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## **Analysis of *eEF1B $\gamma$* interactome in the nuclear fraction of A549 human lung adenocarcinoma cells**

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**Aim.** To study the translation elongation factor eEF1B gamma (eEF1B $\gamma$ ) in the nucleus of lung carcinoma cells. **Methods.** The protein partners of eEF1B $\gamma$  in the nuclear fraction of A549 cells were identified by co-immunoprecipitation (co-IP) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The protein interaction network for nuclear eEF1B $\gamma$  was determined by Cytoscape 3.2.0 program using the MCODE plugin. Additional analysis of the eEF1B $\gamma$  partners was conducted by Map of the cell database. **Results.** 234 proteins interacting with eEF1B $\gamma$  in the nuclear fraction of A549 cells were identified. Possible functional networks involving these contacts were analyzed by two bioinformatic approaches. **Conclusions.** Splicing of pre-mRNA and regulation of mRNA stability are assumed to be the main processes in which nuclear eEF1B $\gamma$  can be involved. We hypothesize that a portion of eEF1B $\gamma$  leaves the cytoplasm-localized EF1B complex during carcinogenesis and enters the nucleus to regulate certain mRNAs by affecting the splicing of their pre-mRNA and/or stability of mRNA.

**Keywords:** eEF1B $\gamma$ , protein-protein interactions, nucleus, A549 cell

### **Introduction**

The high molecular weight complex of translation elongation factors, which is called eEF1B, provides efficient GDP/GTP exchange in the molecule of another translation factor, eEF1A [1]. eEF1A\*GTP binds aminoacyl-tRNA of any specificity and brings it to the pre-A site of the 80S ribosome [2, 3]. Hydrolysis of GTP finalizes the step of codon-anticodon recognition permitting an aminoacyl-tRNA to be fully established in the A site.

After some pause, length of which may depend on tRNA specificity [4], eEF1A\*GDP leaves the ribosome. In principle, the exchange of GDP for GTP in eEF1A can occur spontaneously as the cellular concentration of GTP is much higher than that of GDP. Nevertheless, the eEF1B complex accelerates the nucleotide exchange process leading to the formation of a new eEF1A\*GTP\*aminoacyl-tRNA complex [5].

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eEF1B comprises eEF1B $\alpha$ , eEF1B $\beta$  and eEF1B $\gamma$  subunits. eEF1B $\gamma$  is the only subunit of eEF1B which does not catalyze nucleotide exchange in eEF1A. Instead, it is believed to serve as “glue” holding all subunits of eEF1B together [6]. However, the existence of free eEF1B $\gamma$  was detected in human cancer tissues. Moreover, cancer cells showed a sign of nuclear localization of eEF1B $\gamma$  [7, 8]. As there is a general belief that protein synthesis occurs exclusively in cytoplasm [9], the cyto-nucleo transfer of eEF1B $\gamma$  implies some non-translational role of the subunit.

Here, in an attempt to clarify novel functions of the eEF1B $\gamma$  subunit in cancer cells, we carried out bioinformatic and experimental analysis of the protein partners of this subunit in the nuclear fraction of human lung cancer cells A549 and envisaged non-translational processes and networks involving eEF1B $\gamma$ .

As a result, we propose the splicing of pre-mRNA and regulation of mRNA stability as two main processes in which nuclear eEF1B $\gamma$  can be involved.

## Materials and Methods

### *Preparation of nuclear fraction of human lung cells*

Human lung cancer cells A549 were grown up to  $7.5 \times 10^6$  cells/ml and harvested with Trypsin-EDTA. The nuclear fraction was isolated as in [10]. Briefly, cells were lysed in 1.5 volume of the buffer containing 10 mM HEPES pH7,9; 1,5 mM MgCl<sub>2</sub>; 10 mM KCl; 0,5 % NP-40; 0,2 mM PMSF; 0,5 mM DTT for 20 min on ice. The centrifugation was performed (400g, 10 min) and the precipitate was resuspended in 4.5 volumes of the buffer (10 mM HEPES,

0.25 mM sucrose, 1.5 mM MgCl<sub>2</sub>, 10 mM KCl, 0.1 % NP-40, 0.5 mM DTT, 0.2 mM protease inhibitor PMSF) on ice for 10 min to provide the protein extraction. The sucrose cushion (2M) centrifugation was performed at 400g for 10 min. Nuclei were re-suspended in the lysis buffer and centrifuged at 1500g for 10 min. The procedure was double-repeated. The nuclear pellet was re-suspended in a half of initial volume of the nucleus lysis buffer (20 mM HEPES pH 7.9, 25 % glycerol, 0.42 M NaCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM DTT, 0.2 mM EDTA, 0.2 mM PMSF), incubated on ice for 30 min and centrifuged at 16000g for 30 min. The supernatant was used as a protein the nuclear fraction. The absence of the cytoplasmic fraction admixture was controlled by Western blot with anti-Tubulin antibodies (Abnova, Taiwan) [8].

### *Co-immunoprecipitation*

The nuclear extract was pre-cleared with Protein G Sepharose (Sigma, USA) for 1 hour at 4 °C. Anti-eEF1B $\gamma$  antibodies (Abnova, Taiwan) (1.5  $\mu$ g of antibodies per 1 mg of total protein) were added to pre-cleared lysate for overnight incubation at 4 °C. After addition of the Protein G Sepharose slurry the incubation persisted for 2 hours at 4 °C with continuous shaking. The eluted proteins were loaded on 12 % PAGE [11]. The protein bands of interest were cut and processed for mass-spectrometry analysis (LC-MS/MS).

### *Mass-spectrometry LC-MS/MS*

The nuclear extract incubated with plain G-Sepharose was used as a control of nonspecific binding. The electrophoretic bands that were not present in the control or were much

more extensive than in the control were cut and processed for mass spectrometry analysis at the Mass Spectrometry Laboratory of the Institute of Biochemistry and Biophysics (Warsaw, Poland) as described before [12].

### *Bioinformatic analysis*

Cytoscape 3.2.0 Program [13] interaction database BIOGRID was supplemented with newly identified nuclear protein partners of eEF1B $\gamma$  and analyzed by MCODE plugin which finds highly interconnected regions (clusters) in any network loaded into Cytoscape. These clusters have been shown to represent protein complexes and/or parts of pathways [14]. For the sake of clarity, the known protein partners of eEF1B $\gamma$ : eEF1A1, eEF1A2 and UBC (polyubiquitin-C) were excluded from the database [12]. Also, we limited analysis by the first (direct) partners of the eEF1B $\gamma$  partners. MCODE analysis was performed on the hybrid supercomputer "SCIT-4" of the Glushkov Institute of Cybernetics (GIC) of National Academy of Sciences of Ukraine (<http://icybcluster.org.ua>).

Analysis of the nuclear protein partners which are co-fractionated with eEF1B $\gamma$  was done using the Mapofthecell program (<http://www.mapofthecell.org>). All protein partners of the nucleus-localized eEF1B $\gamma$  identified by co-precipitation studies were tested for a possibility of their co-fractionation with eEF1B $\gamma$  as described in [10] for eEF1B $\beta$ .

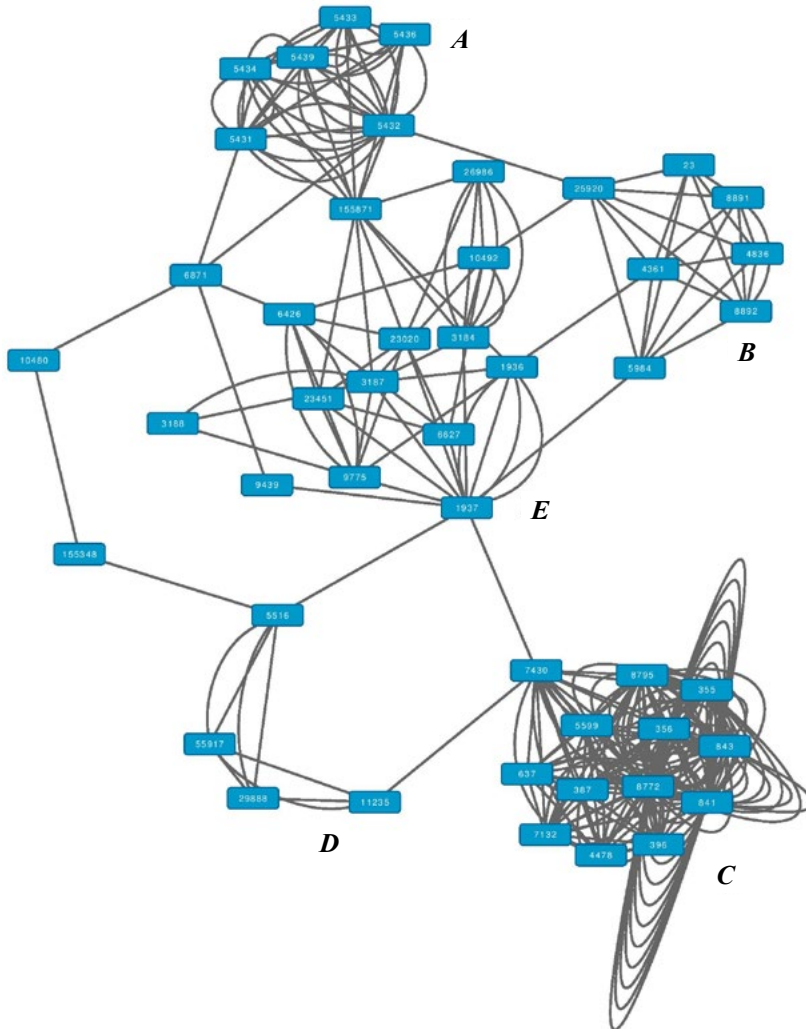
## **Results and Discussion**

Having established a procedure for the cyto-nucleo fractionation of human lung carcinoma A549 cells [10] we isolated the nuclear fraction and conducted co-immunoprecipitation of

the eEF1B $\gamma$  protein partners using anti-eEF1B $\gamma$  antibodies. Subsequent LC-MS/MS identification of precipitated proteins has delivered 234 interacting partners of eEF1B $\gamma$ .

These partners were used for further analysis by the Cytoscape program as described before [15]. Based on published data the Cytoscape protein interaction network of eEF1B $\gamma$  encompassed 11 proteins (including eEF1B $\gamma$ ) [15].

The novel protein interaction network was generated by MCODE after complementing the BioGrid database with newly identified 234 nuclear partners of eEF1B $\gamma$  in A549 cells (Fig. 1). The resulting network contained 47 proteins (including eEF1B $\gamma$ ). Several functional clusters comprising both experimentally defined and predicted by MCODE partners were observed. Cluster A comprised different polypeptides of polymerase (RNA)II (DNA directed) (POLR2B, POLR2C, POLR2D, POLR2G, POLR2E, POLR2J) involved in the transcription process. Importantly, direct interaction of eEF1B $\gamma$  with POLR2C was shown earlier [16] which presents independent evidence for eEF1B $\gamma$  contacts with the polymerase (RNA)II (DNA directed) complex. Cluster B included proteins ABCF1, EIF2B3, NMT1, EIF2B2, MRE11A, RFC4 and NELFB that are mainly involved in translation, transcription and DNA replication/repair processes, Cluster C (TNFRSF10B, TNFRSF1A, FAS, FASLG, FADD, CASP10, CASP8, BID, MAPK8, RHOA, ARHGDI, MSN, EZR) represented the membrane-related proteins involved in apoptosis, cell regulation and cytoskeleton-membrane interaction, Cluster D (PPP2CB, CTTNBP2NL, STRN4, PDCD10) comprised the proteins involved in



**Fig.1.** Protein network containing eEF1B $\gamma$ , generated by MCODE in the Cytoscape 3.2.0 Program from Human BioGRID database supplemented with co-IP/MS-MS experimental data.

cell regulation, and Cluster E (eEF1B $\beta$ , eIF4A3, eIF3M, PABPC1, SYNCRIP, HNRNPD, HNRNPH1, SNRNP20, MED23, SF3B1, SNRPA1, HNRNPH2, SRSF1, TADA2A, tat, gag-pol) included the proteins involved in translation, mRNA splicing, metabolism and transport, transcription activation as well as two proteins related to HIV-1 infection.

A complementary approach to identify the cellular processes potentially involving

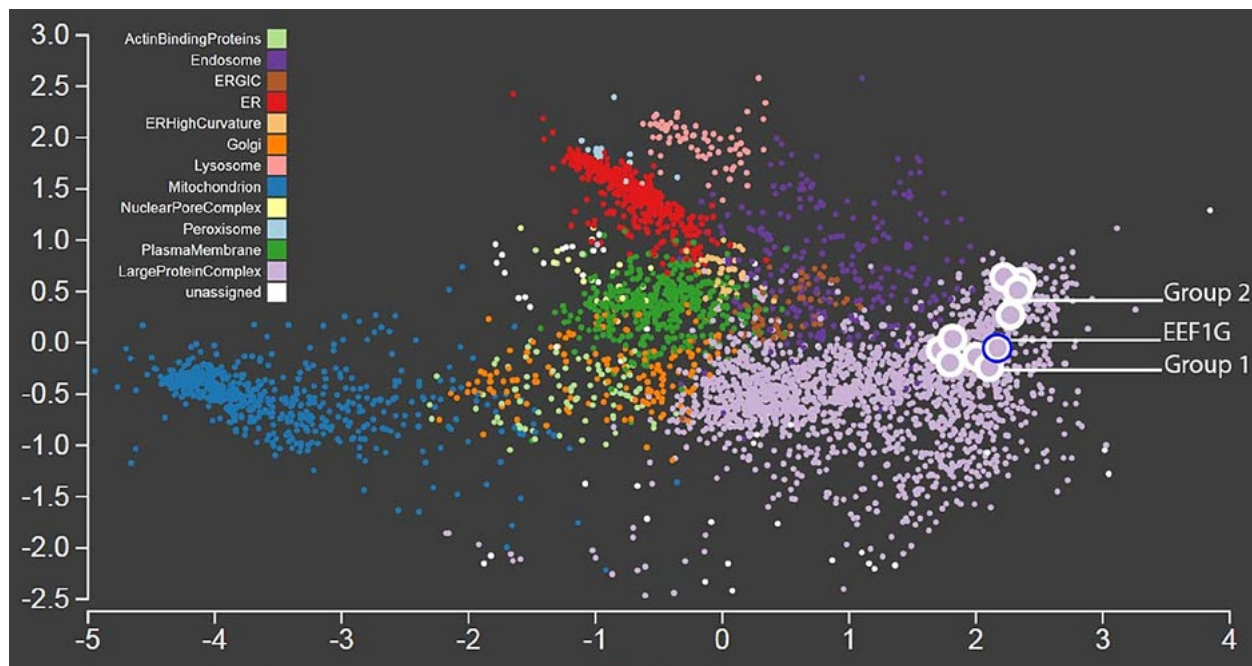
eEF1B $\gamma$  is to combine our eEF1B $\gamma$  co-immunoprecipitation data with the proteomic data derived from precise co-fractionation of the cellular proteins with eEF1B $\gamma$ , with subsequent analysis of the functions of the proteins common for both data sets. Precise co-fractionation is considered an alternative way to estimate a possibility of protein-protein interaction in cell [17]. Recently we used this approach to identify the proteins co-fractionated with the

eEF1B $\beta$  subunit of eEF1B in human cancer cells [10]. For this aim the Mapofthecell database of the subcellular localization of the proteins in cancer human cells was employed [17].

Here we utilized the same approach to find out the proteins of the nuclear fraction of human cancer cells, co-fractionated with the eEF1B $\gamma$  subunit. To do this we used the whole list of 234 protein partners of eEF1B $\gamma$  identified by Co-IP and MS in the nuclear fraction to check a possibility of their co-fractionation with eEF1B $\gamma$  according to the Mapofthecell database. Indeed, nine protein partners were found to co-localize with eEF1B $\gamma$  during precise co-fractionation (Fig. 2). All these proteins showed mixed cyto-nuclear localization according to the Subcellular localization database COMPARTMENTS (<https://compartments.jensenlab.org>) which is consistent with their pres-

ence in the nuclear fraction of A549 cells. Interestingly, two groups of the eEF1B $\gamma$  partners separated by fractionation were observed on the map (Fig. 2). ELAVL1, TIA1, CD2AP, PCBP2 and DAZAP1 represented Group 1, while SERBP1, YTHDC2, YTHDF2 and GIGYF2 represented Group 2. Peculiarly, all but one proteins possessed RNA-binding properties.

Interestingly, these proteins showed quite different abundancy in human cancer cells [17]. The copy number of the eEF1B $\gamma$  estimated as number of molecules per cell (11 587 782) was comparable with the amount of the PCBP2, SERBP1 and FLAVL1 proteins. The copy number of YTHDF2 and DAZAP1 was 15-fold, CD2AP and TIA1 about 40-fold, GIGYF2–64-fold and YTHDC2–141-fold lower that the amount of the eEF1B $\gamma$  molecules per cell.



**Fig.2.** The experimentally defined partners of nuclear eEF1B $\gamma$  (EEF1G) selected by “Mapofthecell” co-fractionation approach. Map 2 of “Mapofthecell” database was used.

The characteristics of the eEF1B $\gamma$  partners are given below.

Protein **PCBP2** (Poly(rC) binding protein 2) is a splicing factor [18], it participates in signal transduction pathways [19]. PCBP2 is a negative regulator of IRES-mediated mRNA translation [20]. PCBP2 is a well-known iron chaperon [21], it participates in regulation of antioxidant defence [22]. It is involved in apoptosis [23] and innate immune response systems [24,25].

PCBP2 is linked to the cancer development, in particular, it is overexpressed in pancreatic ductal adenocarcinoma [26], glioma [27], hepatocellular carcinoma [28]. It exemplifies pro-viral activity [29,30].

CD2AP (CD2 associated protein) is known actin/cytoskeletal regulator controlling actin organization and cellular migration [31]. It interacts with actin capping protein, directs it to different subcellular locations and modulates its activity via allosteric effects [32]. It regulates exosome cargo protein trafficking through the Golgi complex [33].

Mice with CD2AP deficiency showed such signs of glomerular disease as effacement and disorganization of the slit diaphragm, significant membrane dynamics and disrupted podocyte and endothelial integrity [34]. CD2AP participates in spermatogenesis [35]. This protein could be an important factor for Alzheimer's disease development [36]. In particular, it controls A $\beta$  generation in dendritic early endosomes [37]. Also, CD2AP exemplifies pro-viral activity, for instance it binds unstructured subunits of Chikungunya virus replicase [38] and stimulates chronic hepatitis C virus propagation and steatosis by disrupting insulin signaling [39].

**TIA1** (T cell intracellular antigen-1) is prion-related RNA-binding protein [40]. During arsenic stress inducing global shortening of 3'UTRs, TIA1 preferentially interacts with shorter 3'UTR sequences through U-rich motifs, correlating with stress granule association and mRNA decay of long 3'UTR isoforms [41]. TIA1 protein essentially contributes to the fidelity of mRNA maturation, translation, and RNA-stress-sensing pathways in human cells [42]. TIA1 is a key component of stress granules which is regulated by Zn<sup>2+</sup> ions [43]. DNA damage due to mitogens activation promotes mRNA relocation and translation in part due to dissociation of Tia1 from its mRNA targets [44].

TIA1 promotes cancer progression in different tissues [45–48]. It involved in tau-mediated neurodegeneration [49,50]. TIA1 is linked to Amyotrophic Lateral Sclerosis [51] and Welander distal myopathy [52]. TIA1 exemplifies pro-viral activity [53].

Interestingly, both CD2AP and TIA1 are involved in HIPPO signaling system [54,55].

**ELAVL1** (ELAV-like protein 1) which is also called **HuR** (human antigen R), is an RNA-binding protein involved in differentiation and stress response that acts primarily by stabilizing mRNA targets [56–58]. It binds 3'-untranslated region of BECN1/Beclin1 mRNA regulating ferroptosis, recently recognized form of controlled cell death that is characterized by lipid peroxidation, in liver fibrosis [59]. Its binding to PARG mRNA positively affects DNA repair and increases resistance to PARP inhibitors [60]. HuR/ELAVL1 binding to SCN5A mRNA increases its stability, with subsequent reduction of arrhythmic risk in heart failure [61]. HuR/

ELAVL1 is involved in telomerase function, as it associates with TERC and promotes the assembly of the TERC/TERT complex by facilitating TERC C106 methylation [62]. It participates in spermatogenesis [63].

There are a number of reports, which link pro-tumor activity of HuR/ELAVL1 with cancer of different localizations [64–68]. Surprisingly, HuR/ELAVL1 binding to different long non-coding RNAs induces opposite effect on the proliferation of different tumor cells [69,70].

HuR/ELAVL1 demonstrates pro-viral activity [71]. This protein is linked to Parkinson's disease [72]. Disruption of ELAVL1/HuR nuclear export is consistent with the effects of inborn errors of vitamin B12 (cobalamin) metabolisms on brain development, neuroplasticity and myelin formation [73].

**DAZAP1** (DAZ-associated protein 1) is an RNA-binding protein involved in mammalian development and spermatogenesis [74]. Knockdown or over-expression of DAZAP1 causes a cell proliferation defect while phosphorylation of its C-terminal domain which is sufficient to activate splicing is essential for the nuclear/cytoplasmic translocation of DAZAP1 [75]. DAZAP1 affects splicing of pre-mRNA [76]. It can regulate translation of mRNA as well [77].

**SERBP1** (Serpine1 mRNA Binding Protein 1) binds different protein partners which participate in different cellular processes. For instance, it interacts with signaling protein RACK1 involved to signal transduction, mRNA splicing and translation and the cytoskeleton [78]. It interacts with SPIN1, a maternal protein containing Tudor-like domains, which is involved in regulating maternal

transcripts to control meiotic resumption by controlling mRNA stability and/or translation [79]. One of the inhibitory mechanisms in this case is the occupation of the ribosomal mRNA entrance channel [80]. It binds dimers of activation-induced cytidine deaminase (AID) which may contribute to DNA-cleavage and recombination [81]. SERBP1 also affects DNA repair [82]. It is involved in transcriptional complex [83]. During stress SERBP1 is distributed simultaneously to cytoplasmic stress granules and nucleoli, two ribonucleoprotein-enriched subcellular compartments [84]. Cytonuclear distribution can be regulated by methylation of its arginine residues [85].

SERBP1 was markedly upregulated in prostate cancer tissues and was significantly associated with tissue metastasis and Gleason score. The loss of miR-26a-5p promotes proliferation, migration, and invasion through targeting SERBP1 [86].

**GIGYF2** (GRB10 Interacting GYF Protein 2) is a specific RNA-binding protein linked to repression of translation. It shows at least two distinct mechanisms of repression: one depends on 4EHP binding and mainly affects translation; the other is 4EHP-independent and involves the CCR4/NOT complex and its deadenylation activity [87]. Protein GIGYF2 is also a regulator of miRNA-mediated translation repression [88]. GIGYF2 is an autophagy regulator controlling neuron and muscle homeostasis [89] which is possibly involved in the regulation of signaling at endosomes [90]. It is also involved in mammalian development [91].

GIGYF2 mutations may be associated with increased risk of Parkinson's disease [92, 93] and macrocephaly [94]. Increased expression

of GIGYF2 might contribute to the development of diabetes-associated cognitive disorder via negatively regulating IGF1R signaling pathway [95].

**YTHDC2** (YTH Domain Containing 2) protein is a N6-methyladenosine (m6A) reader that specifically recognizes and binds modified nucleotides in RNA. YTHDC2 enhances the translation efficiency of its targets and also decreases their mRNA abundance [96]. Recently it was shown that YTHDC2 interacts with the small ribosomal subunit in close proximity to the mRNA entry/exit sites and controls specific mRNAs by recruitment of the RNA degradation machinery to regulate the stability of m6A-containing mRNAs and by utilizing its distinct RNA-binding domains to bridge interactions between m6A-containing mRNAs and the ribosomes to facilitate their efficient translation [97]. Regulation of gene expression by YTHDC2 is considered an evolutionarily ancient strategy for controlling the germline transition into meiosis [98, 99]. YTHDC2 is found to regulate mammalian spermatogenesis [96]. YTHDC2 promotes cancer metastasis [100].

**YTHDF2** (YTH N6-Methyladenosine RNA Binding Protein 2) is another m6A reader which reduces the stability of target transcripts [101]. Due to this, YTHDF2 plays a role in maternal-to-zygotic transition during the early life of embryos [102, 103]. YTHDF2 may recognize and bind the m6A site of FAM134B that plays a pivotal role in lipid homeostasis to reduce its mRNA lifetime and reduce its protein abundance [104]. It binds to the peroxisome proliferator-activated receptor  $\alpha$  to mediate its mRNA stability to regulate lipid metabolism [105]. It recognized and decayed

methylated mRNAs of Cyclin-A2 and kinase CDK2, thereby prolonging cell cycle progression and suppressing adipogenesis [106]. YTHDF2 plays an important role in regulating hematopoietic stem cells ex vivo expansion by regulating the stability of multiple mRNAs critical for HSC self-renewal of these cells [107]. Ythdf2 modulates neural development by promoting m6A-dependent degradation of neural development-related mRNA target [108]. YTHDF2 is mainly present in the cytosol, however, nearly all YTHDF2 translocated from the cytosol into the nucleus after heat shock [109].

YTHDF2 is a negative regulator of interferon response as it facilitates the fast turnover of interferon mRNAs and consequently helps viral propagation [110]. YTHDF2 plays positive roles in viral gene expression and HIV-1 particle assembly, suggesting that HIV-1 interacts with mRNA decay components to successfully accomplish viral replication [111, 112]. Overexpression of YTHDF2 induces more rapid viral replication, and larger viral plaques, in SV40 infected BSC40 cells [113].

YTHDF2 directly binds the m6A modification site of EGFR 3'-UTR to promote the degradation of EGFR mRNA in HCC cells acting as a tumor suppressor to repress cancer cell proliferation and growth [114,115].

The information is summarized in Table 1.

Supplementation of the bioinformatic Cytoscape approach with the experimental pull-down data permitted to pinpoint a number of different cellular processes potentially involving eEF1B $\gamma$  in nucleus. Among those could be the main molecular biological processes: replication/repair, transcription and translation. According to Cytoscape, eEF1B $\gamma$



may take part in apoptosis, cell regulation and cytoskeleton-membrane interaction, including exosomal trafficking. Use of the Mapofthecell database relying on precise cellular sub-fractionation, provided a basis for narrowing the wide functional variety of potential functions of eEF1B $\gamma$  expected from a number of its cellular partners in nuclear fraction. Consequently, we analyzed the functions of the experimentally proved eEF1B $\gamma$  partners which were predicted by both Cytoscape and Mapofthecell approaches. We reasoned that the coincidence of the functions of the eEF1B $\gamma$  partners identified by different approaches increases the

probability of eEF1B $\gamma$  involvement in fulfillment of this function.

Peculiarly, two functions based on the mRNA-binding properties of the proteins were markedly represented in both datasets. The first one is the splicing of mRNA presented by HNRNPH1, HNRNPD, SNRNP20, MED23, SF3B1 (Cytoscape) and PCBP2, DAZAP1 (Mapofthecell). Fig. 3 shows a possibility that HNRNPD, DAZAP1 and eEF1B $\gamma$  on one side and HNRNPH1, SNRNP20, MED23, SF3B1 and PCBP2 on another side can be involved in different entities in the cell so, the interaction of eEF1B $\gamma$  with the second formation can

**Table 1. The Co-IP/MS identified protein partners, which were co-fractionated with eEF1B $\gamma$ .**

№	Gene names	Protein names	Copy number/cell	RNA-binding ability	Localization		Relation to diseases
					Cyto	Nucl	
1	PCBP2	Poly(RC) Binding Protein 2	13,998,300	+	++++	++++	Pro-tumor activity, pro-viral activity
2	CD2AP	CD2 Associated Protein	276,039		++++	++++	glomerular disease, Alzheimer's disease, pro-viral activity
3	TIA1	Cytotoxic Granule Associated RNA Binding Protein	282,879	+	++++	++++	Pro-tumor activity, Alzheimer's disease, Amyotrophic Lateral Sclerosis, Welander Distal Myopathy
4	ELAVL1 (HUR) 53	ELAV Like RNA Binding Protein 1	3,678,805	+	++++	++++	Pro-tumor activity, pro-viral activity, Parkinson's Disease, Inherited disorders of cobalamin metabolism
5	DAZAP1 48	DAZ Associated Protein 1	778,860	+	++++	++++	azoospermia
6	SERBP1	SERPINE1 mRNA Binding Protein 1	9,859,640	+	++++	++++	Pro-tumor activity
7	YTHDC2	YTH Domain Containing 2	82,311	+	+++	+++	Pro-tumor activity
8	GIGYF2	GRB10 Interacting GYF Protein 2	180,458	+	++++	+++	Macrocephaly, Parkinson disease
9	YTHDF2	N6-Methyladenosine RNA Binding Protein 2	723,343	+	++++	++++	tumor suppressor, pro-viral activity

be dynamic rather than stable. Thus, nucleus-localized eEF1B $\gamma$  can be involved in splicing events. Importantly, the experimental proteomic data have suggested that eEF1B $\gamma$  could be a member of pre-mRNA 3' processing complex [116].

The second function is the regulation of stability of mRNA which involves HNRNPD, HNRNPH1 (Cytoscape) and GIGYF2, YTHDC2, YTHDF2, TIA1, ELAVL1 (MapoftheCell). MapoftheCell of these partners shows that HNRNPH1 does not seem to belong to the stable complex (Fig. 4) which cannot exclude, however, the existence of the dynamic interaction between HNRNPH1 and other members of the entity. One may suggest that eEF1B $\gamma$  can be bound to different mRNAs in nucleus contributing to their stability and transport. Notably, eEF1B $\gamma$  has been described to bind the 3' UTRs of vimentin [117] and some other mRNA [16].

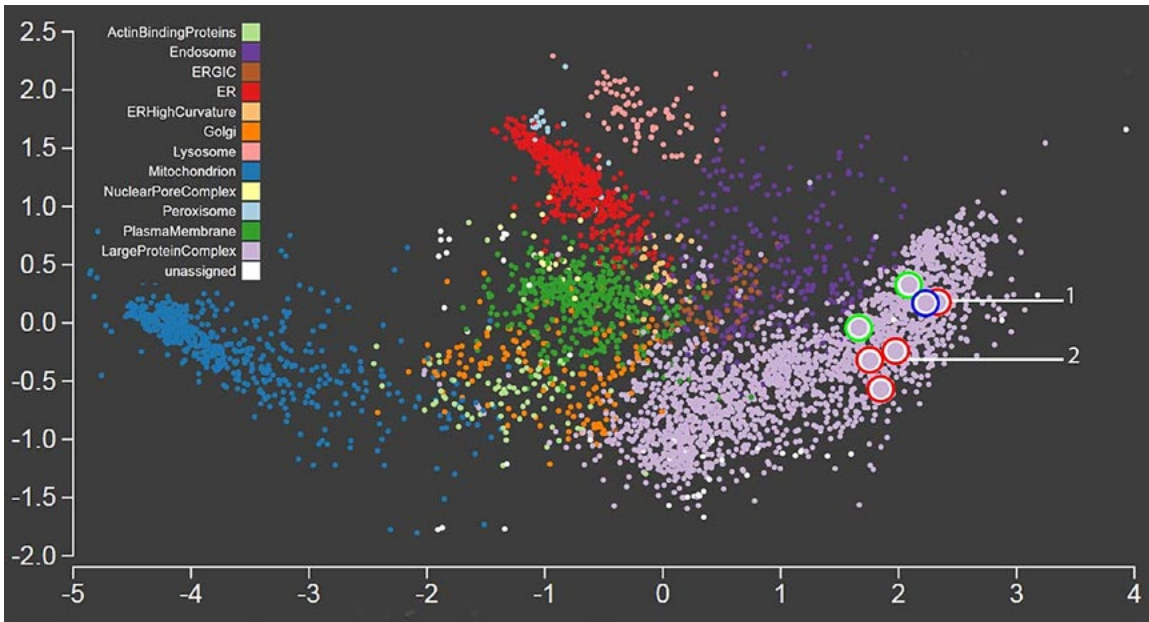
Another function of eEF1B $\gamma$  partners identified by both approaches is related to cytoskeleton-membrane link and cellular trafficking. The group comprises EZR and HNRNPH1 (Cytoscape) and CD2AP (MapoftheCell). It is widely accepted that ezrin participates in anchoring membrane proteins to the cortical actin network [118]. Nuclear localization of ezrin was also reported, however, the role of nuclear ezrin is not yet deciphered [119]. HNRNPH1 and CD2AP participate in exosome trafficking [33,120]. It is worthy to mention that a role of eEF1B $\gamma$  in organelle transport has been shown [16,121]. eEF1B $\gamma$  was also co-immunoprecipitated with an essential component of ER-Golgi transport vesicles [122]. Also, it is known that eEF1B $\gamma$  interacts with cytoskeleton [123] and membranes [124]. All

these facts permit to suggest that eEF1B $\gamma$ , along with its partners, may contribute to the cytoskeleton-membrane interaction and perform a transport role.

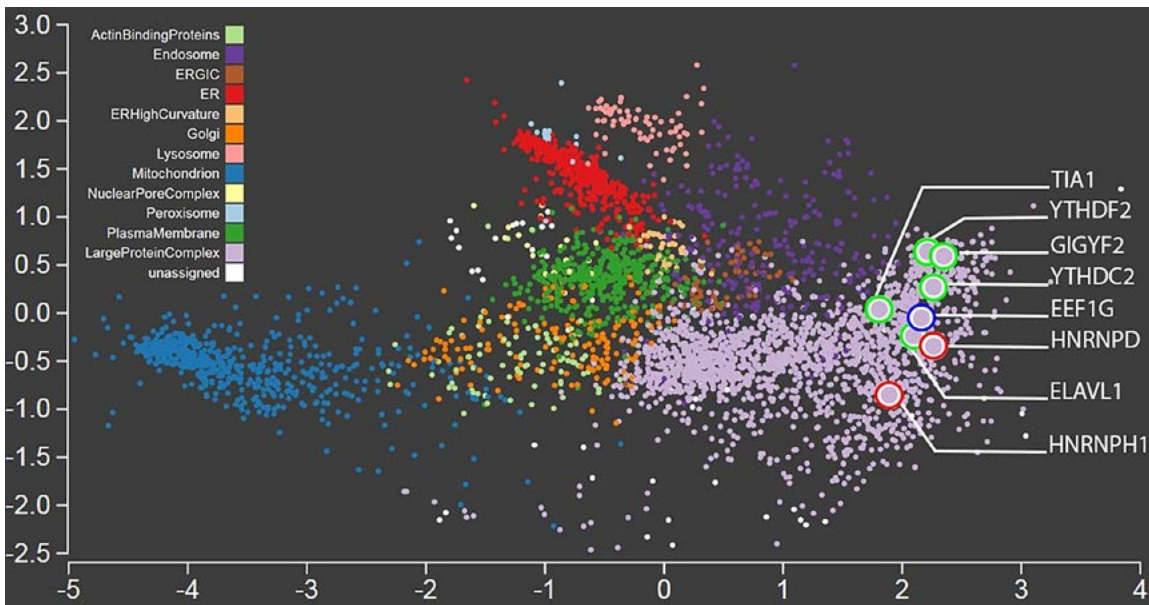
Importantly, the majority of described protein partners of eEF1B $\gamma$  in the nuclear fraction are strongly linked to cancer. Cancer-related functions of PCBP2, TIA-1, HuR/ELAVL1, SERBP1, YTHDC2, YTHDF2 (MapoftheCell database) were described above. All experimentally defined partners of eEF1B $\gamma$  picked up by Cytoscape to build molecular networks, are linked to cancer as well [2,125–132]. Based on these data one may suggest that eEF1B $\gamma$  is also associated with cancer and may play a central hub role to link together various cancer-related processes. There are a few experimental facts indicating such a possibility [7,8,133]. One may suggest that translation function of eEF1B $\gamma$  is linked to its involvement to eEF1B complex, while the induced by cancer appearance of free eEF1B $\gamma$  has regulatory consequences relying on its ability to influence pre-mRNA splicing and mRNAs stability. Subsequently, eEF1B $\gamma$  could be a novel perspective target for molecular therapy of cancer.

Another function of eEF1B $\gamma$  and its partners can be associated with viral propagation. Interestingly, 55 % of the eEF1B $\gamma$  partners picked up by MapoftheCell and 37 % of the eEF1B $\gamma$  partners picked up by Cytoscape showed pro- or anti-viral activity. Peculiarly, eEF1B $\gamma$  by itself is involved in viral infection and propagation [134–136].

Interestingly, no indication of the nuclear eEF1B $\gamma$  partners link to retinoblastoma was found, contrary to what was observed for cytoplasmic eEF1B $\gamma$  [15]. In contrast, a number



**Fig.3.** Protein partners of nuclear eEF1B $\gamma$  involved in splicing of pre-mRNA. Experimentally defined partners of eEF1B $\gamma$  (depicted in blue) predicted by Cytoscape (in red) and Mapofthecell (in green). Numbers show different groups of the partners described in the text. Map 3 of “Mapofthecell” database was used.



**Fig. 4.** Protein partners of nuclear eEF1B $\gamma$  involved in regulation of mRNA stability. Experimentally defined partners of eEF1B $\gamma$  (depicted in blue) predicted by Cytoscape (in red) and Mapofthecell (in green). Map 3 of “Mapofthecell” database was used.

of eEF1B $\gamma$  partners in the nuclear fraction were shown to be related to neurodegenerative disorders (Alzheimer's disease, Parkinson's disease, epilepsy, intellectual disability) so, possible involvement of nuclear eEF1B $\gamma$  in genesis of these diseases should be elucidated in future studies.

## Conclusions

234 proteins were identified by co-immunoprecipitation and LC-MS-MS as interacting with eEF1B $\gamma$  in the nuclear fraction of lung cancer cells A549. Possible functional networks involving eEF1B $\gamma$  and its partners were built with the use of the Cytoscape 3.2.0 program. The networks were related to the DNA replication/repair, transcription, translation, cell regulation and cytoskeleton-membrane interaction, mRNA splicing and intracellular transportation processes. Additional analysis with Mapofthecell engine based on precise protein co-fractionation, permitted to pinpoint two main processes in which nuclear eEF1B $\gamma$  may be involved. They are splicing of mRNA and regulation of mRNA stability. According to our data, eEF1B $\gamma$  may also take part in cytoskeleton-membrane linking and cellular trafficking.

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**Аналіз інтерактому eEF1B $\gamma$  в ядерній фракції клітин аденокарциноми легені людини A549**

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**Мета.** Виявити нові функції фактора елонгації трансляції eEF1B $\gamma$  (eEF1B $\gamma$ ) в ядерній фракції клітин карциноми легені людини A549. **Методи.** Білки-партнери eEF1B $\gamma$  у ядерній фракції клітин аденокарциноми легені людини A549 були ідентифіковані за допомогою ко-іммунопреципітації із наступною рідинною хроматографією та тандемною мас-спектрометрією (LC-MS/MS). Білкові мережі, до яких входить локалізований у ядрі білок eEF1B $\gamma$ , визначали за допомогою програми Cytoscape 3.2.0 із плагіном MCODE. Додатковий аналіз партнерів ядерного eEF1B $\gamma$  проводили за допомогою бази даних Mapofthecell. **Результати.** Ідентифіковано 234 білки, що взаємодіють із eEF1B $\gamma$  в ядерній фракції клітин A549. Мережі білок-білкових взаємодій, до яких залучені ці білки, були проаналізовані за допомогою двох біоінформатичних підходів. **Висновки.** Висунуто передбачення, що сплайсинг пре-мРНК та регуляція стабільності мРНК можуть бути основними процесами, у яких бере участь ядерно локалізований eEF1B $\gamma$ . Під час карциногенезу частина молекул eEF1B $\gamma$  залишає локалізований у цитоплазмі комплекс eEF1B і переходить до ядра, де регулює кількість специфічних мРНК через контроль сплайсингу відповідних пре-мРНК та вплив на стабільність мРНК.

**Ключові слова:** eEF1B $\gamma$ , білок-білкові взаємодії, ядро, клітини A549.

**Анализ интерактома eEF1B $\gamma$  в ядерной фракции клеток аденокарциноми легкого человека A549**

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**Цель.** Выявить новые функции фактора элонгации трансляции eEF1B $\gamma$  в ядерной фракции клеток карциномы легкого человека A549. **Методы.** Белки-партнеры eEF1B $\gamma$  в ядерной фракции клеток аденокарциномы легкого человека A549 были идентифицированы с помощью ко-иммунопреципитации с последующей жидкостной хроматографией и тандемной масс-спектрометрией (LC-MS/MS). Белковые сети, в состав которых входит локализованный в ядре eEF1B $\gamma$ , определяли с помощью программы Cytoscape 3.2.0 с плагином MCODE. Дополнительный анализ партнеров ядерного eEF1B $\gamma$  проводили, используя базу данных Mapofthecell. **Результаты.** Идентифицированы 234 белка, которые взаимодействуют с eEF1B $\gamma$  в ядерной фракции клеток A549. Сети белок-белковых взаимодействий, в которых участвуют данные белки, были проанализированы с помощью двух биоинформатических подходов. **Выводы.** Выдвинуто предположение, что сплайсинг пре-мРНК и регуляция стабильности зрелой мРНК могут быть основными процессами, в которых участвует локализованный в ядре eEF1B $\gamma$ . Во время карциногенеза, некоторая часть молекул eEF1B $\gamma$  оставляет локализованный в цитоплазме комплекс eEF1B и переходит в ядро, где регулирует количество специфических мРНК посредством контроля сплайсинга соответствующих пре-мРНК и влияния на их стабильность.

**Ключевые слова:** eEF1B $\gamma$ , белок-белковые взаимодействия, ядро, клетки A549.

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