In addition to the key role in biosynthesis some aminoacyl-tRNA synthetases provide non-canonical functions. Particularly, separated fragments of tyrosyl-tRNA synthetase (TyrRS) involved into angiogenesis and inflammation. Meanwhile, the vascular inflammation and endothelial dysfunction are central characteristics of the pathogenesis of essential hypertension (EH). The latest studies highlight a role of antibodies in physiopathology of EH. **Aim.** To investigate the full-length TyrRS and its domains as autoantigens in sera of the persons with EH (n = 25), the healthy individuals with family history of the pathology (n = 12), and in the control group of healthy subjects (n = 32).

**Methods.** The recombinant TyrRS and its separated domains coupled with His-tags and generated by *Escherichia coli* were purified by chromatography on Ni-NTA-agarose. The levels of specific autoantibodies (aAbs) in sera of volunteers were measured by ELISA and confirmed in an immunoblotting assay. **Results.** Some subjects with elevated levels of aAbs against the full-length enzyme were detected in the cohort studies. 52% of the persons with EH (n = 25), the healthy individuals with family history of the pathology (n = 12), and in the control group of healthy subjects (n = 32). **Methods.** The recombinant TyrRS and its separated domains coupled with His-tags and generated by *Escherichia coli* were purified by chromatography on Ni-NTA-agarose. The levels of specific autoantibodies (aAbs) in sera of volunteers were measured by ELISA and confirmed in an immunoblotting assay. **Results.** Some subjects with elevated levels of aAbs against the full-length enzyme were detected in the cohort studies. 52% of the persons with EH (n = 25), the healthy individuals with family history of the pathology (n = 12), and in the control group of healthy subjects (n = 32). 52% of the persons with EH (n = 25), the healthy individuals with family history of the pathology (n = 12), and in the control group of healthy subjects (n = 32). 50% against CTD (p = 0.002) were identified. In 50% of the healthy individuals with family history of EH (p = 0.037) the levels of anti-CTD aAbs were elevated. **Conclusions.** The increased levels of aAbs against miniTyrRS and CTD in sera of the persons with EH potentially may be used as a prognostic marker of the disease severity or therapy effectiveness. Moreover, the immunoreactivity of healthy individuals with family history of EH against CTD may be an early marker of hypertension.

**Keywords:** aminoacyl-tRNA synthetases, miniTyrRS, cytokine, endothelial dysfunction, cardiovascular disease, prognostic marker.

Introduction

Tyrosyl-tRNA synthetase (TyrRS) is one of 20 conservative ancient enzymes that are critical at the initial stage of protein synthesis. The aminoacylation reaction, catalyzed by aminoacyl-tRNA synthetases, attaches each amino acid to its cognate tRNA [1, 2]. In addition to the aminoacylation, tRNA synthetases perform other non-canonical functions due to the interactions with various cellular partners [3]. New missions can be associated with their cytoplasmic forms as well as with nuclear and secreted extracellular forms that affect the signaling, immune response, and pathways of the cardiovascular development [4, 5]. Considering the functional versatility of ARSs, their expanded functions and expression may be associated with the pathology of various human diseases [6]. These enzymes are implicated into the neuronal diseases [7–9], tumorogene-
sis, [10], autoimmune diseases, namely, antisyntethases syndrome [11–13], and heart failure [14].

The native mammalian TyrRS is a procytokine [15]. Under apoptotic conditions, it can be secreted and split by leukocyte elastase into two fragments with distinct cytokine properties [16, 17]. The N-terminal catalytic fragment (miniTyrRS) with a Glu-Leu-Arg (ELR) cytokine motif is a potent promoter of the angiogenesis [18] and polymorphonuclear leukocytes recruitment [19, 20]. The C-terminal domain (CTD) has a high sequence similarity to the mature form of the pro-inflammatory cytokine-like protein known as human Endothelial-Monocyte-Activating Polypeptide (EMAP II) [21] endowed with angiogenic properties [22, 23].

Considering that the functional diversity of ARSs is often associated with pathological conditions, and that the separated domains of TyrRS are actively involved in the angiogenesis, we inferred a role of TyrRS in the cardiovascular diseases. Moreover, taking into account the capacity of the enzyme fragments to endothelio-
cytes recruitment [24–27], probably TyrRS is patho-
logically associated with essential hypertension (EH). The latest studies are focused on the activities of TyrRS domains in the treatment of cardiovascular dis-
ceses (CVD) [28–30], but they do not consider the im-
portance of specific autoantibodies (aAbs) against TyrRS and its natural fragments. Meanwhile, the role of antibodies in the pathogenesis of CVD, including EH, is highlighted [31]. Therefore, the investigation of aAbs against TyrRS and its natural fragments can improve our understanding of an importance of the en-
zyme and its separated domains in health and patholo-
gies in general, EH in particular. The purpose of this study was to identify the autoantibodies against TyrRS and its individual modules in sera of the persons with EH, the healthy individuals with family history of EH, and the normal healthy subjects in a control group.

Materials and Methods

Patients and Sera

128 persons with EH were examined (35.3 % females and 64.7 % males; mean ± SD ages 48.4 ± 27.6). All of them had high blood pressure from 7 to 20 years. Serum samples were selected from 25 well-charac-
terized persons with EH and with or without target or-
gan damage, 12 healthy individuals with family history of EH, and 32 healthy volunteers. The males were re-
cruited into all of the cohort studies in a higher propor-
tion. This research was conducted in compliance with the declaration of Helsinki and was approved by the local ethics committee in the National Scientific Center «M.D. Strazhesko Institute of Cardiology» of NAMS of Ukraine. All subjects were informed of the study purposes, and their informed consents were obtained.

Production and purification
of TyrRS and its separated domains

Generation of the recombinant full-length His-TyrRS (528 aa), His-miniTyrRS (362 aa), and His-CTD (166 aa) of Bos taurus has been performed according to our previous report [14]. The use of the bovine proteins is valid because of a high identity to the human TyrRS and its natural fragments (~95 %). The constructed plasmid vectors designated as pET-30a-TyrRS, pET-30a-miniTyrRS, and pET-30a-His-CTD were trans-
formed into E. coli BL21 (DE3) pLysE cells (Novagen, Madison, WI) grown in Lysogeny broth (LB) at 37 °C to optical density (OD) of 0.7–0.9 (600 nm). The ex-
pression of recombinant proteins with 1 mM isopro-
pyl-β-D(2)-thiogalactopyranoside (IPTG, Fermentas, Cambridge, United Kingdom) was induced for 4 h. Affinity purification of the recombinant proteins from the cultural media using nickel-nitriloacetic acid (Ni-
NTA resin, Thermo Scientific, USA) was carried out according to the manufacturer’s recommendations. The purity of His-TyrRS, His-mini-TyrRS, and His-CTD was confirmed by sodium dodecyl sulfate-poly-
acrylamide gel electrophoresis (SDS-PAGE) using a mixture of marker proteins (Fermentas, Lithuania).

ELISA assay

Specific binding of serum IgG aAbs to the recombi-
nant enzyme or its separated domains was analyzed using direct solidphase ELISA. TyrRS, miniTyrRS, or CTD (1 μg/well) in a phosphate-buffered saline (PBS, pH 7.4) were incubated in 96-well polysty-
rene plates. Then the plates were washed ten times with PBS containing 0.1 % Tween-20 (PBS-T), and, in order to block non-specific binding, the samples
were incubated for 1 h at 37 °C with 100 μl of PBS-T added to each well. Subsequently, the wells loaded with 1 : 50 diluted aliquots of sera were incubated for 18 h at 4 °C after washing with PBS-T. 100 μl of horseradish peroxidase (HRP)-conjugated goat anti-human IgG antibodies (Sigma, USA) were added to each well and incubated for 1 h at 37 °C. The plates were washed again with PBS-T, then with the substrate solution, containing 0.02 % H₂O₂, 0.1 M citrate-phosphate buffer (pH 5.8), and 0.5 mg/mL 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) sodium salt (Sigma, USA), was added to each well. After 20 min of incubation at 37 °C, the absorbance was measured at 405 nm in ELISA reader. PBS, pre-immune serum of rabbit, and secondary antibodies served as negative controls. The sera from immunized by the full-length TyrRS rabbits were used as positive controls. For each sample, the OD of the uncoated well was subtracted from the OD of the coated well and then subjected to the data analysis. The OD values greater than the mean + 2 SD values of the normal controls were considered as positive.

Western blot analysis of recombinant peptides
The bacterially expressed His-TyrRS, His-miniTyrRS, and His-CTD recombinant proteins were boiled, resolved by 12 % SDS-PAGE, and electrotransferred to the nitrocellulose membrane (Amersham Bioscience, Germany). The membrane was divided into strips and blocked by 5 % non-fat milk in PBS-T for 1 h at room temperature followed by a triple wash with PBS containing 0.1 % Tween-20. The strips with PBS-T, immunized by TyrRS rabbit serum (1 : 75), and sera of the control healthy individuals, or the healthy subjects with family history of CVD, or the patients with EH (1:100) were incubated for 18 h at 4 °C. The peroxidase-conjugated secondary antibodies (Sigma, USA) to the strips after washing and incubated were added for 1 h at a room temperature. The strips were washed, and the immunoreactivity was detected by ChemiDoc System (Bio-Rad Laboratories, USA).

Statistical analysis
All statistical analyses were performed using the Statistica software, version 7.0. Differences in non-parametric data were tested by the Mann–Whitney test. A P-value less than 0.05 was considered as statistically significant.

Results
Production and characterization of recombinant TyrRS, miniTyrRS, and CTD
To generate the recombinant full-length TyrRS, mini-TyrRS, and CTD, we expressed them in the E. coli BL21 (DE3) pLysE cells using the pET-30a expression system and His-tag sequence in order to facilitate the purification of the recombinant proteins. After induction with IPTG we observed high levels of the His-TyrRS, His-miniTyrRS, and His-CTD expression in bacterial cells. Then recombinant proteins were purified on Ni-NTA agarose under denaturing conditions. The required purity (more than 95 %) of the proteins was confirmed by SDS-PAGE (data not shown).

Detection of anti-TyrRS aAbs, anti-mini-TyrRS, and anti-CTD by ELISA
The presence of anti-CTD, anti-mini-TyrRS, and anti-TyrRS aAbs in the serum samples of the persons with EH,
Fig. 2. Anti-mini-TyrRS (A, C, E) and anti-CTD (B, D, F) aAb levels in sera from persons with EH, healthy individuals with family history of EH and healthy controls shown as OD values. The lines (A, B) show the cut-off values and mean median +2SD values for the healthy controls. Data (D–F) are presented as box plots, where the boxes represent the 25th to 75th percentiles, the points within the boxes represent the median, and the lines outside the boxes represent the minimum and maximum values. Differences were analyzed by the Mann–Whitney U-test.
Autoantibodies against tyrosyl-tRNA synthetase and its separated domains at essential hypertension

the healthy individuals with family history of EH, and the normal controls was assessed by ELISA. The serum samples with OD values greater than the mean +2SD values of the controls were considered as positive.

Anti-TyrRS (6.2 %), anti-miniTyrRS (15.6 %), and anti-CTD (24 %) aAbs were detected in serum samples of the healthy volunteers (data not shown).

Anti-TyrRS positive sera in 12 % (3 of 25) of the persons with EH were observed (Fig. 1). The elevated levels of anti-miniTyrRS antibodies were detected in 52 % (13 of 25) of the persons with EH (Fig. 2, A). Anti-CTD positive sera were shown in 60 % (15 of 25) of the persons with EH and 50 % (6 of 12) of the individuals with family history of EH (Fig. 2, B). The serum anti-miniTyrRS concentrations were significantly higher in the persons with EH compared with those in the Ab-negative normal healthy volunteers (p < 0.001). The serum levels of anti-CTD aAbs were elevated not only among the persons with EH (p = 0.002), but also among the healthy individuals with family history of the pathology (p = 0.037).

Detection of anti-TyrRS, anti-mini-TyrRS, and anti-CTD aAbs by Western blotting

The ELISA representative serum samples (positive, poorly and moderate reactive) of each group were subsequently confirmed by Western blotting against the full-length TyrRS (Fig. 3, A) and its natural fragments (Fig. 3, B).

Discussion and Conclusion

A lot of studies postulate a role of autoantibodies in the pathogenesis of hypertension [32]. For several decades it was known that EH is associated with the elevated serum levels of IgG and IgM autoantibodies [33, 34]. They can be involved into pathogenic reactions by binding to antigens expressed on the surface of endogenous cells, that leads to the destruction of cells via complement- or leukocyte-dependent interactions (type II hypersensitivity reaction) [35]. aAbs may form «immune complexes», that can be deposited in various tissues and cause the local inflammatory responses (type III hypersensitivity) [36]. They also can act as non-immunogenic agonists to the receptors (so-called «type V hypersensitivity»).

![Fig. 3. Immunoreactivity of healthy subjects, individuals with family history of EH, and persons with EH against TyrRS (40 μg for each protein) (A), miniTyrRS (B), and CTD (C), obtained by Western blotting. Lines 1–3 represent the high, moderate, and low reactive sera of healthy volunteers against relevant protein according to ELISA; lines 4–6, by analogy, for individuals with family history of EH; lines 7–9 for persons with hypertension. C is control line, incubated with serum of rabbit immunized by full-length TyrRS; M is protein MW marker](image)

The numerous studies demonstrate the importance of autoantibodies against the angiotensin II type-1 receptors, [37, 38], alpha-1 adrenergic receptors [39], beta-1 adrenergic receptors [40], L-type voltage gated
calcium channels [41], and heat shock proteins 70 and 60 [42–44] in the hypertension pathogenesis. The role of TyrRS and its natural fragments as well as autoantibodies against them in EH remains unknown.

EH is a multifactorial disease with indefinite etiology, its pathogenesis is clearly associated with the development of vascular endothelial dysfunction, characterized by pro-trombotic, pro-inflammatory and pro-constrictive vessel status [45]. Meanwhile, the endothelialcytes recruitment and the pro-inflammatory effects are the key properties of both distinct domains of TyrRS. [5, 17]. Considering all these facts, the involvement of natural fragments of the enzyme into pathogenesis of EH is quite possible.

This is the first investigation of the serum immunoreactivity of the persons with EH, the healthy individuals with family history of EH, and the healthy subjects against TyrRS and its separated domains. The study demonstrates the presence of persons with elevated levels of autoantibodies against the full-length enzyme, miniTyrRS, and CTD in the normal healthy cohort. Such kind of immunoreactivity can be stipulated by some undetected inflammatory process that resulted in the secretion of TyrRS or the appearance of the full-length protein in the intercellular space due to apoptosis. Our data demonstrate the significant immunoreactivity against mini-TyrRS (52 %) and CTD (60 %) among the persons with EH. Surprisingly, we found out the increased levels of autoantibodies against CTD in sera of 50 % of the healthy individuals with family history of the pathology.

Difference in the values of aAbs against the full-length enzyme and its natural fragments can be explained by the potential existence of the epitopes which are responsible for the cytokine activities and are sequestered in a native form by each other. The regions of cytokine activity – ELR on miniTyrRS and heptapeptide (Arg13-Thr19) on the N-terminus of CTD – in the full-length TyrRS have «face-to-face» orientation, conditioned by the electrostatic interactions [46]. Under the apoptotic conditions, specifically inflammation, the native enzyme is secreted into intercellular space and can be cleaved by an extracellular protease such as leukocyte elastase. Moreover, the regions of cytokine activity can be masked in a cell via tRNA connection [46]. As a result, both sequences providing the cytokine activity, become available and two distinct fragments obtain new cytokine functions [16]. It seems absolutely logically to assume normally hidden sequences to be extremely immunogenic. The immunoreactivity of the persons with EH as well as of the individuals with family history of pathology suggests the presence of natural fragments of TyrRS, the formation of which is possible in terms of inflammation [17], known to be a component of the EH pathogenesis.

Earlier we have demonstrated the TyrRS and its separated domains as autoantigens in heart failure caused by the dilated cardiomyopathy, myocarditis and ischemic heart disease [14]. The highest immunoreactivity for the full-length enzyme and the lowest for CTD were revealed. According to our recent findings in sera of the patients with hypertension the highest levels of aAbs were found against the CTD and the lowest against the full-length enzyme. The data comparison suggests potential involvement of aAbs against CTD and mini-TyrRS into the EH pathogenesis.

A practical significance of TyrRS and its separated domains is a «double-edged weapon». On the one hand, TyrRS is one of eight aminoacyl-tRNA synthetases involved into the antisynthetase syndrome [12], its mutant form occurs in Charcot-Marie-Tooth hereditary neuropathy [47]. On the other hand, mini-TyrRs is a potential drug in the myocardial ischemia treatment [30]. Its particular role also was demonstrated in the platelet recovery in the patients suffering from the life-threatening thrombocytopenia or the bone marrow failure [28]. For recovery of cardiac function after myocardial infarction [29] CTD can be applied as an antiangiogenic stimulus [27].

However, the influence of aAbs on TyrRS and its separated domains in the disease pathogenesis as well as in the treatment strategies remains to be elucidated. Potentially, the elevated levels of autoantibodies against mini-TyrRS and CTD in sera of the persons with EH may be used as prognostic markers of the EH severity or the therapy effectiveness. Moreover, the immunoreactivity of healthy individuals with family history of EH against CTD may be an early marker of hypertension.
Declaration of Interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES


Аутоантитіла проти тирозил-тРНК синтетази та її окремих доменів при гіпертонічній хворобі

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Окрім ключової ролі у біосинтезі білка певні аміноаціл-тРНК синтетази виконують також неканонічні функції. Зокрема, для доменів тирозил-тРНК синтетази залучені до антігенезу та запального реакцій. Тим часом, судинні за-палення і дисфункція ендотелію є центральними характеристиками патогенезу гіпертонічної хвороби (ГБ). Останні дослідження підкреслюють роль аутоантитіл в патофізіології ГБ. Мета. Дослідити повно-розмірну TyrRS і її окремі доме-ни як аутоантигени в сироватах осіб з ГБ (n = 25), у здорових осіб зі сімейною історією патології (n = 12), і в контрольній групі здорових осіб (n = 32). Методи. Рекомбінантна TyrRS і її окремі домени, пов’язані з His-тегами, експресува-лися в клітинах E.coli, та очищувалися хроматографією на Ni-NTA-агарозу. Рівні специфічних аутоантитіл (aAbs) в сироват-вах добровольців були виміряні методом IFA та підтвер-джено в імуноблотингу. Результати. Некотрі пацієнти з підвищеним рівнем aAbs проти повно-розмірного ферменту були виявлені в когортних дослідженнях. 52 % осіб з ГБ були ідентифіковані як імунореактивні проти miniTyrRS (p < 0,001) і 50 % проти CTD (p = 0,002). У 50 % здорових осіб
Autoantibodies against tyrosyl-tRNA synthetase and its separated domains at essential hypertension

з сімейною історією ГБ (р = 0,037) рівні анти-CTD aAbs були підвищені. Висновки. Збільшення рівнів aAbs проти miniTyrRS і CTD у сироватці осіб з ГБ потенційно може бути використане як прогностичний маркер ступеня тяжкості захворювання та ефективності терапії. Крім того, імунореактивність проти CTD здорових людей з сімейною історією ГБ може бути раннім маркером гіпертонії.

Ключові слова: аміноаціл-тРНК синтетази, mini-TyrRS, цитокін, ендотеліальна дисфункція, серцевосудинні захворювання, прогностичний маркер.

Автоантитела проти тирозил-тРНК синтетази и её отдельных доменов при гипертонической болезни

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Кроме ключевой роли в процессе биосинтеза белка ряд аміноаціл-тРНК синтетаз также исполняют, так называемые, неканонические функции. В частности, отдельные домены тирозил-тРНК синтетазы вовлечены в ангиогенез и воспалительные реакции. Между тем, сосудистой воспаление и дисфункция эндотелия являются центральными характеристиками патогенеза гипертонической болезни (ГБ). Последние исследования подчеркивают роль антител в патофизиологии ГБ. Цель. Исследовать полноразмерну TyrRS и ее отдельные домены как автоантigliны в сыворотках лиц с ГБ (n = 25), у здоровых лиц с семейной историей патологии (n = 12), и в контрольной группе здоровых лиц (n = 32). Методы. Рекомбинантная TyrRS и ее отдельные домены, связанные с His-тегами и экспрессированные в E. coli, очищали хроматографией на Ni-NTA-агарозе. Уровни специфических автоантител (aAbs) в сыворотке добровольцев были измерены методом ИФА и подтверждены в иммуноблоттинге. Результаты. Некоторые пациенты с повышенным уровнем aAbs против полноразмерного фермента были обнаружены в когортных исследованиях. 52 % лиц с ГБ были идентифицированы как иммунореактивые против miniTyrRS (р < 0,001) и 50 % против CTD (р = 0,002). У 50 % здоровых лиц с семейной историей ГБ (р = 0,037) уровни анти-CTD аAbs были повышены. Выводы. Увеличение уровней aAbs против miniTyrRS и CTD в сыворотке у лиц с ГБ потенциально может быть использовано в качестве прогностического маркера степени тяжести заболевания и эффективности терапии. Кроме того, иммунореактивность против CTD здоровых людей с семейной историей ГБ может быть ранним маркером гипертонии.

Ключевые слова: аміноаціл-тРНК синтетази, mini-Tyr RS, цитокін, дисфункція ендотелія, серцевосудисте забalenня, прогностичний маркер.

Received 20.05.15