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Isoforms of elongation factor eEF1A may be differently regulated at post-transcriptional level in breast cancer progression

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Eukaryotic translation elongation factor 1A exists as two 98 % homologous isoforms: eEF1A1 (A1) and eEF1A2 (A2) which are tissue and development specific. Despite high homology in an open reading frame (ORF) region, mRNAs coding for eEF1A1 and eEF1A2 are different in their untranslated regions (UTR), suggesting a possibility of their dissimilar post-transcriptional regulation. ***Aim****. To analyze the existence of cis-acting motifs in the UTRs of EEF1A1/A2 mRNAs, to confirm the possibility of post-transcriptional control of eEF1A1 and eEF1A2 expression.* ***Methods****. An ensemble of bioinformatic methods was applied to predict regulatory motifs in the UTRs of EEF1A1/A2 mRNAs. Dual-luciferase reporter assay was employed to detect post-transcriptional regulation of eEF1A1/A2 expression.* ***Results****. Numerous regulatory motifs in the UTR of EEF1A1/A2 mRNAs were found bioinformatically. The experimental evidence was obtained for the existence of negative regulation of EEF1A1 and positive regulation of EEF1A2 mRNA in the model of breast cancer development.* ***Conclusions****. EEF1A1 and EEF1A2 mRNAs contain distinct motifs in the UTRs and are differently regulated in cancer suggesting the possibility of their control by different cellular signals.*

Keywords: EEF1A1, EEF1A2, UTR, miRNA, breast cancer.

Introduction. The main function of eukaryotic translation elongation factor 1A (eEF1A) is delivery of aminoacylated tRNA to the A-site of ribosome during the elongation step of protein biosynthesis [1]. eEF1A exists as two isoforms that are tissue and development specific. While the A1 isoform is expressed ubiquitously, expression of the A2 isoform is restricted to the cardiac, muscle and neuronal tissues [2]. In abovementioned tissues, during postnatal period a switch from eEF1A1 to eEF1A2 expression occurs. This change of their expression is crucial, as the mice with a partial deletion of the EEF1A2 gene die on the 28th day after birth [3]. Despite the same catalytic efficacy during the elongation step of protein biosynthesis, the isoforms seem to be different in their non-canonical functions [4–6]. eEF1A1 demonstrates proapoptotic properties, while eEF1A2 has anti-apoptotic, pro-oncogenic characteristics [7–9]. The isoforms possess 98 % similarity of protein sequences [10]. However, mRNAs coding for the A1 and A2 isoforms have different 5' and 3'UTRs. It is well known that through UTRs occurs the post-transcriptional regulation of gene expression [11]. Numerous indirect data showed that mRNAs of eEF1A1 and eEF1A2 may be post-transcriptionally regulated [12–16]. Thus, our aims were to find different regulatory motifs in EEF1A1/A2 mRNAs by *in silico* approach and to demonstrate a possibility of

distinct post-transcriptional regulation of the A1 and A2 isoforms expression experimentally.

Materials and methods. *Reporter plasmids construction*. cDNA was synthesized from RNA of human leukocytes using oligodT primer and M-MuLV Reverse Transcriptase («ThermoScientific», USA) according to the manufacture recommendations. The 3'UTR of EEF1A1 mRNA was amplified using following primers: forward – 5'-GCTCTAGAATATTATCCCTAATACC TGC-3'; reverse – 5'-GCTCTAGACATTGCCAGTCA CTTTAAGA-3'. The PCR mixture contained 2.5 units of PFU polymerase («ThermoScientific»), 2.5 mM $MgCl_2$, 1 μM oligonucleotides, and 1μl of cDNA. The PCR program was: 3 min – initial denaturation at 95 °C followed by 30 cycles of 30 s – denaturation at 95 °C, 30 s – annealing at 55 °C, extension – at 72 °C 3 min; final extension at 72 °C – 5 min.

The 3'UTR of EEF1A2 mRNA was amplified from genomic DNA extracted from human leukocytes using following primers: forward – 5'-GCTCTAGAGCCCG CGGCGCGACCCTCCC-3'; reverse – 5'-GCTCTAG AGAGCGTGGCGAGCGCTGGGC-3. The PCR mixture contained 2.5 units of Taq polymerase («Thermo Scientific»), 2 mM $MgCl_2$, 1μM oligonucleotides, 100 ng of DNA and 8 % of DMSO. The PCR program was: 15 min – initial denaturation at 95 °C followed by 30 cycles of 30 s – denaturation at 95 °C, 30 s – annealing at 69.8 °C, extension – at 72 °C 30 s; final extension at 72 °C – 7 min.

PCR products were digested with *Xba*I restriction enzymes («ThermoScientific») and inserted into *pGL4.13* reporter plasmid («Promega», USA). Orientation of the insert was confirmed by sequencing.

Cell lines. MCF10A, MCF-10AT/MCF-10ANeoT, MCF-10CA1a, MCF-10ANeoT-YB-1, MCF-10ANeoT-MSCV cell lines were maintained in DMEM/F12 medium supplemented with 5 % horse serum, 1 mM Na-Pyruvate, 10 μg/ml insuline, 20 ng/ml EGF, 200 ng/ml hydrocortisone, 100 ng/ml cholera toxin («Sigma-Aldrich», USA), 100 μg/ml streptomycin, 100 U/ml penicillin («Gibco», USA) and cultured in humidified atmosphere at 37°C and 5 % CO_2.

Reporter gene assay. The day before the experiment the cells were seeded into 96-well plates at a concentration calculated to reach 80 % confluence at the time of experiment. The next day the cells were transfected with 25 ng of *pGA1_3UF* or *pGA2_3UF* reporter plasmid using Lipofectamine-2000 according to the manufactures protocol. Luciferase level was quantified 24 h later, using a Dual-Luciferase reporter assay system («Promega») on a TriStar LB 941 reader («Berthold Technologies», USA). Signal of *Firefly* luciferase was normalized to *Renilla* luciferase.

mRNA sequences analysed in the work. EEF1A1 mRNAs: *Homo sapience* (*Hs*) NM_001402, *Bos taurus* (*Bt*) XM_001249987, *Canis familiaris* (*Cf*) XM_845314, *Gallus gallus* (*Gg*) NM_204157, *Equus caballus* (*Ec*) NM_001081781, *Mus musculus* (*Mm*) NM_010106, *Pan troglodytes* (*Pt*) NM_001009165, *Rattus norvegicus* (*Rn*) NM_175838.

EEF1A2 mRNAs: *H. sapience* NM_001958, *B. taurus* NM_001037464, *G. gallus* XM_001233517, *M. musculus* NM_007906, *R. norvegicus* NM_012660, *E. caballus* XM_001915406, *Oryctolagus cuniculus* (*Oc*) NM_001082031.

Results and discussion. Untranslated regions are known to play crucial roles in the post-transcriptional regulation of gene expression [19]. Thus, we concentrated our efforts on elucidation of a possible post-transcriptional control of the A1/A2 expression via UTRs.

The 5'UTR of EEF1A1 and EEF1A2 mRNAs. The 5'UTR plays an important role in regulation of the initiation of protein biosynthesis. We compared several most critical properties of the 5'UTR of both EEF1A1 and EEF1A2 mRNAs. Accordingly to the leaky scanning mechanism, most of mammalian AUG start codons should be in the Kozak context for efficient recognition by ribosome. Strong Kozak context for mammalian and plant mRNAs is defined as a purine at position –3 and a guanine at position +4 [18]. We found that in mRNAs coding for the eEF1A1 and eEF1A2 isoforms AUG start codons are in strong Kozak context (Fig. 1, *A*, *B*). Additionally a hairpin structure located in the region 7–11 bp after the AUG codon may cause ribosome delay on the start codon thus facilitating the initiation step [19]. Using the AUG-hairpin program [20] we examined the sequences of EEF1A1/A2 mRNA 5'UTRs and found that both mRNAs contain a hairpin-like structures in the abovementioned regions (Fig. 1, *A*, *B*), suggesting that the A1 and A2 mRNAs are translated with high efficiency. To find any known regulatory motifs, the sequences of the 5'UTRs of EEF1A1/A2 mRNAs were also analy-

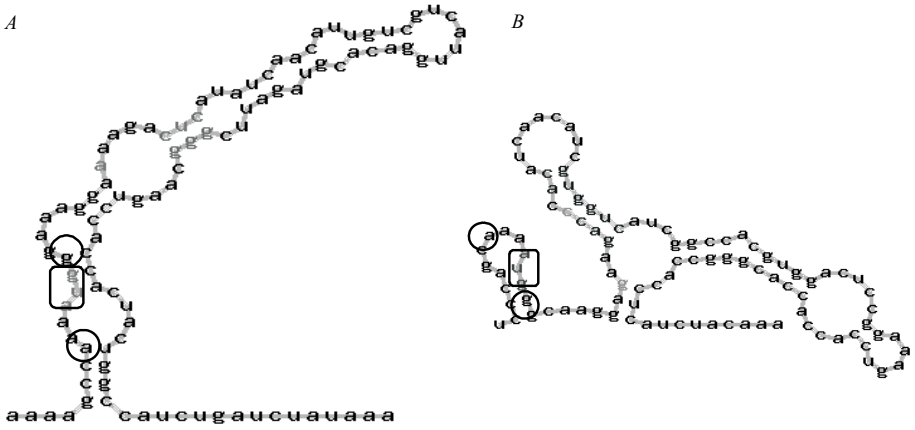

Fig. 1. Secondary structure of the region located downstream to the AUG start codon of the EEF1A1 (*A*) and EEF1A2 (*B*) mRNAs, predicted by AUG_hairpin server. Start codon marked by rectangle, Kozak context marked by circles

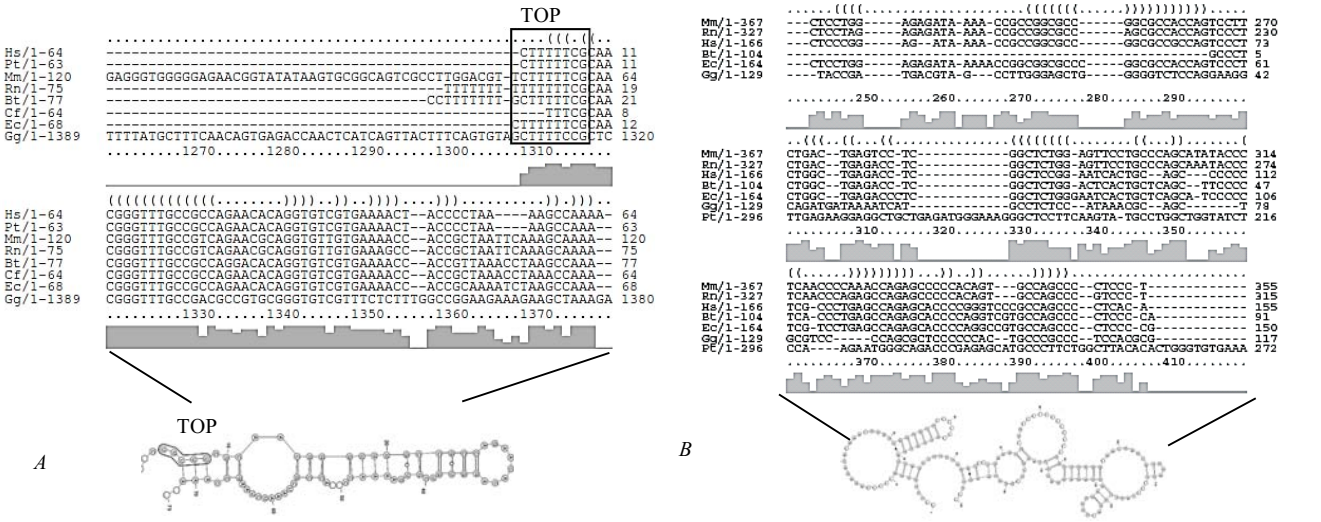

Fig. 2. Conserved secondary structure of EEF1A1 (*A*) or EEF1A2 (*B*) mRNA.The sequences of 5'UTRs of mRNAs from different species were aligned using ClustalW [27].The consensus secondary structure was predicted by RNAalifold [26]. Terminal oligopyrimidine tract (TOP) in the 5'UTR of EEF1A1 mRNA is marked with rectangle in the alignment and localized in the structure. Grey bars represent the level of conservation

zed using UTR scan [21] server. Any known motifs were not found in the 5'UTR of EEF1A2 mRNA, however, a terminal oligopyrimidine tract (TOP) was found in the 5'UTR of the mRNA coding for EEF1A1 isoform, in agreement with earlier investigations [22] (Fig. 2, *A*). TOP is a stretch of pyrimidins that is found in the 5'UTRs of the TOP class mRNAs. This class of mRNAs is actively translated in response to the mTOR signal cascade activation [23]. We found that EEF1A1 but not EEF1A2 mRNA belongs to the TOP class of mRNAs. Finally, we predicted the conserved secondary structure of the 5'UTRs of mRNA coding for the isoforms based on alignment of mRNAs of several mRNAs using RNA alifold [24–26].

Interestingly, the TOP element of EEF1A1 mRNA is folded into a stem-loop structure, with the oligopyrimidine sequence located at the 5'stem (Fig. 2, *A*), so the secondary structure of the TOP element may be important for its functioning. The 5'UTR of EEF1A2 mRNA also contains a conserved secondary structure (Fig. 2, *B*). However, structural and sequence alignment of the predicted structures of the 5'UTR of EEF1A2 mRNA with Rfam database [27] did not give statistically relevant results, thus, to establish its function and trans-factors that often bind to such stem-loops further experimental investigations are needed.

The 3'UTR of EEF1A1 and EEF1A2 mRNAs. m i c r o R N A. microRNAs are small RNAs, approximately 25–20 nucleotides (nt) long, that play a significant role in the post-transcriptional regulation of gene expression by destabilization of mRNA or inhibition of its translation. They are involved in the regulation of almost all cellu-

lar processes [28]. The difference in the 3'UTRs of the mRNAs coding for eEF1A isoforms hints at the possibility of regulation of these mRNA by different miRNAs. Nowadays, a variety of algorithms are available for the miRNA binding sites predictions that are based on the sequence complementarity, conservation and site accessibility. Using combination of servers: PicTar [29], TargetScan [30], MicroT [31], miRANDA [32] and PITA [33] we predicted miRNAs that can differentially target the expression of the A1 and A2 isoforms. To decrease the level of false-positive results, the only miRNAs predicted by more than three algorithms were selected for analysis. Importantly, the EEF1A1 and EEF1A2 mRNAs possess a number of target sequences in their 3' UTR (Table 1). The 3'UTR of EEF1A1 comprises also so-called Brd-box motif. Brd-box is a «AGCTTTA» motif that was initially discovered in *Drosophila melanogaster* [34] and later its functionality was shown in mammals [35]. This sequence is complementary to the 5' region of special Brd-box class miRNAs. MiRANDA algorithm predicted 5 miRNAs (miR-590-5p; miR-21; miR-320a, b, c, d) that can potentially target the EEF1A1 transcript in the Brd-box motif.

Polyadenylation signal. Polyadenylation is an essential step of mRNA processing that occurs in nucleus. According to the NCBI reference database [36], the sequences of the both isoforms have canonical polyadenylation signal (polyA signal). However, using Poly_A _SVM [37] program, we predicted 2 alternative polyA signals (1726–1731bp; 2626–2631bp) in the mRNA coding for eEF1A1 isoform that located upstream to the canonical polyA signal (Fig. 3, *A*). To confirm the functionality of these signals we analyzed the available cDNAs sequences of EEF1A1 mRNA in UCSC Genome Browser [38]. A number of transcripts with shortened 3'UTRs corresponding to the mRNAs proceeded by the alternative polyA signal (1726–1731bp) were found (Fig. 3, *A*). The data obtained confirm the functionality of the predicted polyA signal *in vivo*. Moreover, this alternative polyA signal is conserved among other vertebrates (Fig. 3, *B*) and acts as the only functional polyA signal in some organisms distinct from human. In such species mRNA coding for eEF1A1 have much shorter 3'UTRs. It is possible that the appearance of a longer 3'UTR in the EEF1A1 mRNA may give an evolutionary advantage providing more abilities to regulate the eEF1A1 isoform, especially during embryonic development, where both fast and fine tuning of the gene expression are essential.

Cytoplasmic polyadenylation element binding protein. Polyadenylation of transcripts is not limited to the nucleus. Recently, a cytoplasmic polyadenylation element binding protein (CPEB) has been discovered [39]. Initially, a role of CPEB in regulation of cell cycle was investigated in Xenopus. It regulates the length of poly A tail of the cyclin B mRNA, that influences a stability of this mRNA. Later, a similar mechanism of regulation of the cell cycle, senescence and cancer progression has been found in mammals [40–42]. CPEB is recruited by the cytoplasmic polyadenylation element binding sequence (CPE) located in the 3'UTR of mRNAs. CPE sequences are U-rich. Their examples are: UUUU UAU (CDK2), UUUUAAU (Cyclin B1), UUUUUAU AAAG (G10) [43]. However, to reveal functional activity, CPE needs the downstream presence of nuclear polyA signal (AAUAAA) in close proximity, as CPEB acts in complex with the cleavage and polyadenylation specificity factor (CPSF) and polyA polymerase. Therefore, we examined the 3'UTRs of the EEF1A1/A2 mRNAs for the presence of the CPE sites that are close to the PolyA signal. Analysis of the eEF1A2 mRNA did not return any results in contrast to the eEF1A1 mRNA where 2 CPE sites were detected (Fig. 4, *A*, *B*).The first CPE site (1675–1682bp) is located 43 bp upstream of a predicted non-canonical polyA signal (Fig. 4, *A*). The second CPE site (2810–2817bp) is located quite far, 180 bp, upstream from the second predicted polyA signal (Fig. 4, *B*).

However, the functionality of the second CPE site may still be in place, as the secondary or tertiary structure of mRNA may reveal some folds leading to the sterical proximity of the CPE and polyA signals.

AU-rich elements. mRNA decay is one of the mechanisms of post-transcriptional regulation of gene expression that controls approximately 5–10 % of transcripts [44].The rate of mRNA decay is regulated by the interaction of *cis*-acting elements in the mRNAs and sequence-specific RNA-binding proteins. One of the most studied cis-acting elements is the AU-rich element (ARE) present in the 3'UTRs of several unstable mRNAs [45]. Analyses of the 3'UTRs of mRNAs coding for both isoforms [46] revealed presence of five ARE elements

Table 1
The microRNA binding sites in the 3'UTR of EEF1A1 or EEF1A2 mRNA

Brd-box miRNAs	PicTar	microT 3.0	TargetScan 5.2	miRANDA	PITA
			EEF1A1		
miR-590-5p	hsa-miR-33	hsa-miR-133a, b	hsa-miR-133a, b	hsa-miR-586; hsa-miR-33a, b	hsa-miR-608
miR-21	hsa-miR-181a–c	hsa-miR-590-3p	hsa-miR-33a, b	hsa-miR-543; hsa-miR-323-5	hsa-miR-33a, b
miR-320a–d	–	hsa-miR-543	–	phsa-miR-548n; hsa-miR-382	hsa-miR-543
–	–	–	–	hsa-miR-450a; hsa-miR-520g	hsa-miR-586
–	–	–	–	hsa-miR-520h; hsa-miR-608	hsa-miR-181a, b
–	–	–	–	hsa-miR-495; hsa-miR-451	hsa-miR-133a, b
–	–	–	–	hsa-miR-373; hsa-miR-1278	hsa-miR-590-3p
–	–	–	–	hsa-miR-876-3p; hsa-miR-516b	–
–	–	–	–	hsa-miR-511; hsa-miR-520c-3p	–
–	–	–	–	hsa-miR-20a; hsa-miR-20b	–
–	–	–	–	hsa-miR-520b; hsa-miR-888; hsa-miR-93	–
–	–	–	–	hsa-miR-1237; hsa-miR-606	–

microT 3.0	TargetScan 5.2	miRANDA	PITA
		EEF1A2	
hsa-miR-663	hsa-miR-663	hsa-miR-663	hsa-miR-663; hsa-miR-671-5p
–	hsa-miR-774	hsa-miR-671-5p	hsa-miR-661; hsa-miR-658
–	hsa-miR-675	hsa-miR-658	hsa-miR-1254; hsa-miR-324-5p
–	hsa-miR-342-5p	hsa-miR-1182	hsa-miR-637; hsa-miR-608
–	hsa-miR-31	hsa-miR-661	hsa-miR-615-5p; hsa-miR-330-5p
–	hsa-miR-1238	hsa-miR-675	hsa-miR-492; hsa-miR-744
–	hsa-miR-671-5p	hsa-miR-566	hsa-miR-639; hsa-miR-31
–	hsa-miR-658	hsa-miR-744	hsa-miR-886-5p; hsa-miR-566
–	hsa-miR-492	hsa-miR-208a	hsa-miR-596; hsa-miR-612
–	hsa-miR-566	hsa-miR-432	hsa-miR-342-5p; hsa-miR-1238
–	hsa-miR-661	hsa-miR-639	hsa-miR-652; hsa-miR-548b-3p
–	–	hsa-miR-342-5p	hsa-miR-1324; hsa-miR-145
–	–	–	hsa-miR-326; hsa-miR-553; hsa-miR-1285

N o t e. miRNAs predicted by more than 3 algorithms are underlined.

in the 3'UTR of the EEF1A1 mRNA while no ARE was detected in the 3'UTR of the EEF1A2 mRNA (Fig. 4, *C*). Moreover, the first one and the last two ARE elements are conserved (Suppl., Fig. 1), suggesting a functionality of predicted motifs. Thus, the EEF1A1 mRNA seems to be less stable than the EEF1A2 mRNA.

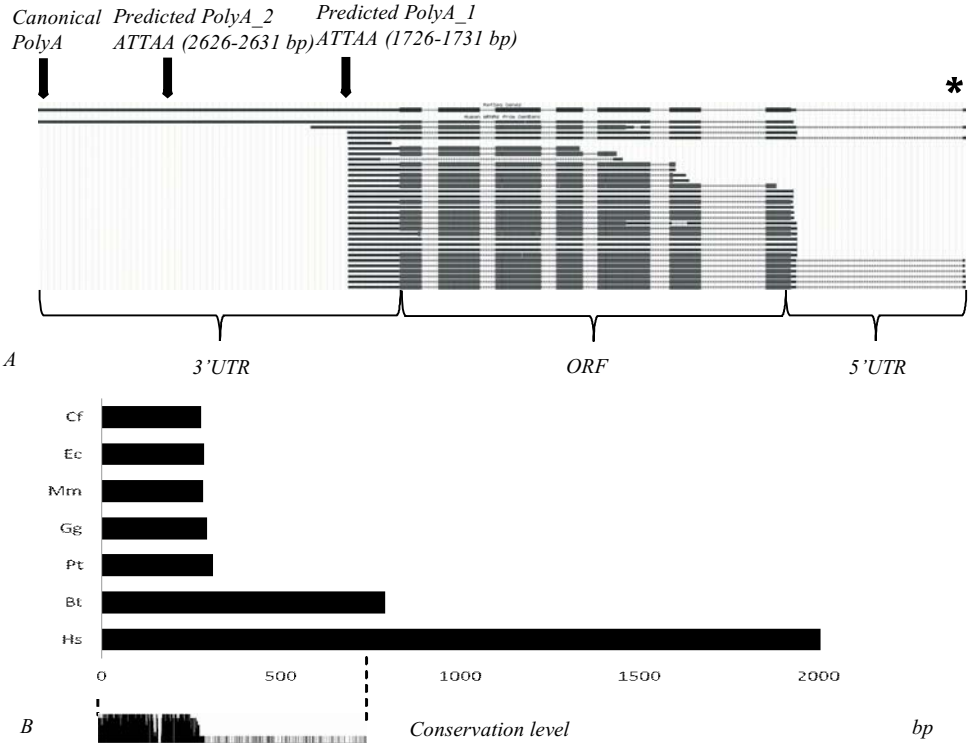

Fig. 3. *A* – graphical representation of alignment of available in GeneBank mRNA transcripts produced from EEF1A1 gene (reference mRNA is marked with a star; canonical and predicted polyadenilation sites are shown with arrows; introns marked by thin line while exons marked in bold); *B* – comparative analysis of the length of the EEF1A1 mRNA 3'UTR in different species (X-axis represents quantity of base pairs; lower diagram schematically demonstrates the level of conservation)

G-quadruplexes. G-quadruplexes are the nucleic acid sequences that are rich in guanine and capable of forming a four-stranded structure. Majority of the articles on g-quadruplexes are devoted to the g-quadruplexes in telomers [47]. However, the g-quadruplexes located in the UTR regions of mRNA have attracted much attention recently [48]. The possibility of the regulation of gene expression by g-quadruplex located within the 5'UTR of the BCL-2 mRNA was shown [49]. Also, the g-quadruplex downstream from the polyA signal was found to modulate the p53 pre-mRNA 3'-end processing [50]. GRS server was used to detect possible g-quartets in the UTRs of the EEF1A1/A2 mRNAs [51]. Contrary to the 5'UTR, where no g-quartets were found, two possible quadruplexes in the 3'UTR of the both isoforms were identified (Table 2). Thus, the expression of eEF1A1/A2 may be regulated by g-quadruplexes.

Experimental evidence for the post-transcriptional regulation of EEF1A1/A2 mRNA expression during breast cancer development. The acquired data suggest that the isoforms of eEF1A1/eEF1A2 may be differently post-transcriptionally regulated. As was mentioned earlier, the eEF1A1 isoform is proapoptotic [9], while eEF1A2 is antiapoptotic and pro-oncogenic [9, 52]. The upregulation of the eEF1A2 isoform in a non-specific tissue is associated with cancer progression [8]. Thus, we decided to examine a possibility of the post-transcriptional control of the isoforms on the model of breast cancer development in MCF-10A derived cells. The eEF1A2 isoform is not expressed in normal breast cells [7]. MCF-10A is non-malignant human mammary epithelial cell line, derived from the mortal extensive fibrocystic disease cells (marked as MCF10 on the graph) [53]. MCF-10AT/MCF-10ANeoT cells are H-Ras oncogene transformed MCF-10A cells, that are not tumorigenic, but form preneoplastic lesions (marked as NeoT) [54]. Subsequent passaging of MCF-10AT/MCF-10ANeoT cells through mouse xenografts resulted in the formation of tumorigenic MCF-10CA1a cells (marked as CA1A) [55]. The transformation of MCF-10AT/MCF-10ANeoT cells with YB-1 protein leads to the metastatic MCF-10ANeoT-YB-1cells generation (marked as YB1) that possess the mesenchymal stem cells properties. As a control to the 10ANeoT-YB-1 cells, the MCF-10ANeoT-MSCV (marked as MSCV) cells that maintain the expression of epithelial cell markers, were created [56]. Thus, all abovementioned cell lines recapitulate different stages of cancer development, from the non-cancer cells (MCF10A) through the tumorigenic (CA1A) to the metastatic cells (YB-1).

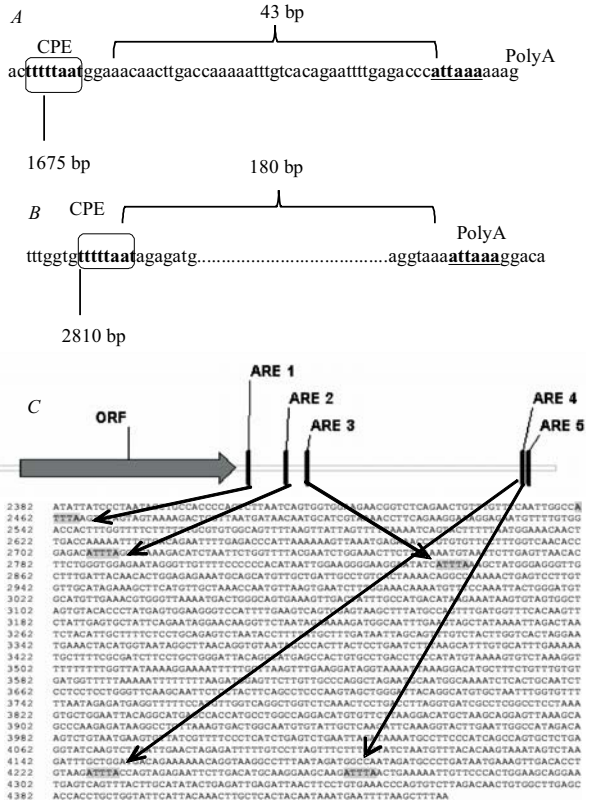

Fig. 4. *A*, *B* – potential CPE sites in the 3'UTR of EEF1A1 mRNA (CPE sites are marked in bold and outlined with rectangles; start of a CPE site and its distance to the PolyA signal are shown); *C* – AU-rich elements (ARE) in the 3'UTR of EEF1A1 mRNA (ARE elements were plotted to the 3'UTR of EEF1A1 mRNA; location of each element in the 3'UTR sequence is shown by arrows)

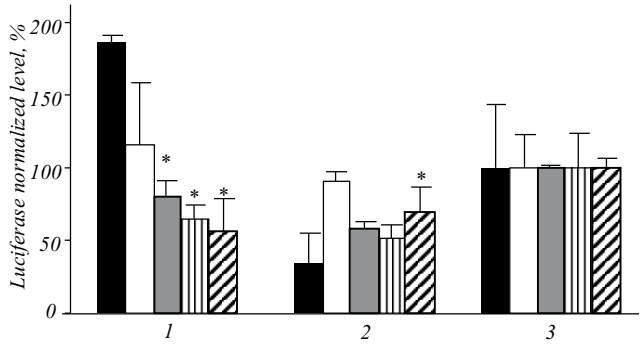

Fig. 5. Post-transcriptional regulation of the EEF1A1 and EEF1A2 mRNA expression in the MCP10A derived cell lines. MCP10A cells were transfected with *1 – pGL4.13* + EEF1A1 mRNA 3'UTR; *2 – pGL4.13* + EEF1A2 mRNA 3'UTR or *pGL4.13* reporter plasmid alone. After 24 h of expression, luciferase level was measured. Error bars represent SDs. *P < 0.05 vs control; *n* = 3. Student's t test

To demonstrate a possibility of different post-transcriptional controls of the eEF1A1/A2 expression, we expressed luciferase ORF with fused 3'UTR of EEF1A1 (pGA1_3UF) or EEF1A2 (pGA2_3UF) mRNAs in all abovementioned cells.

Table 2

G-quadruplexes in the 3'UTRs of EEF1A1 and EEF1A2 mRNAs predicted by QGRS server

Position	Length, bp	Quadruplex sequence
		EEF1A1
1893	12	GGAAGGGGAAGG
2826	25	GGTTTTTCCATGTTGGTCAGGCTGG
		EEF1A2
1564	30	GGGCGCCCGCGGCGCGACCCTCCCCGGCGG
1735	30	GGTCCAGTGGAAGTTCTTCAAGAGGAAAGG

N o t e. The start position and length of g-tetrads are shown. Guanines that assist in planar tetramers formation are underlined.

A trend to the loss of negative post-transcriptional control of pGA2_3UF in the MCF10A derived cells compared to pGA1_3UF was found (Fig. 5). The data obtained strongly argue for the different post-transcriptional regulation of the EEF1A1/A2 mRNAs in the model of breast cancer progression.

Conclusions. The data presented show that mRNAs coding for the A1 and A2 isoforms of eukaryotic translation elongation factor 1 possess distinct regulatory motifs in their 5' and 3'UTR's. We have provided experimental evidence that the EEF1A1 and EEF1A2 mRNAs may be differently regulated at post-transcriptional level, at least, in the case of model breast cancer development.

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Можливість різної посттранскрипційної регуляції ізоформ фактора елонгації eEF1A при раку молочної залози

Резюме

Евкаріотний фактор елонгації трансляції 1А представлений двома тканиноспецифічними ізоформами А1 (eEF1A1) і А2 (eEF1A2), які гомологічні на 98 % і експресуються на різних стадіях розвитку організму. Незважаючи на високу гомологію кодуючої ділянки, мРНК ізоформ значно відрізняються за 5'- і 3'-нетрансльованими послідовностями (НТП). На основі даного факту можна припустити, що посттранскрипційний контроль експресії ізоформ А1 і А2 відбувається за різними механізмами. ***Мета****. Експериментально перевірити вміст цис-регуляторних мотивів у НТП мРНК EEF1A1/ А2, а також наявність посттранскрипційного контролю експресії ізоформ.* ***Методи****. Регуляторні мотиви у НТП мРНК EEF1A1/A2 передбачено із застосуванням біоінформатичних методів. Існування посттранскрипційної регуляції експресії eEF1A1/ А2 підтверджено методом репортерних генів.* ***Результати****. Ви-*

явлено численні мотиви в НТП мРНК EEF1A1/A2. Отримано експериментальні дані, які засвідчують інгібування експресії мРНК EEF1A1 та стимулювання експресії мРНК EEF1A2 на посттранскрипційному рівні у клітинній моделі розвитку раку молочної залози. ***Висновки****. мРНК ізоформ фактора елонгації EEF1A містять відмінні один від одного мотиви в НТП і по-різному регулюються в процесі злоякісного перетворення клітин, що передбачає ймовірність регулювання їхньої експресії на посттранскрипційному рівні у відповідь на клітинні сигнали.*

Ключові слова: EEF1A1, EEF1A2, НТП, мікроРНК, рак молочної залози.

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Возможность различной посттранскрипционной регуляции изоформ фактора элонгации eEF1A при раке молочной железы

Резюме

Эукариотический фактор элонгации трансляции 1А представлен двумя тканеспецифичными изоформами А1 (EEF1A1) и А2 (EEF1A2), гомологичными на 98 % и экспрессирующимися на разных стадиях развития организма. Несмотря на высокую гомологию в кодирующей области, мРНК изоформ имеют существенно различные 5'- и 3'-нетранслируемые последовательности (НТП). На основе данного факта можно предположить, что посттранскрипционный контроль экспрессии изоформ А1 и А2 осуществляется по различным механизмам. ***Цель****. Экспериментально проверить содержание цис-регуляторных мотивов в НТП мРНК EEF1A1/A2, а также наличие посттранскрипционного контроля экспрессии изоформ.* ***Методы****. Регуляторные мотивы в 3'НТП мРНК EEF1A1/1A2 предсказаны с применением биоинформатических подходов. Существование посттранскрипционной регуляции экспрессии мРНК eEF1A1/A2 подтверждено методом репортерных генов.* ***Результаты****. Выявлены многочисленные мотивы в НТП мРНК EEF1A1/A2. Получены экспериментальные данные, свидетельствующие об ингибировании экспрессии мРНК EEF1A1, а также о стимулировании экспрессии мРНК EEF1A2 на посттранскрипционном уровне в клеточной модели рака молочной железы.* ***Выводы****. мРНК изоформ фактора элонгации eEF1A содержат различные мотивы в НТП и по-разному регулируются в процессе злокачественной трансформации клеток, что предполагает возможность регуляции их экспрессии на посттранскрипционном уровне в ответ на клеточные сигналы.*

Ключевые слова: EEF1A1, EEF1A2, НТП, микроРНК, рак молочной железы.

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