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Non-canonical interactions of the β subunit of the translation elongation complex eEF1B and analysis of their possible functional role

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Aim. To predict protein networks which may comprise the β subunit of the translation elongation complex eEF1B in lung carcinoma cell line. **Methods.** The protein partners of eEF1Bβ from cytoplasmic extract of A549 cells were identified by co-immunoprecipitation (co-IP) combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS). The molecular interaction network for eEF1Bβ was predicted and visualized by a Cytoscape 3.2.0 program using an MCODE plugin. GO analysis of cellular distribution was performed by a STRAP program. **Results.** 162 high-scored proteins interacting with eEF1Bβ in the cytoplasm of lung carcinoma cells A549 have been identified by mass-spectrometry. Possible functional networks involving these contacts were predicted bioinformatically. **Conclusions.** Four protein networks are identified as possible targets of eEF1Bβ in lung cancer cells. These groups are involved in the cell cycle regulation; DNA replication and repair; chromatin remodeling; chaperoning and signal transduction. The data allow to narrow down further search for non-canonical cancer-related function of eEF1Bβ.

Keywords: eEF1Bβ, protein-protein interactions, A549 cells

Introduction

The human translation elongation machinery is responsible for the elongation of a polypeptide chain by the 80S ribosome. The multisubunit complex eEF1B provides nucleotide exchange in the eEF1A molecule. The latter, being in GTP form, supplies aminoacyl-tRNA to the ribosomal A site. Correct codon-anticodon recognition persuades the GTP hydrolysis, after which eEF1A*GDP leaves the ribosome for another round of the GDP/GTP exchange. eEF1B comprises three subunits, among which eEF1Bα and eEF1Bβ are functional whereas a role of eEF1Bγ is not yet deciphered.

For a long time the eEF1B complex was considered very stable, however, recently we have found that some pool of its subunits could exist separately from the complex in different human cancer tissues [1–3]. The role of non-complexed subunits remains elusive. Few studies have suggested that eEF1Bβ may play a role in carcinogenesis, by currently unknown means [4–7]. Some non-canonical duties of eEF1Bβ have been revealed recently [8–10]. We reason that eEF1Bβ, either involved in eEF1B or not, may stably or transiently join other protein complexes, fulfilling in such way its cancer-related functions. One of the ways to approach these functions is the identification of non-translational contacts of the

eEF1B subunits in the cancer cell. Here, we present the combination of our experimental and bioinformatics data permitting to predict for the first time possible non-translational interaction networks of the eEF1Bβ subunit in the cytoplasm of lung carcinoma cells A549.

Materials and Methods

Obtaining cytoplasmic fraction

A549 cells were cultured in DMEM (Sigma, USA) growth medium with 10% FBS (Sigma, USA) and 1 % penicillin/streptomycin (Sigma). The cells were grown up to 7×10^6 cells/ml and harvested with Trypsin-EDTA. Cytoplasmic and nuclear fractions were obtained as described in [11] with modifications. The cells were resuspended in 1.5 volume of lysis buffer (10 mM HEPES pH7.9, 1.5 mM MgCl2, 0.5 % NP-40, 0.2 mM PMSF, 0.5 mM DTT) and incubated on ice for 20 min. The suspended cells were centrifuged at 400 g for 10 min following with supernatant centrifugation at 16000 g for 30 min. The obtained fraction was used as cytoplasmic extract. The quality of the cytoplasmic fraction was analyzed by Western blot with primary mouse anti-Tubulin (cytoplasmic marker) and rabbit anti-PARP and anti-Histone 3.3 (nuclear markers) antibodies.

Co-immunoprecipitation

The cytoplasmic extract from A549 cells was incubated with Protein G Sepharose (Sigma, USA) for 1 hour at 4 °C for pre-clearing. Mouse anti-eEF1Bβ antibodies (Abnova, Taiwan) (1.5 μg of antibodies per 1 mg of total protein) were added to pre-cleared lysates and the incubation persisted overnight at 4 °C. To precipitate the antibody-protein complex, Protein G Sepharose was added according to the manufacturer's protocol and incubated for 2 hours at 4 °C. All incubations have been done with orbital shaker. The samples were analyzed by 12 % PAGE analysis. Gel was stained with the colloidal CBB-G250 [12]. The protein bands of interest were cut and processed for mass-spectrometry analysis (LC-MS/MS).

LC-MS/MS

The cytoplasmic extract incubated just with G-Sepharose was used as a control of nonspecific binding. Only the 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). The proteins from each band were digested with trypsin. MS analysis was performed using a LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific) coupled with a nanoAcquity (Waters Corporation) LC system. Spectrometer parameters were as follows: polarity mode, positive; capillary voltage, 1.5 kV. A sample was first applied to the nanoACQUITY UPLC Trapping Column (Waters) using water containing 0.1 % formic acid as the mobile phase. Next, the peptide mixture was transferred to the nanoACQUITY UPLC BEH C18 Column (Waters, 75 μm inner diameter; 250 mm long) and an ACN gradient (5–40% over 100 min) was applied in the presence of 0.1 % formic acid with a flow rate of 250 nl/min and eluted directly to the ion source of the mass spectrometer. Each LC run was preceded by a blank run to avoid sample carry-over between the analyses.

The acquired MS/MS data were pre-processed with Mascot Distiller (version 2.3.2.0, Matrix Science, London, UK). The initial search parameters were set as follows: enzyme, trypsin; variable modifications, carbamidomethyl, oxidation; peptide mass tolerance, ± 100 ppm; fragment mass tolerance, ± 0.1 Da; max missed cleavages, 1; ions score or expect cut-off, 30; max missed cleavages – 1, Swiss-Prot database with the taxonomy restricted to Homo sapiens (20348 sequences); fragmentation mode, HCD; significance threshold, $p<0.05$.

Bioinformatic analysis

To find and visualize the molecular interaction network for eEF1Bβ we used Cytoscape 3.2.0 Program. Cytoscape3.2.0 is a new powerful open source bioinformatics software platform for visualizing molecular interaction networks and integrating with

gene expression profiles and other state data [13]. The Cytoscape 3.2.0 interaction database BIOGRID was supplemented with newly identified protein partners of eEF1Bβ and analyzed by MCODE plugin which finds clusters (highly interconnected regions) in any network loaded into Cytoscape. Clusters in a protein-protein interaction network have been shown to be protein complexes and parts of pathways [14].

For the sake of clarity, such known protein partners of eEF1Bβ as eEF1A1, eEF1A2 and UBC (polyubiquitin-C) were excluded from the database as they interact with a huge number of cell proteins and create a very complicated network of protein-protein interactions that is not associated with eEF1Bβ directly. As the Human BioGRID Database (18107 proteins, 217927 protein interactions) was too huge for analysis by MCODE program, we simplified the task by taking for analysis only the first (direct) partners of eEF1Bβ 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).

Cellular component GO analysis of proteins in cluster (complex) containing eEF1Bβ was performed by STRAP (Software Tool for Researching Annotation of Proteins) Program [15].

Results and Discussion

The aim of this study was to identify protein partners of eEF1Bβ in the cytoplasmic fraction of A549 cells and to place them in the context of known protein networks in order to decipher possible novel functional routes of the eEF1B complex/free eEF1Bβ subunit in the cytoplasm of cancer cells. For identification of possible partners of eEF1Bβ we used the method of co-immunoprecipitation, with subsequent mass-spectrometric identification of the interacting proteins. 162 proteins were identified as the interacting partners of eEF1Bβ as described in Materials and Methods section.

First, we used the existing human BioGRID database to generate by MCODE the protein network containing eEF1Bβ as a target partner. The resulting network contained 35 proteins. Two of them showed direct interactions with eEF1Bβ (Fig. 1). Those are eEF1Bγ and FLNC (filamin C, gamma) and through them eEF1Bβ is connected with other functional groups. There were six evident protein groups that represent G-proteins (GNAl1, GNAl2, GNB4 – subcluster A) connected with melatonin receptor 1A; mRNA processing (CSTF2, CPSF2 – subcluster B); transcription regulation (TAF1, TAF2, TBP, RANBP2, HIST1H4A, TBL1X – subcluster C); signal transduction (CCND3, CHUK, PPP1CA, PPP1CB, IKBKB, CTNNB1, UBE2D1, NPEPPS, RYK, SLC9A3R1 – subcluster D); cell cycle regulation (BTG1, FBXL3, FBXW2, BTG2 – subcluster E); and desmosomal cadherin-plakoglobin complex (CSTA, IVL, DSP, JUP – subcluster F) (Fig. 1).

Then, to show yet unknown networking clusters of eEF1Bβ we supplemented the BioGRID database with newly discovered interacting partners of eEF1Bβ, identified by us in co-IP/MS experiments and performed the same analysis by the Cytoscape 3.2.0 program. The resulting protein cluster contained 71 proteins and showed different organization (Fig. 2; Table 1). It was possible to differentiate several functional protein groups among the main cluster (Fig. 2). The proteins of these subclusters participate in the translation (aminoacyl-tRNA synthetases IARS, RARS, MARS – subcluster A); chaperoning and signal transduction (DNAJA1, HSPA1A, HSPA1B, HSPA8, YWHAB, YWHAQ, PRKDC, CDK4 – subcluster B); mRNA processing (SRSF2, SRSF3, SON, U2AF1 – subcluster C); transcription regulation (PHC1, PHC2, PHC3, CBX4 – subcluster D); cell cycle regulation (ANAPC2, ANAPC5, ANAPC7, APC2, CDC16, CDC23, CDC26, BUB1B, TRIM33 – subcluster E); gene expression regulation and chromatin remodeling (HDAC1, MBD2, NCOR2, IRF5, TOP2B, PIAS1, SP1, BCL11B, JUN, HIST3H3, ARID1A, ARID1B, SMARCD1, SMARCD2, SMARCE 1, MORF4L2, MRGBP – subcluster F); DNA replication and repair (ORC4, ORC5, ORC6, DBF4, MCM7, TONSL, MMS22L – subcluster G) (Fig. 2). There are some

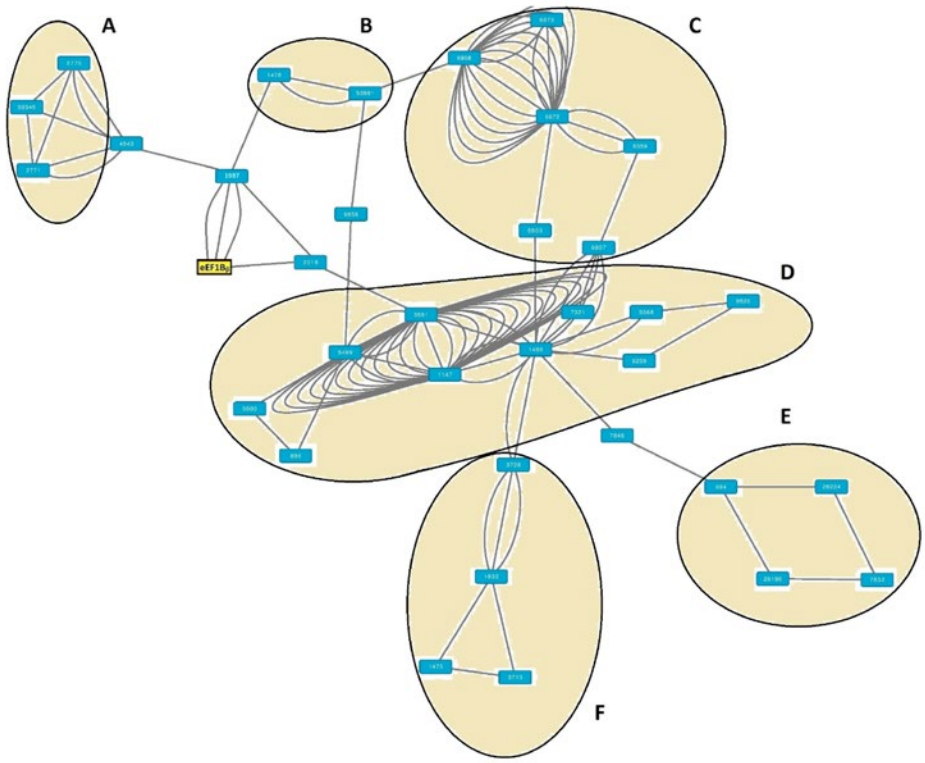

Fig. 1. Protein cluster containing eEF1Bβ generated by MCODE in Cytoscape 3.2.0. Program from Human BiaoGRID database. (Subclusters: *A* – G-proteins, *B* – mRNA processing, *C* – transcription regulation, *D* – signal transduction, *E* – cell cycle regulation, *F* – desmosomal cadherin-plakoglobin complex).

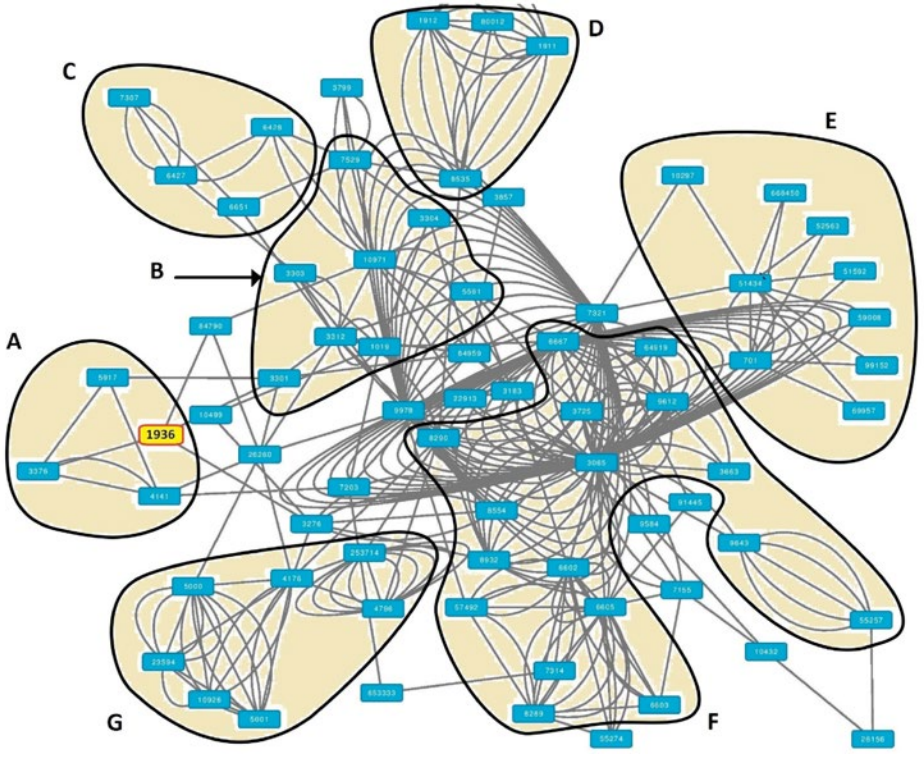

Fig. 2. Protein cluster containing eEF1Bβ (Gene ID 1936) generated by MCODE in Cytoscape 3.2.0. Program from Human BioGRID database supplemented with co-IP/MS experimental data. (Subclusters: *A* – translation related proteins, *B* – chaperones and signal transduction associated proteins, *C* – mRNA processing, *D* – transcription regulation, *E* – cell cycle regulation, *F* – gene expression regulation and chromatin remodeling, *G* – DNA replication and repair).

Table 1. **Proteins of the eEF1Bβ cluster generated by MCODE after adding to BioGRID database experimental co-I P/MS results.**

№	Entry name	Gene names	Protein names	Localization [of] Cyto	Nucl	Subclaster (according [to] Fig. 2)
1	1433B	YWHAB	14-3-3 protein beta/alpha	+	+	**B**
2	1433T	YWHAQ	14-3-3 protein theta	+		**B**
3	APC2	APC2 APCL	Adenomatous polyposis coli protein 2	+	+	**E**
4	ANC2	ANAPC2 APC2 KIAA1406	Anaphase-promoting complex subunit 2 (APC2)	+	+	**E**
5	APC5	ANAPC5 APC5	Anaphase-promoting complex subunit 5 (APC5)	+	+	**E**
6	APC7	ANAPC7 APC7	Anaphase-promoting complex subunit 7 (APC7)	+	+	**E**
7	SYRC	RARS	Arginine--tRNA ligase, cytoplasmic	+	+	**A**
8	ARI1A	ARID1A BAF250 BAF250A C1orf4 OSA1 SMARCF1	AT-rich interactive domain-containing protein 1A		+	**F**
9	ARI1B	ARID1B BAF250B DAN15 KIAA1235 OSA2	AT-rich interactive domain-containing protein 1B	+	+	**F**
10	BC11B	Bcl11b Ctip2 Rit1	B-cell lymphoma/leukemia 11B		+	**F**
11	CDC16	CDC16 ANAPC6	Cell division cycle protein 16 homolog	+	+	**E**
12	CDC23	CDC23 ANAPC8	Cell division cycle protein 23 homolog	+	+	**E**
13	CDC26	CDC26 ANAPC12	Cell division cycle protein 26 homolog	+	+	**E**
14	CDK4	CDK4	Cyclin-dependent kinase 4	+	+	**B**
15	MCM7	MCM7 CDC47 MCM2	DNA replication licensing factor MCM7	+	+	**G**
16	TOP2B	TOP2B	DNA topoisomerase 2-beta	+	+	**F**
17	PRKDC	PRKDC HYRC HYRC1	DNA-dependent protein kinase catalytic subunit	+	+	**B**
18	**DNJA1**	**DNAJA1 DNAJ2 HDJ2 HSJ2 HSPF4**	**DnaJ homolog subfamily A member 1**	+	+	**B**
19	CBX4	CBX4	E3 SUMO-protein ligase CBX4		+	**D**
20	PIAS1	PIAS1 DDXBP1	E3 SUMO-protein ligase PIAS1		+	**F**
21	RBX1	RBX1 RNF75 ROC1	E3 ubiquitin-protein ligase RBX1	+	+	
22	RN185	RNF185	E3 ubiquitin-protein ligase RNF185			
23	TRI33	TRIM33 KIAA1113 RFG7 TIF1G	E3 ubiquitin-protein ligase TRIM33		+	**E**
24	EF1D	EEF1D EF1D	Elongation factor 1-delta	+	+	**A**
25	FBX25	FBXO25 FBX25	F-box only protein 25		+	
26	HS71A	HSPA1A HSPA1 HSX70	Heat shock 70 kDa protein 1A	+	+	**B**
27	HS71B	HSPA1B	Heat shock 70 kDa protein 1B	+	+	**B**
28	HSP7C	HSPA8 HSC70 HSP73 HSPA10	Heat shock cognate 71 kDa protein	+	+	**B**

№	Entry name	Gene names	Protein names	Localization [of] Cyto	Nucl	Subclaster (according [to] Fig. 2)
29	HNRPC	HNRNPC HNRPC	Heterogeneous nuclear ribonucleoproteins	+	+	
30	HDAC1	HDAC1 RPD3L1	Histone deacetylase 1	+	+	**F**
31	H31T	HIST3H3 H3FT	Histone H3.1t		+	**F**
32	IRF5	IRF5	Interferon regulatory factor 5	+	+	**F**
33	**SYIC**	**IARS**	**Isoleucine--tRNA ligase, cytoplasmic**	+	+	**A**
34	K1C9	KRT9	Keratin, type I cytoskeletal 9		+	
35	KINH	KIF5B KNS KNS1	Kinesin-1 heavy chain	+	+	
36	MBD2	MBD2	Methyl-CpG-binding domain protein 2	+	+	**F**
37	BUB1B	BUB1B BUBR1 MAD3L SSK1	Mitotic checkpoint serine/threonine-protein kinase BUB1 beta	+	+	**E**
38	MO4L2	MORF4L2 KIAA0026 MRGX	Mortality factor 4-like protein 2		+	**F**
39	MRGBP	MRGBP C20orf20	MRG/MORF4L-binding protein		+	**F**
40	NCOA2	NCOA2 BHLHE75 SRC2 TIF2	Nuclear receptor coactivator 2		+	
41	NCOR2	NCOR2 CTG26	Nuclear receptor corepressor 2	+	+	**F**
42	ORC4	ORC4 ORC4L	Origin recognition complex subunit 4	+	+	**G**
43	ORC5	ORC5 ORC5L	Origin recognition complex subunit 5		+	**G**
44	ORC6	ORC6 ORC6L	Origin recognition complex subunit 6		+	**G**
45	PHF10	PHF10 BAF45A	PHD finger protein 10		+	
46	PHC1	PHC1 EDR1 PH1	Polyhomeotic-like protein 1		+	**D**
47	PHC2	PHC2 EDR2 PH2	Polyhomeotic-like protein 2		+	**D**
48	PHC3	PHC3 EDR3 PH3	Polyhomeotic-like protein 3		+	**D**
49	UBB	UBB	Polyubiquitin-B	+	+	
50	**ANM1**	**PRMT1 HMT2 HRMT1L2 IR1B4**	**Protein arginine N-methyltransferase 1**	+	+	
51	DBF4A	DBF4 ASK DBF4A ZDBF1	Protein DBF4 homolog A		+	**G**
52	MMS22	MMS22L C6orf167	Protein MMS22-like		+	**G**
53	SON	SON C21orf50 DBP5 KIAA1019 NREBP HSPC310 HSPC312	Protein SON		+	**C**
54	F86B2	FAM86B2	Putative protein N-methyltransferase FAM86B2			
55	RL1D1	RSL1D1 CATX11 CSIG PBK1 L12	Ribosomal L1 domain-containing protein 1		+	
56	RBM14	RBM14 SIP	RNA-binding protein 14		+	
57	RBM39	RBM39 HCC1 RNPC2	RNA-binding protein 39		+	
58	RALY	RALY HNRPCL2 P542	RNA-binding protein Raly		+	

№	Entry name	Gene names	Protein names	Localization [of] Cyto	Nucl	Subclaster (according [to] Fig. 2)
59	SRSF2	SRSF2 SFRS2	Serine/arginine-rich splicing factor 2		+	**C**
60	SRSF3	SRSF3 SFRS3 SRP20	Serine/arginine-rich splicing factor 3	+	+	**C**
61	U2AF1	U2AF1 U2AF35 U2AFBP FP793	Splicing factor U2AF 35 kDa subunit		+	**C**
62	SLAP2	SLA2 C20orf156 SLAP2	Src-like-adapter 2	+		
63	SMRD1	SMARCD1 BAF60A	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 1		+	**F**
64	SMRD2	SMARCD2 BAF60B PRO2451	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily D member 2		+	**F**
65	SMCE1	SMARCE1 BAF57	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1		+	**F**
66	TCPG	CCT3 CCTG TRIC5	T-complex protein 1 subunit gamma	+		
67	TONSL	TONSL IKBR NFKBIL2	Tonsoku-like protein	+		**G**
68	JUN	JUN	Transcription factor AP-1	+	+	**F**
69	SP1	SP1 TSFP1	Transcription factor Sp1	+	+	**F**
70	**TBA1C**	**TUBA1C TUBA6**	**Tubulin alpha-1C chain**	+	+	
71	UB2D1	UBE2D1 SFT UBC5A UBCH5 UBCH5A	Ubiquitin-conjugating enzyme E2 D1	+	+	

In bold – experimentally identified partners.

ubiquitination related proteins that work mostly as connecting links between the subclusters (UBE2D1, RBX1, UBB, UBASH3B, FBXO25).

Interestingly, the only one protein (except eEF1Bβ) was common for the cluster obtained from Human BioGRID database supplemented with co-IP/MS experimental data and the cluster obtained from only BioGRID database. It is ubiquitin-conjugating enzyme E2 D1 (UBE2D1), a member of the E2 ubiquitin-conjugating enzyme family. It participates in the ubiquitination of the tumor-suppressor protein p53 and the hypoxia-inducible transcription factor HIF1 alpha by interacting with the E1 ubiquitin-activating enzyme and the E3 ubiquitin-protein ligases. Therefore, it is possible that a eEF1Bβ takes part in the cell signaling events where p53 and/or HIF1 are involved.

Peculiarly, the only 4 out of 162 novel protein interactions were automatically selected by the Cytoscape program to build reliable protein networks, those are isoleucyl-tRNA synthetase (IARS); protein arginine methyltransferase 1 (PRMT1); DnaJ (Hsp40) homolog, subfamily A, member 1 (DNAJA1) and tubulin alpha 1c (TUBA1C). This fact may reflect the limitations of the current version of the Cytoscape program. At the same time we cannot exclude that these proteins demonstrated the strongest networking ability among the 162 partners.

Isoleucine-tRNA synthetase (Gene ID 3376) is the class-I aminoacyl-tRNA synthetase which catalyzes the aminoacylation of $tRNA^{Ile}$ by isoleucine. It belongs to the translation related proteins subcluster A (Fig. 2.). Despite eEF1Bβ was shown to interact with several aminoacyl-tRNA syhthetases, the

valyl-tRNA synthetase being a the most prominent example [16, 17], no interaction with isoleucyl-tRNA syhthetase was shown before. In the case this interaction is confirmed by other experimental means, it may shed a light on the novel structural feature of the organization of translation compartment in human cells. Interestingly, the translation-related subcluster comprised both several tRNA synthetases (IARS, RARS, MARS) and eEF1Bβ itself, the latter observation favors a possibility of forming eEF1Bβ oligomers [18].

Peculiar is the existence of a direct contact of eEF1Bβ with the protein arginine methyltransferase 1 (PRMT1) (Fig. 2). This protein (Gene ID 3276) in our scheme does not belong to any group, rather it serves as a connection link among three subclusters – translation related proteins (A), gene expression regulation and chromatin remodeling (F) and DNA replication and repair (G). PRMT1 might potentially methylate one of 16 arginine residues of eEF1Bβ. Amino acid sequence of eEF1Bβ was analyzed by GPS-MSP (Methyl-group Specific Predictor) 1.0 Program [19]. Indeed, arginine 123 (R123) was predicted as a potential methylation site with a rather high score. No arginine methylation sites were predicted in eEF1Bα and eEF1Bγ, which, together with eEF1Bβ, constitute the eEF1B complex. Thus, the interaction with PRMT1 could be important for methylation of R123 in eEF1Bβ. PRMT1 is known to be responsible for the majority of cellular arginine methylation activity [20]. Moreover, its disregulated expression may play a role in many types of cancer [21]. Recently it has been shown that PRMT1 is an important regulator of epithelial-mesenchymal transition (EMT), cancer cell migration, and invasion in non-small cell lung cancer [22]. On the other hand, there is an evidence that eEF1Bβ can participate in modulation of proliferation and EMT in cancer cells [23]. Taking into consideration these facts, a possibility of synergic action of eEF1Bβ and PRMT1 in cancerogenesis may be suggested.

Alpha tubulin TUBA1C has GTP-binding activity. GTP hydrolysis may inhibit microtubule nucleation by destabilizing the nascent plus ends required for persistent elongation. Interestingly, eEF1Bβ is a GDP/GTP exchange factor. Among TUBA1C-related pathways are transport to the Golgi and subsequent modification. This protein is also a partner of eEF1A1, which we excluded from the input data; therefore, we can suggest that eEF1Bβ interacts with TUBA1C together with eEF1A1. In the cluster (Fig. 2) TUBA 1C (Gene ID 84790) connects translation related subcluster (A) and chaperones and signal transduction associated subcluster (B).

DNAJA1 (Gene ID 3301) is a co-chaperone for chaperones HSPA8/Hsc70, HSPA1A and HSPA1B which all are present in the corresponding subcluster (B) (Fig.2). It stimulates ATP hydrolysis, but not the folding of unfolded proteins mediated by HSPA1A. Recently, eEF1A1/HSC70 have been shown to cooperatively suppress brain endothelial cell apoptosis [24]. Hsp70 and eEF1A1 interacted with HSP23 under calcium overload, showing anti-apoptotic effects [25]. It cannot be excluded that interaction of eEF1Bβ with DNAJA1 can bring to this co-chaperone another co-chaperone eEF1A1, with subsequent inhibition of apoptosis in cancer cells. Thus, the experimentally determined contacts of both TUBA1C and DNAJA1 with eEF1Bβ may be accomplished either directly or via eEF1A.

Several protein networks (B, E, F and G) (Fig. 2.) were identified as possible targets of eEF1Bβ in cancer as they contain the proteins associated with carcinogenesis.

NCOR2 (Gene ID 9612) is a nuclear receptor corepressor 2 that mediates transcriptional silencing of certain target genes and is localized in the gene expression regulation and chromatin remodeling subcluster F (Fig. 2). It is a member of the family of thyroid hormone- and retinoic acid receptor-associated co-repressors. This protein acts as part of a multisubunit complex, which includes histone deacetylases to modify chromatin structure that prevents the basal transcriptional activity of target genes. Aberrant expression of NCOR2 is associated with certain cancers [26].

JUN, jun proto-oncogene, (Gene ID 3725) is a direct partner of NCOR2 and is localized in the same

subcluster F (Fig. 2). This protein is highly similar to the viral protein and interacts directly with the specific target DNA sequences to regulate the gene expression. Its gene is intronless and is mapped to a chromosomal region involved in both translocations and deletions in human malignancies. In the study using non-small cell lung cancers (NSCLC), JUN was found to be overexpressed in 31 % of the cases in the primary and metastatic lung tumors, whereas no JUN expression was observed in the normal conducting airway and alveolar epithelia [27].

BUB1B (BUB1 mitotic checkpoint serine/threonine kinase B) is a kinase involved in the spindle checkpoint function. BUB1B (Gene ID 701) belongs to the cell cycle regulation subclaster E and is a direct partner of NCOR2 (Fig. 2). The protein is localized at the kinetochore and plays a role in the inhibition of the anaphase-promoting complex/cyclosome (APC/C), delaying the onset of anaphase and ensuring proper chromosome segregation. It may play a role in tumor suppression [28].

MMS22L, (MMS22-like, DNA repair protein) (Gene ID 253714) is localized in the DNA replication and repair subcluster G (Fig. 2). It forms a complex with tonsoku-like, DNA repair protein (TONSL). This complex recognizes and repairs DNA double-strand breaks at the sites of stalled or collapsed replication forks. It can also bind the histone-associated protein NFKBIL2 to help to regulate the chromatin state at the stalled replication forks. Finally, this protein appears to be overexpressed in the most lung and esophageal cancers [29].

CDK4, cyclin-dependent kinase 4, (Gene ID 1019) is a member of the chaperones and signal transduction associated subcluster B (Fig. 2). It belongs to the Ser/Thr protein kinase family. CDK4 is a catalytic subunit of the protein kinase complex that is important for [the] cell cycle G1 phase progression. The activity of this kinase is restricted to the G1-S phase, which is controlled by the regulatory subunits D-type cyclins and CDK inhibitor p16 (INK4a). This kinase is responsible for the phosphorylation of retinoblastoma gene product (Rb). Altered expression or functioning of CDK4 was found to be associated with tumorigenesis of a variety of cancers [30]. On the other hand, there are some evidences that eEF1Bβ signaling pathway leads to the modulation of cancer cells proliferation

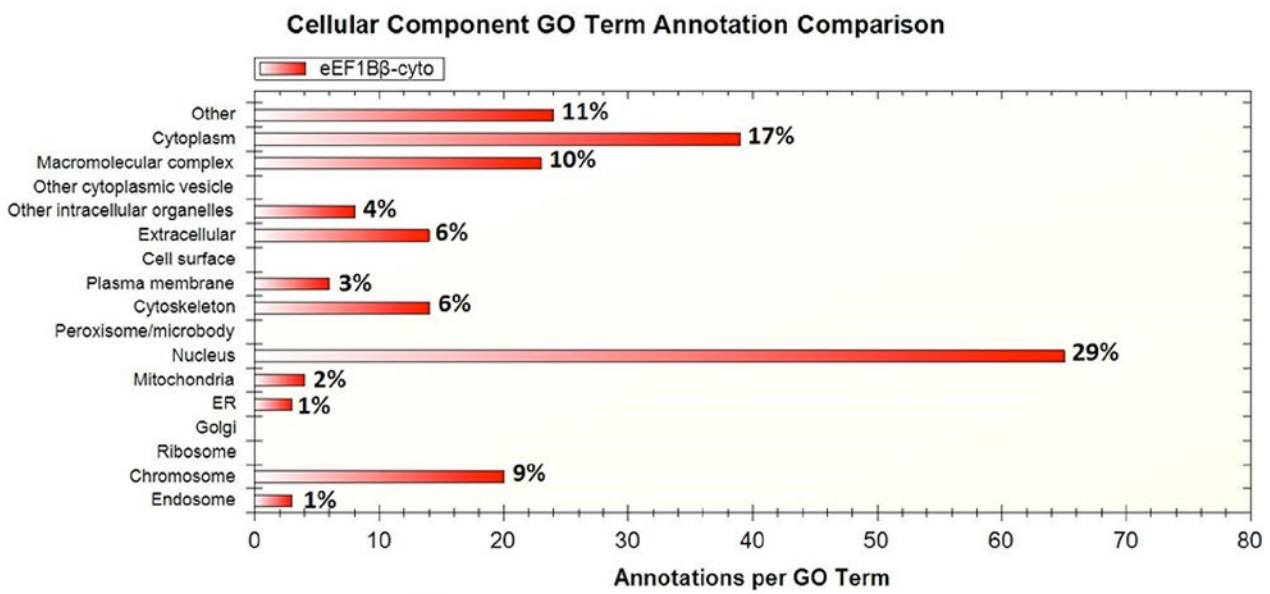

Fig. 3. Cellular component GO analysis of proteins in cluster (complex) containing eEF1Bβ.

via cyclin D1. It was shown that eEF1Bβ knockdown in cancer cells significantly decreased the cell cycling and proliferation, which were concomitant with a decrease in the cyclin D1 expression and RB phosphorylation [23].

To classify the proteins involved into eEF1Bβ networking by the subcellular localization we applied the GO analysis using the STRAP program (Fig. 3). The proteins were mainly localized to the nucleus (66 proteins) and cytoplasm (40 proteins), whereas 37 of them can be found in both cytoplasm and nucleus, and protein eEF1Bβ is among them (Table1). Interestingly, despite our "cyto-nucleo" fractionation was well controlled, some proteins considered by databases to be nuclear were detected among the cytoplasmic partners. It reinforces the recent observations that many proteins believed to be nuclear show cytoplasmic and/or organelle localization as well. There were also 20 proteins associated with chromosomes, 14 proteins with cytoskeleton, 6 proteins with plasma membrane, 4 with mitochondria, 3 with endoplasmic reticulum and 3 with endosome (Fig.3).

Conclusions

162 proteins interacting with eEF1Bβ in the cytoplasm of human cancer cells have been used to construct possible functional networks involving these contacts by the Cytoscape 3.2.0 and STRAP programs.

Four protein networks are identified as possible targets of eEF1Bβ in cancer. The groups are involved in the cell cycle regulation; DNA replication and repair; chromatin remodeling; chaperoning and signal transduction. The data permit to narrow down the field of further search for the non-canonical cancer-related function of eEF1Bβ.

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Пошук неканонічних взаємодій β субодиниці комплексу елонгації трансляції eEF1B і аналіз їх можливої функціональної ролі

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Мета. Передбачити, до яких функціональних кластерів білків клітин карциноми легені А-549 може входити субодиниця β комплексу факторів елонгації трансляції eEF1B. **Методи.** Білки-партнери eEF1Bβ, отримані з цитоплазматичного екстракту клітин А549 методом ко-імунопреципітації (co-IP), були ідентифіковані за допомогою высокоефективної рідинної хроматографії з тандемною мас-спектрометрією (LC-MS/MS). Сітка молекулярних взаємодій eEF1Bβ була передбачена і побудована за допомогою програми Cytoscape 3.2.0 з використанням плагіна MCODE. **Результати.** Методом мас-спектрометрії було ідентифіковано 162 білка, що взаємодіють з eEF1Bβ в цитоплазмі клітин карциноми легені А549. Можливі функціональні сітки, що включають ці контакти, були передбачені біоінформатично. **Висновки.** Чотири білкові сітки були ідентифіковані як можливі мішені eEF1Bβ при раку. Ці групи білків залучені в регуляцію клітинного циклу; реплікацію та репарацію ДНК, ремоделювання хроматину; шаперонну функцію та сигнальну трансдукцію. Отриманні данні дозволяють звузити поле подальшого пошуку неканонічних, пов'язаних з раком функцій eEF1Bβ.

Ключові слова: eEF1Bβ, білок-білкові взаємодії, клітини А549.

Поиск неканонических взаимодействий β субъединицы комплекса элонгации трансляции eEF1B и анализ их возможной функциональной роли

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Цель. Предсказать функциональные кластеры белков, в какие может быть вовлечена β субъединица комплекса факторов элонгации трансляции eEF1B в клетках карциномы легкого A549. **Методы.** Белки-партнёры eEF1Bβ, полученные из цитоплазматического экстракта клеток A549 методом ко-иммунопреципитации (co-IP), были идентифицированы с помощью высокоэффективной жидкостной хроматографии и последующей тандемной масс-спектрометрии (LC-MS/MS). Сеть молекулярных взаимодействий eEF1Bβ была предсказана и построена с помощью программы Cytoscape 3.2.0 с использованием плагина MCODE. **Результаты.** Методом масс-спектрометрии было идентифицировано 162 белка, взаимодействующих с eEF1Bβ в цитоплазме клеток карциномы лёгкого A549. Возможные функциональные сети, включающие эти контакты, были предсказаны биоинформатически. **Выводы.** Четыре белковые сети были идентифицированы в качестве возможных мишеней eEF1Bβ при раке. Эти группы белков вовлечены в регуляцию клеточного цикла; репликацию и реперацию ДНК, ремоделирование хроматина; шаперонную функцию и сигнальную трансдукцию. Полученные данные позволяют сузить поле дальнейшего поиска неканонических, связанных с раком функций eEF1Bβ.

Ключевые слова: eEF1Bβ, белок-белковые взаимодействия, клетки A549.

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