 °C for 2 min and 95 °C for 2 min, this stage was followed by 40 cycles of PCR—95 °C 30 s and 54 °C 1 min. Third stage - dissociation step (default software setting) was set at the end of each run. Ramp rates between all steps were 1.6 °C/s.

The standard curves were set at the start of experiments and used for the entire study. For construction of standard curves, the standard DNA (Taq Man Control Genomic DNA, Applied Biosystems, USA) was used.

Table 1
Control group. F = female, M = male.

Patient No.	Age (years)	BMI	Gestational week/ Mode of delivery	Newborn (g)	Apgar score	Placenta (g)
1	38	22.3	39/spont.	M 3490	9-9-10	535
2	31	21.1	39/S.C.	M 3560	8-9-9	605
3	23	19.4	40/spont.	F 3520	3-6-7	580
4	35	21.5	38/S.C.	F 3175	8-9-9	590
5	31	18.9	40/S.C.	F 3880	8-10-10	603
6	30	25.3	41/S.C.	M 3180	10-10-10	575
7	30	27.8	41/S.C.	M 3821	8-9-9	640
8	38	20.0	40/S.C.	F 3250	10-10-10	460
Mean ± SD	32 ± 4.6	22 ± 2.9	39.8 ± 1.0	3485 ± 255		574 ± 51

Table 2
Diabetic group. The comparison of fetal and placental weigth has shown no significant differences between studied groups. F = female, M = male;* placental chorangiosis.

Patient No.	Age (years)	BMI	Diabetic (years)	GlyHb (mmol/ mol)	Gestational week/ Mode of delivery	Newborn (g)	Apgar score	Placenta (g)
1	34	25.2	20	71	38/S.C.	F 3630	3-9-9	655*
2	35	22.2	6	38	38/S.C.	M 3680	7-8-9	645
3	33	22.7	15	37	40/S.C.	M 3790	9-10-10	620*
4	21	23.7	7	58	37/spont.	M 2920	7-9-10	550*
5	36	21.1	17	40	39/S.C.	F 4070	9-10-10	770
6	23	23.4	1	31	39/spont.	F 3170	10-10-10	490*
7	36	25.0	15	33	38/S.C.	F 2860	7-9-10	475
8	31	31.2	13	45	37/S.C.	M 3570	8-9-9	560
9	28	30.8	14	48	39/S.C.	M 3390	9-9-10	500*
10	24	26.4	11	47	37/S.C.	M 2400	10-10-10	400
11	38	25.2	27	50	38/S.C.	F 4290	10-10-10	680*
12	42	44.6	14	44	36/S.C.	M 4200	2-5-6	695*
13	31	25.5	7	38	40,S.C.	F 2880	10-10-10	615*
14	33	28.1	10	48	39/spont	M 3430	8-10-10	610
15	30	22.5	10	43	40/spont	F 3900	10-10-10	560
16	29	23.9	6	72	38/S.C.	F 4480	6-9-9	620*
Mean ± SD	31.5 ± 5.5	26.3 ± 5.5			38.3 ± 1.2	3541 ± 567		590 ± 91