Monoclonal Antibodies Against Tyrosyl-tRNA Synthetase and Its Isolated Cytokine-Like Domain

Iuliia Kondratiuk,¹ Antonina Khoruzenko,² Olga Cherednyk,² Valeriy Filonenko,² and Aleksander Kornelyuk¹

Tyrosyl-tRNA synthetase (TyrRS) is one of the key enzymes of protein biosynthesis. In addition to its basic role, this enzyme reveals some important non-canonical functions. Under apoptotic conditions, the full-length enzyme splits into two fragments having distinct cytokine activities, thereby linking protein synthesis to cytokine signaling pathways. The NH₂-terminal catalytic fragment, known as miniTyrRS, binds strongly to the CXCR1 chemokine receptor and, like interleukin 8, functions as a chemoattractant for polymorphonuclear leukocytes. On the other hand, an extra COOH-terminal domain of human TyrRS has cytokine activities like those of a mature human endothelial monocyte-activating polypeptide II (EMAP II). Moreover, the etiology of specific diseases (cancer, neuronal pathologies, autoimmune disorders, and disrupted metabolic conditions) is connected to specific aminoacyl-tRNA synthetases. Here we report the generation and characterization of monoclonal antibodies specific to N- and C-terminal domains of TyrRS. Recombinant TyrRS and its N- and C-terminal domains were expressed as His-tag fusion proteins in bacteria. Affinity purified proteins have been used as antigens for immunization and hybridoma cell screening. Monoclonal antibodies specific to catalytic N-terminal module and C-terminal EMAP II-like domain of TyrRS may be useful as tools in various aspects of TyrRS function and cellular localization.

Introduction

THE BASIC FUNCTION OF AMINOACYL-tRNA SYNTHETASES (ARSs) is activation of amino acid and their transfer to the cognate tRNAs.⁽¹⁾ In addition to their key role in protein biosynthesis, these enzymes also participate in other cellular processes. For example, ARSs regulate the expression of some genes, including their own. The regulation is carried out on the level of transcription, mRNA processing, and translation. These enzymes catalyze the synthesis of dinucleoside oligophosphates and thus influence many other cell functions. ARSs also participate in inflammation, angiogenesis, and apoptosis.^(2,3) The non-canonical activities of ARSs depend on the type and cellular location of ARS: tyrosyl-, tryptophanyl-, and lysyl-tRNA synthetases are secreted to trigger signaling pathways; glutamyl-prolyl- and glutaminyl-tRNA synthetases express their non-canonical activities in the cytoplasm, whereas lysyl- and methionyl-tRNA synthetases exert nuclear functions.^(4,5)

The importance of non-canonical functions of ARSs in the development of human diseases has been shown for many enzymes of this group. These enzymes are implicated in neuronal diseases; for example, some mutations in glycyl- and tyrosyl-tRNA synthetases are causally linked to Charcot-Marie-Tooth disease.^(6,7) Lysyl-tRNA synthetase is a possible

cause of amyotrophic lateral sclerosis.⁽⁸⁾ Mutations in mitochondrial aspartyl-tRNA synthetase are associated with leukoencephalopathy.⁽⁹⁾ The aminoacylation activity of methionyl-tRNA synthetase, which is required for translation initiation, is increased in human colon cancer.⁽¹⁰⁾ Preferential expression of the α -subunit of phenylalanyl-tRNA synthetase was observed in lung solid tumors and acute phase chronic myeloid leukemia.⁽¹¹⁾ In addition, ARSs are a cause of many autoimmune diseases. The autoantibodies directed against ARSs have been associated with a clinical picture, including myositis, arthritis, interstitial pneumonia, systemic lupus erythematosus, and other features that have been referred to as the "anti-synthetase syndrome."⁽¹²⁾

These diverse connections of ARSs with various human diseases make them attractive targets for the development of therapeutics, although as therapeutic agents of themselves, and as targets for drugs, tRNA synthetases will yield many opportunities for disease intervention.⁽¹³⁾

Human tyrosyl-tRNA synthetase (TyrRS) is inactive as a cell-signaling molecule, but it can be split into two distinct cytokines under apoptotic conditions.^(15,16) NH₂-terminal fragment (mini TyrRS) harbors the catalytic site of enzyme and C-terminal domain (C-TyrRS) found only in the mammalian cell. Mini TyrRS binds strongly to the interleukin-8 (IL-8) type A receptor and, like this cytokine, functions as a

¹Department of Protein Engineering and Bioinformatics, ²Department of Cell Signaling, Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine, Kyiv, Ukraine.

chemoattractant for polymorphonuclear leukocytes (PMN). All CXC chemokines such as IL-8 have a Glu-Leu-Arg (ELR) cytokine motif. This motif is essential for binding to PMN receptors and for PMN activity. The ELR motif of mini TyrRS is also important for its cytokine activity.⁽⁵⁾ The C-domain of human TyrRS has a high sequence similarity to the mature form of pro-inflammatory cytokine known as human endothelial-monocyte-activating polypeptide (EMAP II). The isolated C-TyrRS has potent chemotactic activity for polymorphonuclear leukocytes and mononuclear phagocytes, and stimulates production of myeloperoxidase, tumor necrosis factor- α , and tissue factor.^(16,17)

Hence, TyrRS may be a potential cause or complicating factor in the development of pathological conditions in humans. Polyclonal and autoantibodies against TyrRS were obtained by our lab previously,^(18,19) but it cannot fully describe the structural and functional features of TyrRS. The use of monoclonal antibodies is often preferred due to their high specificity and significantly less signal-to-noise ratio. In this study, we describe the production and characterization of monoclonal antibodies specific towards TyrRS and its distinct domains. Recombinant His-TyrRS, His-mini TyrRS, and His-C-TyrRS were used as antigens and in hybridoma screening procedures. We showed that 4D4 and 3F9 monoclonal antibodies specifically recognized both recombinant and endogenous mini TyrRS and C-terminal domain of TyrRS according to Western blot and immunoprecipitation data. These antibodies may be useful for studying the non-canonical activities of TyrRS under normal and pathological conditions.

Materials and Methods

Cell culture and cell culture media

Escherichia coli BL21 (DE3) pLysE cells (Novagen, Madison, WI) were grown in Luria-Bertani broth (LB) (bactotrypton, yeast extract, sodium chloride; Helicon, Russia). SP2/0 myeloma cells were obtained from Russian cell culture collection (Institute of Cytology of the Russian Academy of Sciences, St. Petersburg, Russia). Roswell Park Memorial Institute Medium (RPMI) 1640 and fetal calf serum (FCS) were purchased from Gibco (Grand Island, NY). Polyethylene glycol (PEG)-2000 was from Merck (Darmstadt, Germany) and Protein G-Sepharose Fast Flow was from Sigma-Aldrich (Stockholm, Sweden).

Production and purification of His-TyrRS, His-mini TyrRS, and His-C-terminal domain of TyrRS fusion proteins

Generation of recombinant full-length His-TyrRS (528 aa), His-mini TyrRS (362 aa), and His-C-terminal domain of TyrRS (His-TyrRS/C-term, 166 aa) were performed according to our previous report.⁽²⁰⁾ The constructs designated as pET-30a-TyrRS, pET-30a-mini TyrRS, and pET-30a-His-TyrRS/C-term (kindly provided by Dr. Alexey Dubrovskiy, Department of Protein Engineering and Bioinformatics, Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv) were transformed into *E. coli* BL21 (DE3) pLysE cells and grown at 37°C to optical density of 0.7–0.9 (600 nm). The expression of recombinant proteins was induced with 1 mM isopropyl- β -D(2)-thiogalactopyranoside (IPTG, Fermentas, Cambridge, United Kingdom) for 4 h. Affinity purification of recombinant

proteins from the cultural media was carried out using nickel-nitriloacetic acid (Ni-NTA-agarose, Qiagen, Crawley, UK) according to the manufacturer's recommendations. The purity of purified His-TyrRS, His-mini TyrRS, and His-TyrRS/C-term was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Production of hybridomas

Full-length TyrRS, mini TyrRS, and C-domain of TyrRS were used for hybridoma generation according to standard protocol⁽²¹⁾ with some modifications. Female BALB/c mice (8 weeks old) were immunized with 15 μ g of recombinant full-length His-TyrRS fusion protein in complete Freund's adjuvant by intraperitoneal (i.p.) injections every 2 weeks. When the titer of anti-TyrRS antibody in the sera of immunized mice reached 10^{-5} – 10^{-6} , the production of hybridomas was performed according to standard protocol.⁽²⁾ In brief, immunized mice were boosted with 15 μ g of antigen in PBS by i.p. injection. Three days later, splenocytes from immunized mice and SP2/0 myeloma cells cultured in RPMI 1640 containing 20% FCS were fused in the presence of PEG-2000. Primary screening of hybridoma supernatants was performed 7 days later using ELISA.

ELISA assay

TyrRS, mini TyrRS, or C-domain of TyrRS (1 μ g/well) in a phosphate-buffered saline (PBS, pH 7.4) were incubated in 96-well polystyrene plates for 1 h at 37°C. The plates were then washed three times with PBS containing 0.1% Tween-20 (PBS-T) and incubated with 200 μ L of 2% bovine serum albumin (BSA) in PBS added to each well for 1 h at 37°C in order to block non-specific binding. Subsequently, the wells were loaded with 100 μ L aliquots of hybridoma media and were incubated for 1 h at 37°C followed by four washes with PBS-T. After washing, 100 μ L of horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG antibodies (1:5000 v/v, Promega, Madison, WI) were added to each well and incubated for 1 h at 37°C. Plates were washed again four times with PBS-T, then substrate solution, containing 0.02% H_2O_2 , 0.1 M citrate-phosphate buffer (pH 5.8), and 0.5 mg/mL 2, 2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) sodium salt (Sigma), was added to each well. After a 15 min incubation at 37°C, the absorbance was measured at 490 nm in ELISA reader. PBS, hybridoma medium alone, pre-immune serum, and secondary antibody served as negative controls. We used the sera from immunized mice as positive controls.

Western blot analysis of recombinant peptides

Bacterially expressed His-TyrRS, His-mini TyrRS, and His-TyrRS/C-term recombinant proteins were boiled, resolved by a 12% SDS/PAGE, and electrotransferred to nitrocellulose membrane Hybond-C (Amersham, Sweden). The membrane was divided into strips and blocked by 5% non-fat milk in PBS for 1 h at room temperature followed by a single wash with PBS containing 0.1% Tween-20. Strips were incubated with PBS, post-immune serum (1:1000), hybridoma media from clones, or cell culture media alone for 4 h at room temperature. After three washes, a peroxidase-conjugated secondary antibody (Promega) was added to the strips and incubated for 1 h at room temperature. Strips were washed three times, and the

immunoreactivity was detected by an ECL system (Amersham).

Purification of TyrRS MAb, mini TyrRS MAb, and C-terminal of TyrRS MAb

For production of ascetic fluid, BALB/c mice were injected with 0.5 mL of pristane (Sigma) and 7–10 days later inoculated with 5×10^6 hybridoma cells.⁽²²⁾ The ascetic fluid was collected after 7–10 days. The fraction of immunoglobulins was precipitated from ascetic fluid with 50% ammonium sulphate and used for affinity purification by Protein-G-Sepharose chromatography. The IgG fractions were pulled together and dialyzed against PBS. The purified antibodies with 50% glycerol were aliquoted and stored at -20°C .

Cell lysate preparation

HEK and NIH 3T3 cells were lysed in buffer containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 50 mM NaF, 5 mM EDTA, and a mixture of protease inhibitors (Roche Molecular Diagnostics, Cedex, France). Protein concentration was estimated by Bradford protein assay.⁽²³⁾

Immunoprecipitation

Hybridoma media from selected positive clones were incubated with 25 μL of 50% suspension of Protein G-Sepharose for 2 h at 4°C on the wheel. The beads were washed three times in lysis buffer, as described above, and the clarified lysate (300 μg of protein) from HEK and NIH 3T3 cell line was added. After incubation on the wheel at 4°C , beads were washed four times with 1 mL of lysis buffer. Immune complexes were removed from beads by boiling in Laemmli sample buffer and separated by SDS-PAGE. Resolved proteins were visualized by Coomassie staining or transferred onto nitrocellulose membrane for immunoblotting.

Results and Discussion

Searching for the molecular mechanisms of non-canonical activities of TyrRS is impossible without a detailed elucidation of its structural and functional features. Monoclonal antibodies generated against different domains of TyrRS are important tools for solving this problem. Here we describe the generation and characterization of monoclonal antibodies against recombinant TyrRS and their cytokine-like domains.

Production and characterization of recombinant TyrRS, mini TyrRS, and C-domain of TyrRS

To generate recombinant full-length TyrRS, mini TyrRS, and C-domain of TyrRS in sufficient quantities for monoclonal antibody production, we expressed it in *E. coli* BL21 (DE3) pLysE cells with the use of a pET expression system and His-tag sequence in order to facilitate the purification of the recombinant proteins. We observed very high levels of His-TyrRS, His-mini TyrRS, and His-TyrRS/C-term expression in bacterial cells after induction with IPTG (Fig. 1). Then recombinant proteins were purified on Ni-NTA agarose under denaturing conditions. Figure 1 shows that purity of His-TyrRS, His-mini TyrRS, and His-TyrRS/C-term was more than 95%.

FIG. 1. Analysis of induction of expression (A) and purification (B) of recombinant TyrRS, mini TyrRS, and C-domain of TyrRS. (A) Total cell lysates (30 μg) from the IPTG induced (lanes 2, 4, 6) and non-induced (lanes 1, 3, 5) BL21 pLysE cells expressing His-TyrRS (lanes 5, 6), His-mini TyrRS (lanes 3, 4), and His-TyrRS/C-term (lanes 1, 2) were separated by SDS-PAGE and Coomassie staining. (B) Affinity purification of His-TyrRS, His-mini TyrRS, and His-C-terminal domain of TyrRS on Ni-NTA Agarose under denaturing conditions following SDS-PAGE and Coomassie staining. Lane 1, His-C-terminal domain of TyrRS; lane 2, His-mini TyrRS; lane 3, His-TyrRS.

The full-length His-TyrRS has been used as antigen for mice immunization. By ELISA assay, we found this protein to be a good antigen, and the titer of antibodies in the sera of immunized mice reached 10^5 – 10^6 after the third immunization. Spleen cells from the best responder were fused with the myeloma cells Sp2/0 using the PEG method of hybridoma production. The first round of ELISA screening was carried out using His-TyrRS as an antigen and 10 positive clones were identified. Three days later, we performed a second ELISA screen of selected clones using His-mini TyrRS and His-TyrRS/C-term as antigens and confirmed the specificity of eight clones towards TyrRS (six clones were specific for His-mini TyrRS and two clones for His-TyrRS/C-term).

We selected the two strongest clones (4D4 and 3F9) specific for His-mini TyrRS and His-TyrRS/C-term, respectively, for subcloning and further analysis. Western blot analysis of selected antibodies confirmed their specificities towards His-TyrRS, His-mini TyrRS, and His-TyrRS/C-term, respectively (Fig. 2).

FIG. 2. Western blot analysis of HEK 293 cell lysate, His-TyrRS, His-mini TyrRS, and His-TyrRS/C-term by TyrRS-specific hybridoma clones 4D4 (A) and 3F9 (B). Lane 1, HEK 293 cell lysate; lane 2, His-TyrRS; lane 3, His-mini TyrRS; lane 4, His-TyrRS/C-term.

FIG. 3. Application of monoclonal antibody 4D4 (**A**) and 3F9 (**B**) in immunoprecipitation. Western blot analysis of TyrRS immunoprecipitated from HEK 293 and NIH 3T3 cell lysates by 4D4 (**A**) and 3F9 (**B**) MAbs and blotted by the same antibodies. Lane 1, immune complex of NIH 3T3 lysate and MAb 4D4 or 3F9; lane 2, pG Sepharose + supernatants from NIH 3T3 cells; lane 3, pG Sepharose + MAb 4D4 or 3F9; lane 4, NIH 3T3 cell lysate; lane 5, immune complex of HEK293 lysate and MAb 4D4 or 3F9; lane 6, pG Sepharose + HEK 293 lysate; lane 7, pG Sepharose + MAb 4D4 or 3F9; lane 8, HEK 293 lysate.

According to the data presented, the 4D4 clone recognizes specifically both recombinant His-TyrRS and His-mini TyrRS, but not His-TyrRS/C-term (Fig. 2). Conversely, the 3F9 clone recognizes specifically recombinant His-TyrRS and His-TyrRS/C-term, but not His-mini TyrRS (Fig. 2). It is very important that both clones recognized specifically endogenous full-length TyrRS in HEK 293 cell lysates.

In the next step, we tested the ability of the selected clones to immunoprecipitate native TyrRS. In this assay, we incubated 300 μ g of protein extracts from HEK and NIH 3T3 cells with Protein G-Sepharose containing IgGs from selected clones. Immune complexes were washed extensively with cell lysis buffer, resolved by SDS-PAGE, and immunoblotted with TyrRS positive hybridomas. We found that only 4D4 was able to immunoprecipitate specifically TyrRS from HEK and NIH 3T3 cells (Fig. 3). The 3F9 clone was not able to immunoprecipitate TyrRS.

Our study describes the production and characterization of MAbs 4D4 and 3F9, which are specific towards full-length TyrRS and their separate N-terminal mini TyrRS and C-domain of TyrRS in various immunoassays, including ELISA, Western blotting, and immunoprecipitation. MAbs 4D4 and 3F9 may be useful for investigation of tyrosyl-tRNA synthetase in health and disease.

The C-terminal module of TyrRS is attached to the catalytic N-terminal mini TyrRS through an interdomain flexible linker, which contains a PEST-like sequence.⁽¹⁴⁾ PEST sequences enriched by proline, serine, glutamic acid, and threonine usually are natural substrates for cleavage by many intracellular proteinases.⁽²⁴⁾ Consequently, pathological processes that lead to the release of proteinases could trigger proteolytic cleavage of TyrRS at the PEST-like sequence. As a result, the isolated NH₂- and COOH-terminal parts of TyrRS will be released, which reveal completely different angiogenic activities properties. Release of two antagonistics in their angiogenic proteins from cancer cells may be one of the factors responsible for high plasticity of endothelium.⁽²⁵⁾ Since development of malignant phenotype is partially dependent on the rate of angiogenesis, balance between proangiogenic and angiostatic factors may play an important role in the development of cancer, and the shift of balance may lead to malignant cell transformation.

Hence, monoclonal antibodies against the isolated NH₂- and COOH-terminal domains of TyrRS may be useful for studying different aspects of non-canonical activities of TyrRS, especially in some pathologies, including cancer.

Acknowledgments

This work was supported in part by the National Academy of Sciences of Ukraine within the program, Dynamic Aspects

of Functioning of Eukaryotic Tyrosyl-tRNA Synthetase and the Study of Mutation Influence on Aminoacylation of tRNA and of Neurodegenerative Disorders (registration no. 0107 U004938).

Author Disclosure Statement

The authors have no financial interests to disclose.

References

1. Agou F, Waller J-P, and Mirande M: Expression of rat aspartyl-tRNA synthetase in *Saccharomyces cerevisiae*. *J Biol Chem* 1996;271:29295–29303.
2. Park SG, Ewalt KL, and Kim S: Functional expansion of aminoacyl-tRNA synthetases and their interacting factors: new perspectives on housekeepers. *Trends Biochem Sci* 2005;30:569–574.
3. Park SG, and Kim S: Do aminoacyl-tRNA synthetases have biological functions other than in protein biosynthesis? *IUBMB Life* 2006;58:556–558.
4. Wakasugi K, Slike BM, Hood J, Otani A, Ewalt KL, Friedlander M, Cheresch DA, and Schimmel P: A human aminoacyl-tRNA synthetase as a regulator of angiogenesis. *Proc Natl Acad Sci USA* 2002;99:173–177.
5. Yang XL, Schimmel P, and Ewalt KL: Relationship of two human tRNA synthetases used in cell signaling. *Trends Biochem Sci* 2004;29:250–256.
6. Antonellis A, Ellsworth RE, Sambuughin N, Puls I, Abel A, Lee-Lin S-Q, Jordanova A, Kremensky I, Christodoulou K, Middleton LT, Sivakumar K, Ionasescu V, Funalot B, Vance JM, Goldfarb LG, Fischbeck KF, and Green ED: Glycyl-tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. *Am J Hum Genet* 2003;72:1293–1299.
7. Jordanova A, Irobi J, Thomas FP, Dijck PV, Meerschaert K, Dewil M, Dierick I, Jacobs A, Vriendt ED, Guergueltcheva V, Rao CV, Tournev I, Gondim FAA, D'Hooghe M, Gerwen VV, Callaerts P, Bosch LVD, Timmermans J-P, Robberecht W, Gettemans J, Thevelein JM, Jonghe PD, Kremensky I, and Timmerman V: Disrupted function and axonal distribution of mutant tyrosyl-tRNA synthetase in dominant intermediate Charcot-Marie-Tooth neuropathy. *Nat Genet* 2006;38:197–202.
8. Kunst CB, Mezey E, Brownstein MJ, and Patterson D: Mutations in SOD1 associated with amyotrophic lateral sclerosis cause novel protein interactions. *Nat Genet* 1997;15:91–94.
9. Scheper GC, Klok TVD, Andel RJV, Berkel CGMV, Sissler M, Smet J, Muravina TI, Serkov SV, Uziel G, Bugiani M, Schiffmann R, Krägeloh-Mann I, Smeitink JAM, Florentz C,

- Coster RV, Pronk JC, and Knaap MSV: Mitochondrial aspartyl-tRNA synthetase deficiency causes leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation. *Nat Genet* 2007;39:534–539.
10. Kushner JP, Boll D, Quagliana J, and Dickman S: Elevated methionine-tRNA synthetase activity in human colon cancer. *Proc Soc Exp Biol Med* 1976;153:273–276.
 11. Rodova M, Ankilova V, and Safo MG: Human phenylalanyl-tRNA synthetase: cloning, characterization of the deduced amino acid sequences in terms of the structural domains and coordinately regulated expression of the alpha and beta subunits in chronic myeloid leukemia cells. *Biochem Biophys Res Commun* 1999;255:765–773.
 12. Hirakata M: Autoantibodies to aminoacyl-tRNA synthetases. *Int Med* 2005;44:527–528.
 13. Park S, Schimmel P, and Kim S: Aminoacyl-tRNA synthetases and their connections to disease: *PNAS* 2008;105:11043–11049.
 14. Kornelyuk AI: Structural and functional investigation of mammalian tyrosyl-tRNA synthetase. *Biopolym Cell* 1998;14:349–359.
 15. Wakasugi K, and Schimmel P: Highly differentiated motifs responsible for two cytokine activities of a split human tRNA synthetase. *J Biol Chem* 1999;274:23155–23159.
 16. Wakasugi K, and Schimmel P: Two distinct cytokines released from a human aminoacyl-tRNA synthetase. *Science* 1999;284:147–151.
 17. Kornelyuk A, Tas MPR, Dubrovsky A, and Murray JC: Cytokine activity of the non-catalytic EMAP-2-like domain of mammalian tyrosyl-tRNA synthetase. *Biopolym Cell* 1999;15:168–172.
 18. Kondratiuk I, Babaryk MA, Sidorik LL, and Kornelyuk OI: Optimization of biosynthesis process of catalytic module of mammalian tyrosyl-tRNA synthetase and its research by immunochemical methods. *J Taras Shevchenko Natl Univ Kyiv Biol* 2010;56:33–35.
 19. Kondratiuk I, Sidorik LL, Bobyk VI, Ryabenko DV, and Kornelyuk AI: Identification of autoantibodies to tyrosyl-tRNA synthetase in heart dysfunctions. *Biopolym Cell* 2010;26:373–377.
 20. Kondratiuk I, Babaryk MA, and Kornelyuk AI: Optimization of bacterial expression of mammalian tyrosyl-tRNA synthetase on cultivation strain *Escherichia coli* BL21 (DE3) pLysE. *Microbiol Biotechnol* 2009;4:6–12.
 21. Harlow E, and Lane D: *Antibodies: A Laboratory Manual*. Cold Spring Harbor Press, Cold Spring Harbor, NY, 1998.
 22. Hurrell JGR: *Monoclonal Hybridoma Antibodies: Techniques and Application*. CRC Press, Boca Raton, FL, 1982.
 23. Bradford M: Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–254.
 24. Rogers S, Wells R, and Rechsteiner M: Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science* 1986;234:364–368.
 25. Ivakhno SS, and Kornelyuk AI: Cytokine-like activities of some aminoacyl-tRNA synthetases and auxiliary p43 co-factor of aminoacylation reaction and their role in oncogenesis. *Exp Oncol* 2004;26:250–255.

Address correspondence to:

Iuliia Kondratiuk

Department of Protein Engineering and Bioinformatics

Institute of Molecular Biology and Genetics

National Academy of Sciences of Ukraine

150, Zabolotnogo Street

Kyiv 03680

Ukraine

E-mail: kondratiuk_yulya@ukr.net

Received: November 2, 2012

Accepted: February 22, 2013