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Expression of different subunits of eukaryotic translation elongation factor eEF1 in human glial brain tumors.

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Eukaryotic elongation factor 1 (eEF1) mediates the binding of aminoacyl-tRNA to the ribosome in GTP-dependent manner. eEF1 consists of four subunits: eEF1A, eEF1B , eEF1B and eEF1B . eEF1A has two different isoforms: eEF1A1 is present throughout development and is ubiquitously expressed with the exception of adult muscle, while eEF1A2 is developmentally regulated and expressed only in muscle cells and neurons. Expression of eEF1A1, eEF1A2, eEF1B , eEF1B and eEF1B genes was analyzed by Northern blot hybridization of a panel of brain tumor and normal brain tissue RNAs. Totally 23 glioblastoma and 10 normal brain samples were investigated. In gliomas, no meaningful difference in the mRNA content for the eEF1A1, eEF1B and eEF1B subunits as compared to normal brain tissues was found. However, we have observed approximately 2-fold decrease in the eEF1B mRNA expression in human gliomas as compared to normal human brain by Northern blot analysis. Besides, we have shown reduced level of the eEF1A2 mRNA expression in glioblastoma as compared to normal human glia.

Keywords: eEF1, eukaryotic translation elongation factor 1, overexpression of genes, human glial brain tumors.

Introduction. Elongation factor 1 (eEF1) is a multiprotein complex playing a pivotal role in the translation of genetic information in eukaryotic cell. The complex consists of four functionally different subunits: eEF1A is responsible for the delivery of correct aminoacyl-tRNA to the A- site of mRNA-programmed 80S ribosome and supposedly facilitates the transport of deacylated tRNA back to the aminoacyl-tRNA synthetase for recharging [1]. The structurally different subunits eEF1B and eEF1B catalyze the exchange of GDP for GTP in the eEF1A molecule, and the eEF1B subunit is believed to serve as a "glue" to keep all subunits of the eEF1B complex together [2, 3, 4].

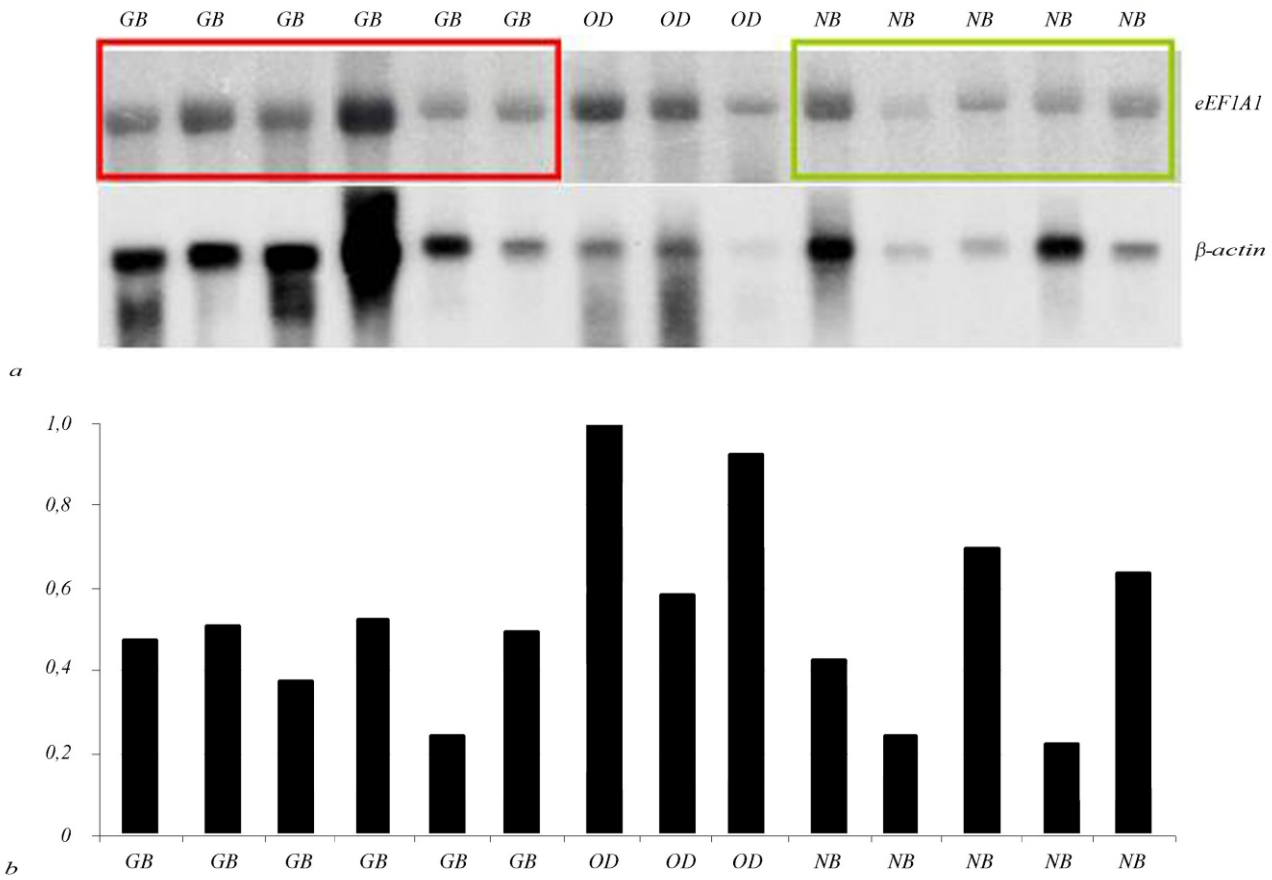

Fig.1. Northern blot hybridization of [^{32}P] marked probe of eEF1A1 cDNA and -actin cDNA with RNA samples of human brain tissue (a: NB – normal human brain; GB – glioblastoma; OD – oligodendroglioma), b – densitometric analysis.

It is becoming increasingly evident that the constituents of mammalian translation machinery play an important role in carcinogenesis. A recent research has shown clearly a potential oncogenic role of the translation elongation factors. Thus, eEF1A besides its well known housekeeping role might participate in the malignant transformation of cells. The transformation ability of eEF1A1 was assumed long time ago [5], but recently the important role in carcinogenesis has been ascribed to another isoform of eEF1A – eEF1A2. The expression of the eEF1A isoforms is mutually exclusive: eEF1A2 is found in brain and muscles while eEF1A1 is present in all other tissues of the organism. The appearance of the eEF1A2 isoform in non-inherent tissues such as ovary or mammary gland is directly linked to carcinogenesis [6, 7]. Since the eEF1A2 isoform is normally expressed in neuronal rather than glial brain tissue it is tempting to investigate if glial types of cancer could be coupled with the admission of eEF1A2.

Overexpression of the eEF1B and eEF1B subunits was also found in certain types of human cancer [8 - 12]. However, no information about expression of the eEF1B complex in brain cancer is available until now.

The aim of present work is to investigate the expression level of mRNAs coding all subunits of the eEF1 complex including the eEF1A2 isoform in human glioblastomas and normal brain.

Materials and methods. The samples of astrocytic gliomas, World Health Organization (WHO) II – IV grade, were obtained from the A.P. Romodanov Institute of Neurosurgery (Ukraine). The tumors were classified on the basis of examination of hematoxilin and eosin stained sections of the surgical specimens according to the WHO criteria. The surgical specimen of histologically normal brain tissue adjacent to the

tumor serves as a source of normal adult human brain RNA.

The samples of embryonic tissues were obtained from the Center of Embryonic Tissues "EM CELL" (Ukraine).

Plasmids with eEF1A1, eEF1A2, eEF1B , eEF1B and eEF1B cDNAs inserts were received from V. Shalak, Institute of molecular biology and genetics NASU, Ukraine, C. Knudsen, Aarhus University, Denmark and G. Sheu, Chung Shan Medical University, Taichung, Taiwan.

Total RNA was isolated from frozen tissues according to Chomczynski and Sacchi [13]. RNA (0.01 mg per lane) was electrophoretically separated in a 1.5% agarose gel containing 2.2 M formaldehyde and then transferred to a Hybond-N nylon membrane (GE Healthcare Bio-Sciences Corp., USA).

[^{32}P]-labeled probe was produced with RediPrime II kit (GE Healthcare Bio-Sciences Corp.) using fragments of the eEF1A1, eEF1A2, eEF1B , eEF1B and eEF1B cDNAs obtained after digestion of the plasmid by corresponding endonucleases [14].

RNA-containing membrane was incubated with [^{32}P]-labeled cDNA probes in 50% formamide, 5 x SSC, 5 x Denhardt's solution, 0.5% SDS and 0.01 mg/ml salmon sperm DNA at 42 ^{0}C overnight.

Extensive washing was performed twice with 2 x SSC, 0.1% SDS for 15 min at room temperature; once with 2 x SSC, 0.1% SDS for 30 min at 65 ^{0}C; and finally with 0.2 x SSC, 0.1% SDS for 30 min at 65 ^{0}C. Subsequently, the membrane was exposed to a radiographic film with an intensifying screen at -70 ^{0}C. The membrane was re-hybridized with the [^{32}P]-labeled human -actin cDNA probe used as a control of RNA gel loading.

RT-PCR was performed using 1 – 5 мg of total RNA and RevertAid M-MuLV Reverse Transcriptase ("Fermentas", Lithuania) according to the manufacture's protocol under the following conditions: 94 °C 3 min, followed by 25 cycles each of 94 °C 30 sec, 58 °C 60 sec and 72 °C 60 sec and after the last one 72 °C 7 min. PCR products were analyzed by separating in 1% agarose gel.

Primer sequences were as follows:

eEF1A1 –

f 5'GCATCCTACCACCAACTCGT 3',

r 5'CAGCATCACCAGACTTCAA 3';

eEF1A2 –

f 5'GAGAAGCGCTACGACGAGAT 3',

r 5'CTTCACCGACACGTTCTTCA 3';

eEF1B –

f 5' GCGTCAGAAAAATGGCTAC 3',

r 5' TCCTCCTCATTGTCACTGCCAAAC 3'.

Densitometric analysis of the hybridization signals was performed by the Scion Image program.

Results and discussion. Expression of the eEF1A1, eEF1A2, eEF1B , eEF1B and eEF1B genes was analyzed by Northern blot hybridization of a panel of brain tumor and normal brain tissue RNAs. Totally 23 glioblastoma and 10 normal brain samples were investigated.

The mRNA, coding for glial tissue eEF1A1 isoform, is found to be highly expressed, and no difference in the eEF1A1 mRNA level was found between normal brain and glioblastoma samples (Fig.1).

Normally the eEF1A2 isoform is expressed in neurons rather than in glial tissue. Since the samples of normal brain represented mix of glia and neurons, a positive signal of eEF1A2 expression was possible to be detected in these samples. It is thought that the appearance of eEF1A2 in non-specific for this isoform tissue is related to the carcinogenesis [6, 7]. Therefore, it is of substantial interest to research if the expression of eEF1A2 commences during glial cancer, namely in astrocytic gliomas of different grades. In control experiments the eEF1A2 cDNA was hybridized with total RNA isolated from the rabbit muscles, brain and liver. A high level of expression was observed in the muscle sample, less intense signal was detected with RNA from the brain tissue and no signal in the liver sample (Fig. 2 a). Importantly, a low occurrence of the eEF1A2 mRNA was observed in the gliomas samples (Fig.2 b).

Thus, the isoforms of eEF1A, which were directly shown oncogenic in several cell lines and tissues, do not reveal elevated level of mRNA in glioma brain tumors.

The genes coding for eEF1B and eEF1B subunits of eEF1B complex were shown to be overexpressed in different types of cancer [8 - 12]. No information about their mRNAs level in the brain tumors is now

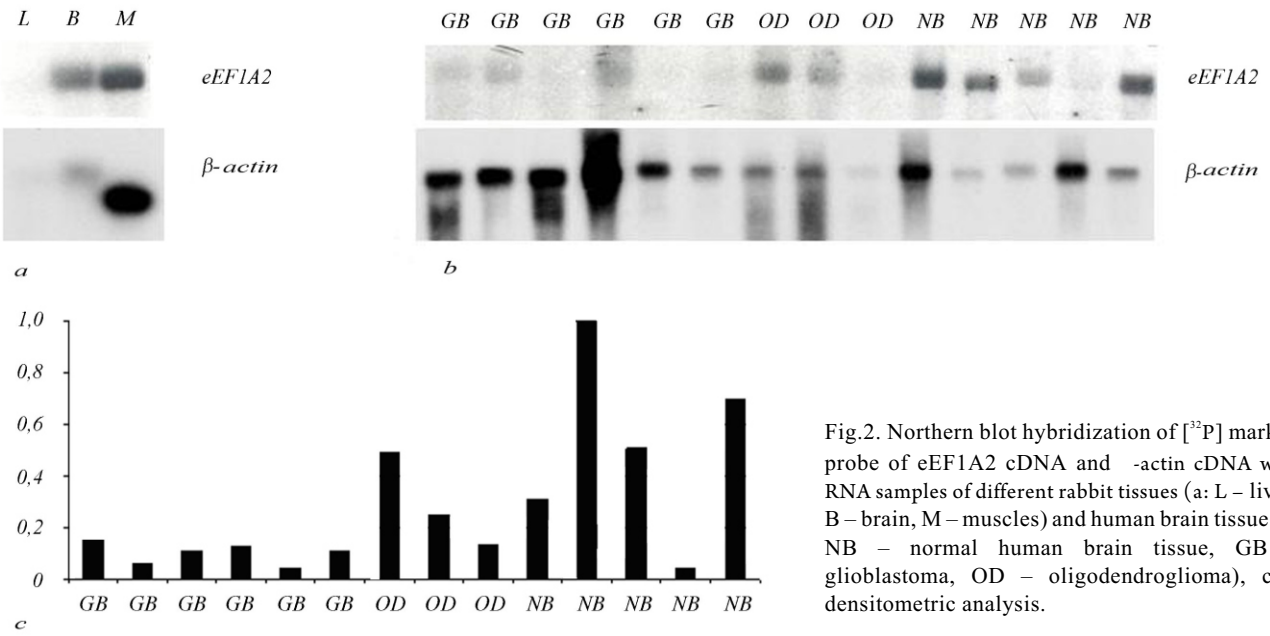

Fig.2. Northern blot hybridization of [^{32}P] marked probe of eEF1A2 cDNA and -actin cDNA with RNA samples of different rabbit tissues (a: L – liver, B – brain, M – muscles) and human brain tissue (b: NB – normal human brain tissue, GB – glioblastoma, OD – oligodendroglioma), c – densitometric analysis.

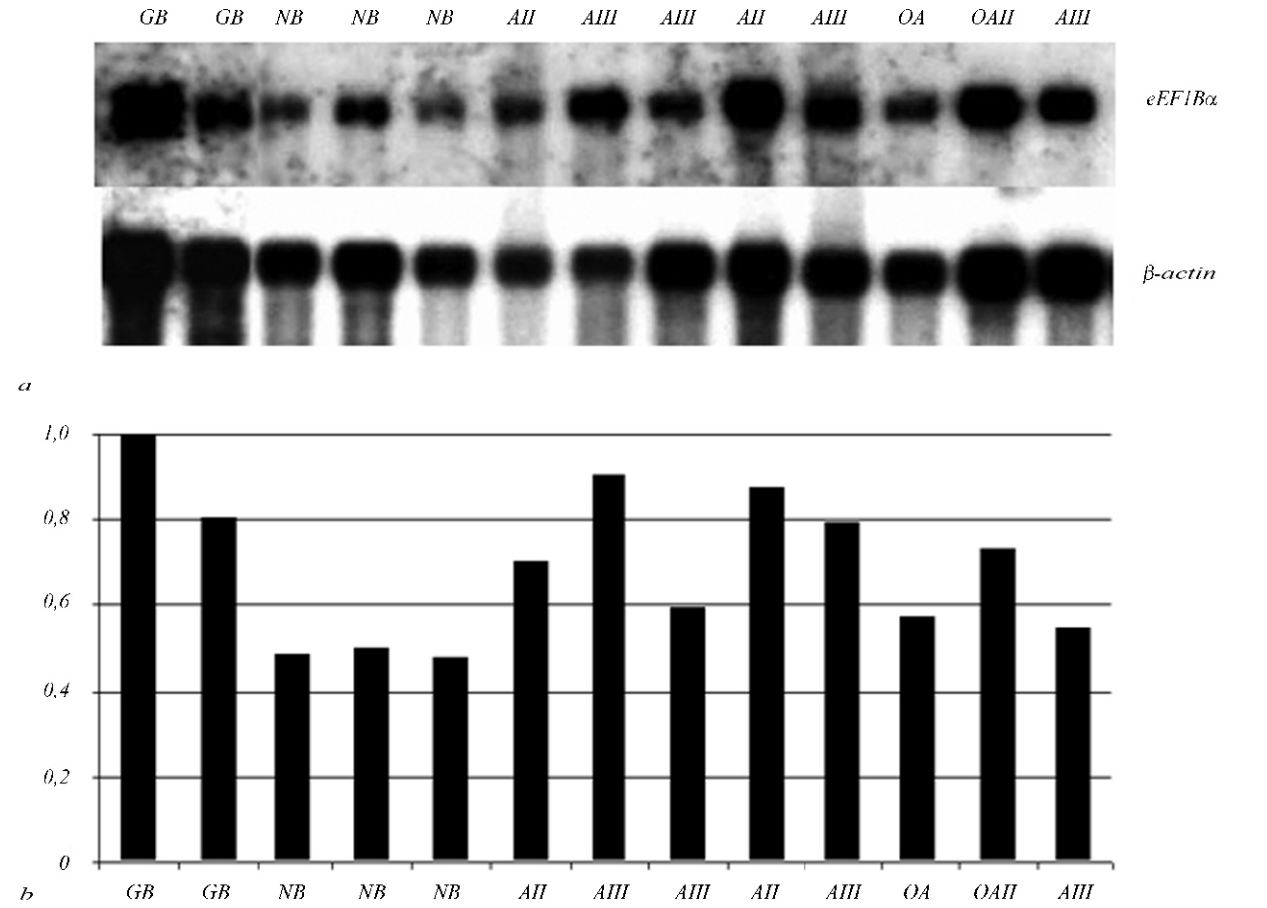

Fig.3. Northern blot hybridization of [^{32}P] marked probe of eEF1B6 cDNA and -actin cDNA with RNA samples of human brain tissue (a: NB – normal human brain, GB – glioblastoma, AII – astrocytoma II grade, AIII – astrocytoma III grade, OA – oligodendroastrocytoma, OAII – oligodendroastrocytoma II grade); b – densitometric analysis.

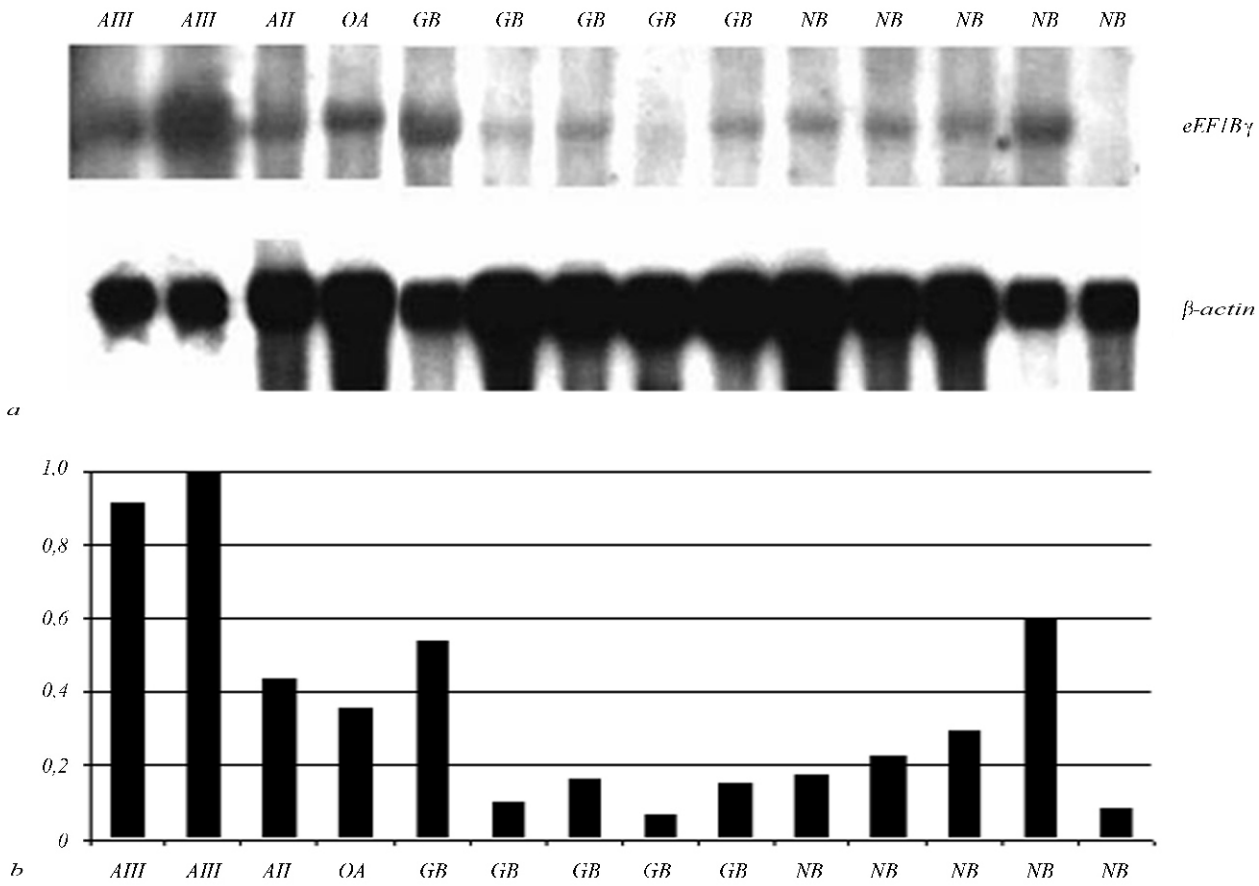

Fig.4. Northern blot hybridization of [^{32}P] marked probe of eEF1Br cDNA and -actin cDNA with RNA samples of human brain tissue (a: NB – normal human brain, GB – glioblastoma, AII – astrocytoma II grade, AIII – astrocytoma III grade, OA – oligodendroastrocytoma); b – densitometric analysis.

available. Interestingly, despite the subunit stoichiometry in the eEF1B complex being 1:1:1 the expression level of different subunits during carcinogenesis is not always coordinated [8 - 12]. In other words, overexpression of the eEF1B subunit is not necessarily accompanied by overexpression of the eEF1B subunit and vise verse. It suggests a novel, non-translational, cancer-related function of the subunits. Therefore, it is important to analyze the level of expression of the eEF1B complex subunits in the normal and cancerous brain.

No differences in the expression level of eEF1B (Fig. 3) and eEF1B (Fig. 4) genes in the brain tumor comparing to the normal brain samples were found by Northern blot hybridization of corresponding cDNAs with panel of RNAs derived from the brain tissue. If the equally high expression of eEF1B in normal and cancer tissues was not surprising (the elevated expression of this subunit was never observed during carcinogenesis), the absence of the eEF1B overexpression suggests its non-involvement in the brain cancer development, contrary to the tumors of different origin [8 - 10].

Unexpectedly, no eEF1B gene expression was detected in both normal brain and glioblastoma by the Northern blot technique (Fig. 5 a). For a positive test of the eEF1B expression we used human embryonic tissues where the eEF1B mRNA was observed (Fig.5 b). Consequently, more sensitive RT-PCR method was used to examine the eEF1B expression in the human brain. The PCR technique indeed revealed the eEF1B expression in normal brain and glioblastomas (Fig. 6). The level of eEF1B mRNA expression is rather low and could be beyond a detection limit of the Northern blot procedure. No difference between the eEF1B

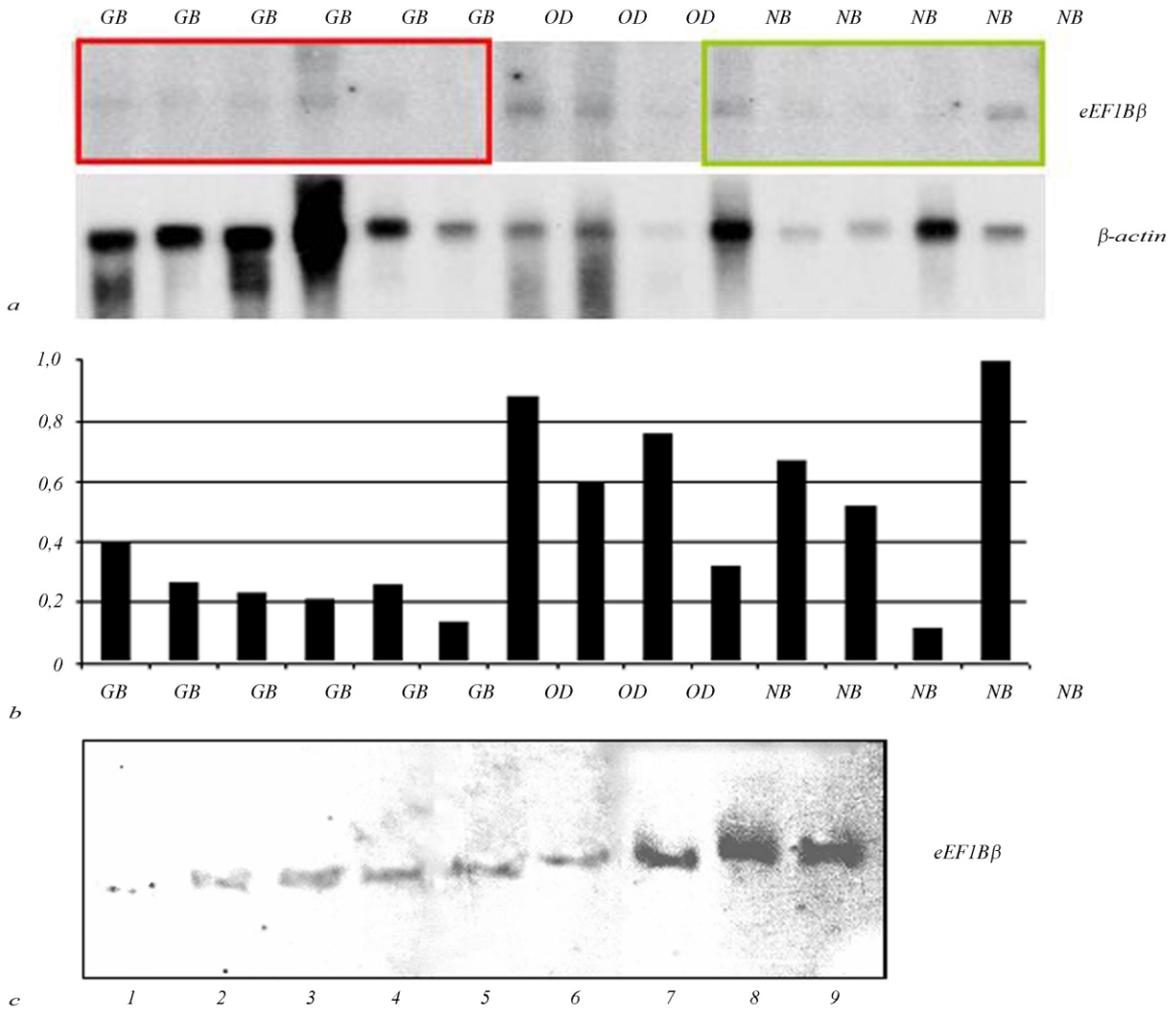

Fig.5. Northern blot hybridization of [^{32}P] marked probe of eEF1B cDNA and -actin cDNA with RNA samples of human brain tissue (a: NB – normal human brain; GB – glioblastoma; OD – oligodendroglioma) and human embryonic tissues (c: 1 – kidney, 6 weeks; 2 – kidney, 7 weeks; 3 – brain, 6 weeks; 4 – brain, 7 weeks; 5 – liver, 6 weeks; 6 – liver, 7 weeks; 7 – liver, 8 weeks; 8 – liver, 9 weeks; 9 – liver, 10 weeks), b – densitometric analysis.

mRNA level in normal brain and glioblastoma could be disclosed at present.

Low quantity of eEF1B mRNA in the brain is a surprising finding taking into account aforementioned 1:1:1 stoichiometry of the eEF1B complex. It was suggested recently that M4 muscarinic receptor could take over the GDP/GTP exchange role for eEF1A in some tissues [16] thus explaining low presence of eEF1B in the brain samples.

It is necessary to note that the differences were observed in the eEF1B subunits expression level between individual tumors, with a few tumors samples exhibiting either high or low amounts of individual transcripts. Such differences in gene expression undoubtedly contribute to the observed heterogeneity in the biological properties of cancers derived from the same organ.

Further investigation of the eEF1B subunits level by Western blotting will help to reveal more accurately the composition of the eEF1B complex in the human brain and a possible cancer-related role of eEF1B subunit.

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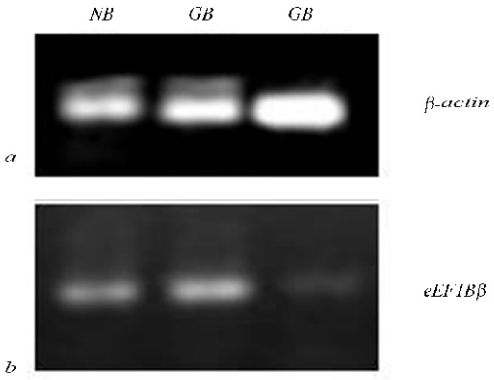

Fig.6. Gel-electrophoresis of PCR products with eEF1B (a) and -actin (b) primers (NB – human normal brain; GB – glioblastoma) in 1% agarose gel.

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Дослідження експресії різних субодиниць еукаріотного фактора елонгації трансляції eEF1 у гліальних пухлинах головного мозку людини

Резюме

Еукаріотний фактор елонгації трансляції 1 (eEF1) є одним із основних компонентів трансляційного апарату клітини, який бере участь в елонгації білкового ланцюга. eEF1 складається з чотирьох субодиниць: eEF1A, eEF1B , eEF1B і eEF1B . Існують дві тканиноспецифічні ізоформи субодиниці eEF1A – A1 і A2. Експресію генів eEF1A1, eEF1A2, eEF1B , eEF1B і eEF1B проаналізовано Нозерн-блот гібридизацією панелі РНК пухлин і нормального головного мозку людини з відповідними олігонуклеотидними зондами. Загалом досліджено 23 зразки гліальних пухлин і 10 зразків нормального головного мозку людини. Нозерн-гібридизацією визначено відсутність відмінностей в експресії мРНК субодиниць eEF1A1, eEF1B , eEF1B та зниження кількості мРНК eEF1B в гліобластомах порівняно з умовною нормою приблизно в два рази. Також показано зниження рівня експресії мРНК eEF1A2 в зразках пухлин у порівнянні з умовно нормальною глією людини.

Ключові слова: eEF1, еукаріотний фактор елонгації трансляції 1, надекспресія гена, гліальні пухлини головного мозку людини.

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Исследование экспрессии разных субъединиц эукариотного фактора элонгации трансляции eEF1 в глиальных опухолях головного мозга человека

Резюме

Эукариотный фактор элонгации трансляции 1 (eEF1) является одним из основных компонентов трансляционного аппарата клетки, участвующий в элонгации белковой цепи. eEF1 состоит из четырех субъединиц: eEF1A, eEF1B , eEF1B и eEF1B . Существуют две тканеспецифические изоформы субъединицы eEF1A (A1 и A2). Экспрессия генов eEF1A1, eEF1A2, eEF1B , eEF1B и eEF1B проанализирована Нозерн-блот гибридизацией панели РНК опухолей и нормального головного мозга человека. Всего исследованы 23 образца глиальных опухолей и 10 образцов нормального головного мозга человека. Нозерн-гибридизацией выявлено отсутствие отличий в экспрессии мРНК субъединиц eEF1A1, eEF1B , eEF1B и снижение количества мРНК eEF1B в глиобластомах по сравнению с условной нормой приблизительно в два раза. Также показано снижение уровня экспрессии мРНК eEF1A2 в опухолевых образцах по сравнению с условно нормальной глией человека.

Ключевые слова: eEF1, эукариотный фактор элонгации трансляции 1, сверхэкспрессия гена, глиальные опухоли головного мозга человека.

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