Isoacceptor tRNA in human placenta tissue as assessed with two-dimensional polyacrylamide gel electrophoresis (2D PAGE)

Izoakceptorowe tRNA w tkance ludzkiego łożyska oceniane za pomocą dwukierunkowej elektroforezy w żelu poliakryloamidowym (2D PAGE)

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Abstract. Transfer ribonucleic acids (tRNAs) play a pivotal role in translation process during protein biosynthesis in ribosomal system, and also take part in many other processes. It has been observed that changes in the cell metabolism are strictly connected with isoacceptor tRNA population. Human placenta seems to be an adequate model for investigation of the isoacceptor tRNA population changes due to morphological and metabolic changes taking place in the course of pregnancy. Placenta tRNA samples were taken by means of phenol-isopropanol extraction and additionally purified by BD-cellulose column chromatography. Then labeled and unlabeled tRNA preparations were divided into individual isoacceptors by two-dimensional polyacrylamide gel electrophoresis. Electropherogram analysis suggests that the isoacceptor tRNA population, as pregnancy progresses, shows only small quantitative changes in content of individual isoacceptors, irrespective of pregnancy duration.
Key words: isoacceptors, placenta, transfer RNA

Streszczenie. Trasnportujące RNA odgrywają kluczową rolę w procesie translacji biosyntezy białka w układzie rybosomalnym. tRNA biorą również udział w wielu innych procesach komórkowych. Zaobserowano, że zmiany metabolizmu komórki są ściśle związane z populacją izoakceptorowych tRNA. Ludzkie łożysko jest odpowiednim modelem do badania zmian populacji izoakceptorowych tRNA ze względu na zmiany morfologiczno-metaboliczne zachodzące w przebiegu ciąży. Próbki tRNA łożyskowego zostały uzyskane poprzez ekstrakcję fenolowo-izopropanolową i dodatkowo oczyszczone chromatograficznie na kolumnie z BD-celulozy. Następnie znakowane i nieznakowane preparaty tRNA były rozdzielane na poszczególne izoakceptory z użyciem techniki elektroforezy dwukierunkowej w żelu poliakrylamidowym. Z analizy elektroforegramów wynika, że populacja izoakceptorowych tRNA w miarę trwania ciąży wykazuje jedynie niewielkie zmiany ilościowe zawartości poszczególnych izoakceptorów, niezależnie od czasu trwania ciąży.
Słowa kluczowe: łożysko, transportujące RNA, izoakceptory

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Introduction

Transfer ribonucleic acids (tRNAs) play a pivotal role in translation process during protein biosynthesis in ribosomal system. Apart from this function, tRNAs take part in many other processes, e.g. tRNA isoacceptor for lysine (Lys_3) is a primer for HIV reverse transcriptase; tRNAs are a part of ubiquitin pathway of protein degradation system, and also in glutamate metabolism [1–3].

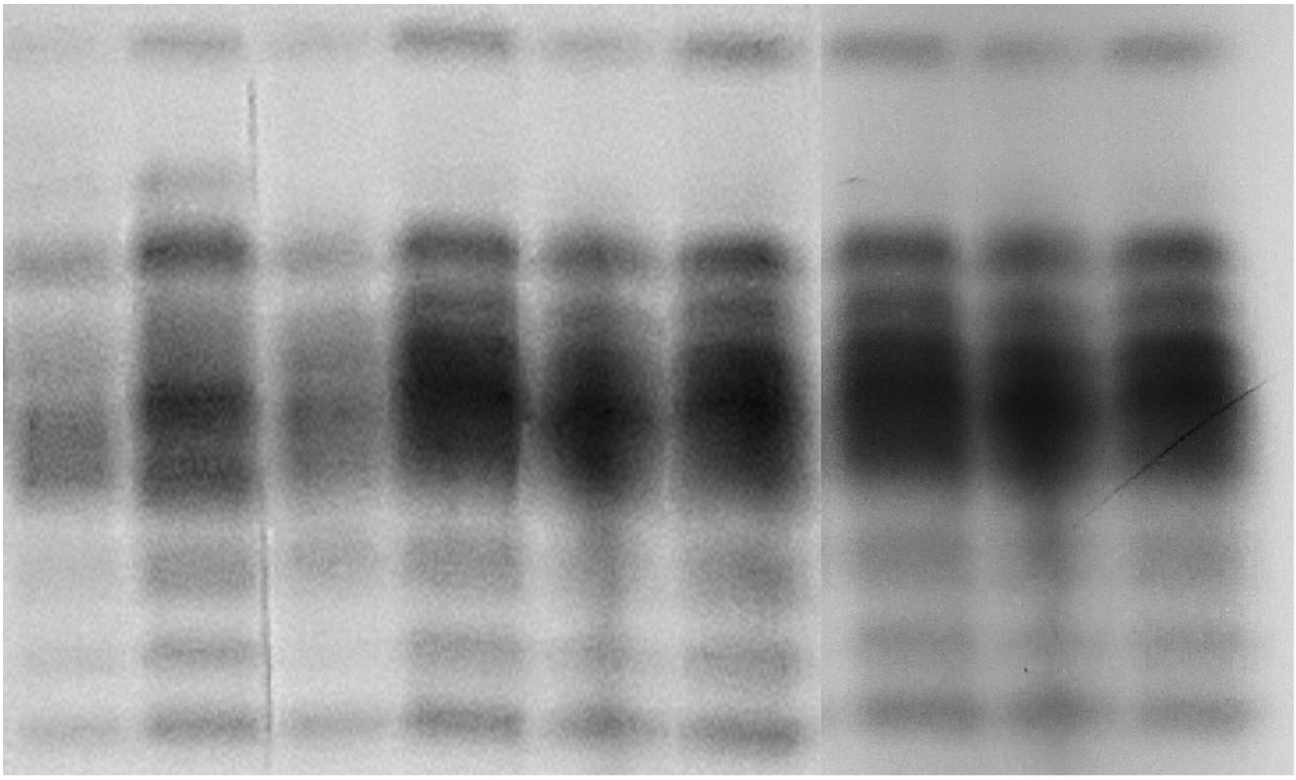

Figure 1. PAGE first dimension electrophoretic patterns of tRNA placental samples from different period of pregnancy
Rycina 1. Elektroforegram pierwszego kierunku łożyskowych próbek tRNA z różnych okresów ciąży

It was previously observed that highly proliferating cells exhibited changes toward tRNA hypomodification [4]. It is believed that these changes are strictly connected with adaptation of tRNA isoacceptors population to specific type of metabolism in proliferating tissues [4,5].

Human placenta tissue seems to be an adequate model for investigation of tRNA isoacceptors population changes starting from early to late stage of pregnancy. Additionally, it was observed previously that term placenta tRNA exhibits unusual deficiency of modified purine base – queuine [6]. Therefore, we undertook our studies to find differences, if any, in pattern of placental tRNA isoacceptors in the second and third trimester of pregnancy.

Material

Human placenta tissues from the second trimester of pregnancy were obtained after late spontaneous abortions caused by cervical incompetence, fetus malformations or uterine abnormalities (bicornual uterus). Third trimester placenta tissues were taken after preterm delivery caused by factors different than placental factors (premature rupture of the membrane, uterine preterm contractility) and other factors leading to spontaneous preterm delivery with no placental pathology. All samples were collected by the clinical staff at the Department of Perinatology and at the Department of Gynecological Surgery, University School of Medicine, Lublin, and at the Department of Obstetrics and Gynecology at County Hospital in Bełżyce.

Methods

Immediately after delivery, 10–20 gram samples of placenta tissues were washed in ice-cold 0.9% sodium chloride solution to remove blood, quickly immersed in liquid nitrogen and stored at -80°C until further processing, but no longer than for 4 weeks. Crude tRNA samples were obtained using phenol-isopropanol method described previously by Sein et al. [7] Additional purification by DEAE-52 column chromatography was necessary in order to remove oligonucleotides, DNA fragments and traces of phenol. Total tRNA samples (15 mg) were labeled at 3'end using nucleotidyltransferase (CCAse – kindly provided by Dr. Pierre Guillemaut, IBMP, Strasbourg, France) and ^{32}P a-ATP (Amersham, United Kingdom). Labeled and unlabeled (50mg) tRNA samples were mixed and tRNA isoacceptor patterns were obtained using two-dimensional polyacrylamide gel electrophoresis

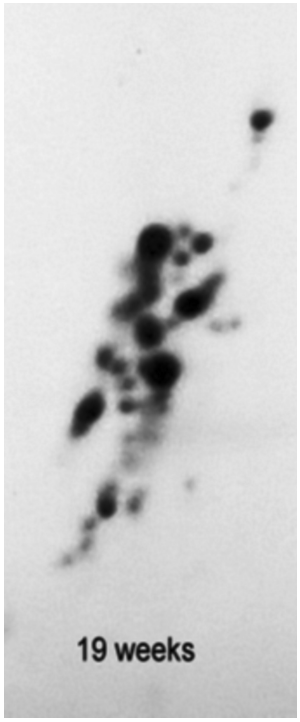

Figure 2. 2D PAGE electrophoretic pattern of tRNA isoacceptors in placenta tissues at 19 weeks

Rycina 2. 2D PAGE elektroforegram izoakceptorów tRNA w tkankach łożyska z 19. tygodnia ciąży

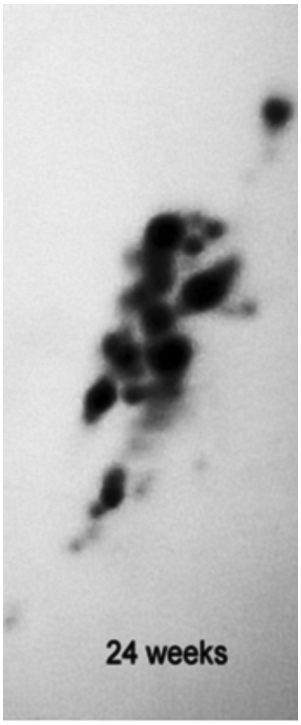

Figure 3. 2D PAGE electrophoretic pattern of tRNA isoacceptors in placenta tissues at 24 weeks

Rycina 3. 2D PAGE elektroforegram izoakceptorów tRNA w tkankach łożyska z 24. tygodnia ciąży

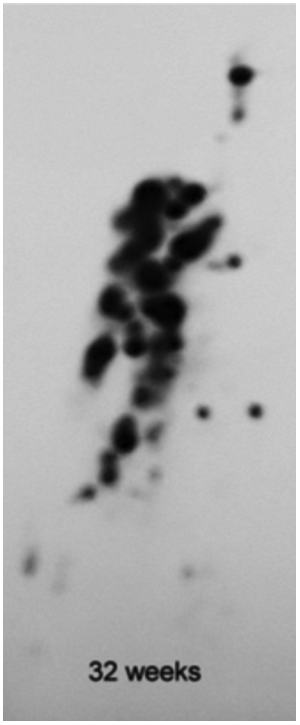

Figure 4. 2D PAGE electrophoretic pattern of tRNA isoacceptors in placenta tissues at 32 weeks

Rycina 4. 2D PAGE elektroforegram izoakceptorów tRNA w tkankach łożyska z 32. tygodnia ciąży

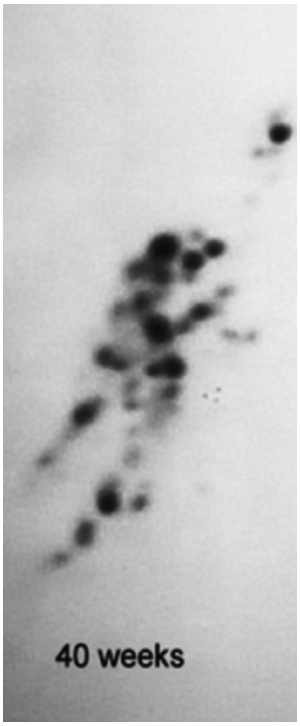

Figure 5. 2D PAGE electrophoretic pattern of tRNA isoacceptors in placenta tissues at 40 weeks

Rycina 5. 2D PAGE elektroforegram izoakceptorów tRNA w tkankach łożyska z 40. tygodnia ciąży

(2D PAGE by methods described by Fradin et al.) [8]. For the first dimension, 10% polyacrylamide gel (PAG) in semidenaturating conditions (4M urea) was used, whereas for the second dimension, 20% PAG and 7M urea were applied. All electrophoretic procedures were conducted at 4°C and 400 Volts. The duration of electrophoresis was monitored using bromophenol blue and xylene cyanol as markers of tRNA migration. Gels were stained using methylene blue solution and autoradiographed (Fuji film) within 6 to 8 hours at room temperature.

Results and discussion

In the first dimension of the electrophoresis we have obtained approximately 12 fractions located within 4S area along 6 cm of the gel for all samples (fig. 1). In second dimension for labeled material we were able to obtain nearly 40 well separated spots representing particular tRNA cluster of isoacceptors (fig. 2). Using "cold" (unlabeled) tRNA samples only 25 spots were clearly visible in the gels. These 25 spots detected by staining with methylene blue, were completely and strictly superpositioned to labeled tRNA isoacceptors.

We did not find any distinct differences among tRNA samples taken from particular weeks of gestation. However, it could be observed that minimal quantitative differences (as far as the amount of tRNA isoacceptor is concerned) exist between tRNA isoacceptors population, irrespective of the advancement of gestation. Probably, this reflects individual fluctuations in tRNA patterns. It seems very reasonable that molecular maturation of placental tissue (in term of tRNA modification) is established at the beginning of the second trimester of gestation, when trophoblastic tissue is matured to placenta. After this point of time, normal metabolism of placenta tissue is already established and is independent of ageing. However, further investigations are necessary to extend these observations to trophoblastic tissue, which is very dynamic in its structure changing (maturation), as well as in its functions (oncofetal proteins production, steroidogenesis).

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References

1. Bjork GR. Biosynthesis and function of modified nucleosides. In: Soll D, RajBhandary U, eds. tRNA: structure, biosynthesis, and function. Am. Society for Microbiology, Washington, 1995: 165–205
2. Li Z, Shalom A, Huang Y, et al. Multiple forms of tRNA(Lys3) in HIV-1. Biochem Biophys Res Commun. 1996; 227 (2): 530–540
3. Levican G, Katz A, de Armas M, et al. Regulation of a glutamyl-tRNA synthetase by the heme status. Proc Natl Acad Sci USA. 2007; 104 (9): 3135–3140
4. Dirheimer G, Baranowski W, Keith G. Variations in tRNA modifications, particularly of their queuine content in higher eukaryotes. Its relation to malignancy grading. Biochimie. 1995; 77 (1–2): 99–103
5. Baranowski W, Dirheimer G, Jakowicki JA, Keith G. Deficiency of queuine, a highly modified purine base, in transfer RNAs from primary and metastatic ovarian malignant tumors in women. Cancer Res. 1994; 54 (16): 4468–4471
6. Baranowski W, Tomaszewski J, Keith G. Unusual deficiency of the modified purine base queuine in transfer ribonucleic acid from the human placenta as tested by enzymatic assay. Am J Obstet Gynecol. 1993; 169 (3): 581–582
7. Sein KT, Becarevic A, Kanazir D. A simple modified method for the extraction of rat liver sRNA. Anal Biochem. 1969; 28 (1): 65–69
8. Fradin A, Gruhl H, Feldmann H. Mapping of yeast tRNAs by two-dimensional electrophoresis on polyacrylamide gels. FEBS Lett. 1975; 50 (2): 185–189