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# Influence of rat progenitor neurogenic cells supernatant on glioma 101.8 cells *in vitro*

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**Aim.** To evaluate the influence of the rat progenitor neurogenic cells supernatant (RPNS) on the transplantable rat malignant brain glioma cells (strain 101.8) under conditions of cultivation. **Methods**. primary cultures were obtained from glioma 101.8 fragments (n = 12) and intact brain of newborn rats (n = 9). RPNS was received from neurogenic cell suspensions of fetal rat brain on 8–11$^{th}$ (E8-11) and 12–16$^{th}$ (E12-16) days of gestation. **Results.** RPNS (E8-11) as well as RPNS (E12-16) showed a cytotoxic effect on the glioma 101.8 cells in short-term cultures, the level of which was dose-dependent and intensified with increasing duration of incubation. RPNS (E12-16) had a more pronounced cytotoxic action on the cells of glioma 101.8 compared with RPNS (E8-11). The cytotoxic index (CI) of RPNS (E12-16) on the glioma 101.8 cells was significantly higher than CI determined in cell suspensions of normal rat brain (CI was (91.99 ± 2.37) % and (22.9 ± 4.97) % respectively over 48 h incubation with RPNS). After RPNS (E8-11) influence on the glioma 101.8 primary cultures the signs of dose-dependent cytotoxic effects were observed: the thinning of growth areas, appearance of dystrophic and necrobiotic changes in tumour cells and decreasing of a mitotic index. These features were strengthened under the RPNS (E12-16) influence. **Conclusions.** fetal RPNS showed dose-dependent cytotoxic and antiproliferative effects on the cultivated glioma 101.8 cells, which were intensified with the increasing of rat brain gestational age and lengthening of the incubation duration . A prerequisite for such effects is likely the NPC ability to produce the substances with antitumour activity.

**Keywords:** progenitor neurogenic cells, rat fetal brain, supernatant, glioma 101.8, cytotoxic index, mitotic index.

## Introduction

Despite intensive studies of malignant gliomas throughout the world, no significant progress in their treatment has been achieved for today because of the invasiveness and high reccurence of gliomas. The combination of surgery, radio- and chemotherapy is the gold standard in the treatment of these tumors, but does not ensure its effectiveness. One of the alternative approaches to solve this problem is the using of neurogenic stem cells and progenitor cells (NSC/NPC) for the tumors targeting therapy due to the NSC/NPC ability to migrate to the site of pathology and integrate into the local microenvironment [1, 2]. NSC/NPC can be used to induce a long-term antitumor response by stimulating the immune system and to deliver in a tumor the cytolytic viruses, enzymatic converters of drugs, proapoptotic genes and genes of cytokines [1–3].

It is known that NPC can migrate to glioblastomas and induce the death of the tumor cells in mice and rats [3]; they prolonged the survival of animals or almost completely inhibited the growth of glioma [4]. The multipotent NPC of human, rat and mouse express and produce proinflammatory as well as sup-

pressor cytokines (IL-1α, IL-1β, IL-6, IL-10, TGF-β1, TGF-β2, TNF-α [5, 6], LIF [7]). However, the mechanism of the NPC antitumor properties remains unclear.

According to the previous studies, the fetal nerve cells of rats of the 18–20$^{th}$ day of gestation exhibited an antitumor effect on the experimental glioma 101.8 cells *in vitro* and *in vivo* after the joint heterologous transplantation under the kidney capsule of mice [8]. Genetic modification of the primary cell cultures of fetal neural tissue of the 10–12$^{th}$ day of gestation using cDNA TGF-α contributed to the strengthening of antitumor effect on the glioma 101.8 cells [9].

The purpose of this study was to evaluate the influence of the rat progenitor neurogenic cells supernatant (RPNS) on the cultured 101.8 glioma cells, which by the histobiological properties are close to glioblastomas, the most malignant human brain gliomas.

## Materials and Methods

The fragments of transplantable rat brain glioma (strain 101.8) (All-Russian Collection of cell cultures, Institute of Human Morphology of Russian Academy of Sciences, Moscow, Russian Federation) (n = 12) served as a material for the cultivation. Brain cells of newborn rats were used (n = 9) for comparative assessment of the studied biological product impact on the intact neural cells.

All manipulations with experimental animals were carried out in compliance with the Law of Ukraine «On protection of animals from cruelty», «European Convention for the protection of vertebrate animals used for experimental and other scientific purpose», based on the principles of bioethics and biosafety rules.

*RPNS* was prepared from a suspension of neurogenic rat brain cells on 8–11$^{th}$ (E8-11) and 16$^{th}$ (E12-16) days of gestation [10]. Native rat brain tissue obtained in these dates, was released from envelopes in buffered saline, transferred to DMEM medium («Sigma», Germany) and suspended by repeated pipetting. Cells were precipitated by centrifugation for 5 min at 1500 rev/min, washed in medium DMEM, and resuspended in fresh DMEM medium. The viability of cells in suspension was determined in a standard cytotoxic test with 0.2 % trypan blue («Merch», Germany). The concentration of cells was adjusted to $6.0\times10^6$ mg/ml, to the resulting cell suspension concanavalin A (0.10 mg/ml) was added and cells were incubated for 2 h in a $CO_2$-incubator at (37.0 ± 0.5) °C, constant humidity of 95 % and 5 % $CO_2$. After incubation, the cells were precipitated by centrifugation for 5 min at 1500 rev/min, washed in medium DMEM, resuspended in fresh DMEM medium and incubated in the same conditions for 24 h. After incubation, the cells were re-precipitated by centrifugation for 5 min at 1500 rev/min, the supernatant was taken and protein concentration in supernatant was determined by the method of Lowry. RPNS was standardized to a concentration of 1.0 mg/ml, divided into aliquots and stored at (–20.0 ± 0. 5) °C.

The composition of the obtained preparation of RPNS was investigated by electrophoresis in 1.5 % PAGE [11]. *Cytokines content* in RPNS was determined by ELISA: tumor necrosis factor alpha (TNF-α) and interleukins (IL-1β, IL-4, IL-10) – by using «Vector-Best» test kits (Novosibirsk, Russia), transforming growth factor beta (TGF-β1) – by using the test system «DRG TGF-β1 ELISA» (USA), the brain neurotrophic factor (BDNF) – by using test kit «Quantikine» (R&D Systems, USA), – according to the manufacturer's instructions.

*Glioma 101.8 model* was reproduced by intracerebral inoculation of 0.02 ml ($3.5 \times 10^5$) glioma strain 101.8 cell suspension into the left hemisphere of the brain of rats [10]. At the peak of the cancer clinical manifestations the animals were anesthetized and the tumor was removed for obtaining cultures.

*Obtaining fresh-isolated cells suspensions.* Tumor tissue or newborn rats intact brain tissue was washed in DMEM medium, released from blood vessels and envelopes, crushed with microscissors in DMEM medium and mechanically dissociated with repeated pipetting. Cells were sedimented by centrifugation for 5 min at 1500 rev/min, washed in DMEM medium, the fresh DMEM medium was added to cells precipitate and resuspended.

*The viability of cells* in suspensions was determined by the permeability of the plasma membrane to 0.2 % trypan blue [12].

*Study of RPNS action on short-term cell cultures*. RPNS (0.01, 0.10 mg/ml) was added to a suspension

of fresh-isolated cells ($2.0 \times 10^6$) which in the volume of 2 ml were incubated in biologically inert glass centrifuge tubes with periodic shaking for 24 h in a $CO_2$-incubator at a temperature of (37.0 ± 0.5) °C, constant humidity of 95 % and 5 % $CO_2$. In the suspensions the number of viable cells before and after incubation with RPNS was determined.

RPNS *cytotoxic effect* was evaluated by cytotoxic index (CI):

$$CI = \frac{VCi - VCi_{+RPNS}}{VCi} \times 100\ \%,$$

where VCi – number of viable cells in the initial suspension; $VCi_{+RPNS}$ – number of viable cells in the suspension after incubation with RPNS.

*Primary cultures* of the brain tumors and newborn rats intact brain cells were obtained under the protocol [12]. The tumor tissue or newborn rats intact brain tissue was washed in DMEM medium, released from blood vessels and envelopes, crushed with microscissors in DMEM medium and mechanically dissociated with repeated pipetting. The *c*ells were sedimented by centrifugation for 5 min at 1500 rev/min, washed in DMEM medium, the fresh DMEM medium was added to the cells precipitate and resuspended. The fresh-isolated cells in the quantity of $1 \times 10^6$ were applied onto the covering adhesive slides coated with polyethylenimine («Sigma», Germany), which were placed in Petri dishes and cultured in medium 199 and DMEM (1 : 1, 2 ml) supplemented with fetal calf serum (10 %), glucose (400 mg) and insulin (0.2 U/ml). The cell cultures were kept in a $CO_2$-incubator (37 °C, 95 % humidity and 5 % $CO_2$) and observed in inverted microscope (Biolam P-3, LOMO, St.-Petersburg, Russia).

*Study of RPNS action in primary cultures.* The cultures with a uniform zone of growth were selected (6-8$^{th}$ day), RPNS was added (0.01, 0.10 mg/ml) and cultures were incubated for 24 and 48 h. For morphological examination cultures were fixed in 10 % formalin, obtained cytological preparations were stained with Carazzi's hematoxylin.

*Methods of obtaining histological preparations.* To investigate the histostructure of experimental glioma 101.8 native tissue the tumor was removed from rat cranial cavity, fixed in 10 % formalin solution, embedded in paraffin. From these blocks the slices of 5–7 μ were prepared by sledge microtome HM430 (MICROM International GmbH, Germany) and stained with hematoxylin-eosin, hematoxylin-picrofuchsin.

*Microscopic examination* and photographic registration of histological and cytological preparations of the primary cultures were performed by light optical photomicroscope Axiophot («OPTON», Germany) with an object micrometer («Carl Zeiss», Germany), which is certified to calibrate the images increase in morphometric studies (object lens × 40, ocular x10, adapter × 2). The preparations were analyzed for the cytological changes in cellular composition and mitotic index (MI) was determined.

A cellular composition of the growth zone of primary cultures of glioma 101.8 was assessed by phenotypic features of the tumour cells and their ability to form the spatial histotypical structures specific to the type of neuroglial growth. The form of cytoplasmic bodies of tumour cells, the presence and severity of processes, the chromatin structure and nuclei form, the character of intercellular connections were evaluated. In each specimen the morphology of experimental cultures was analyzed compared with control. Cytological changes in tumour cell cultures after incubation with RPNS were analyzed by taking into account the standard cytostructural signs of cytopatogenic effect (the appearance of dystrophic and necrobiotic changes of tumour cells). The signs of growth zone overall structure rarefaction of the tested cultures due to desquamation of dead cells were evaluated. In the preserved parts of the growth zone the changes in mitotic activity were analyzed.

MI was determined by counting mitoses in three observations of each culture sample in 10 randomly selected microscope fields of view (×400). In each specimen at least 1000 cells were counted. MI was calculated using the formula:

$$MI = \frac{\text{number of cells with the presence of mitosis}}{1000} \times 100\ \%.$$

*Statistical analysis of the data* was performed using the statistical software package «Statistica 6.0», the reliability of the difference was evaluated using Student t-test.

## Results and Discussion

I. *The study of RPNS influence on the glioma 101.8 short-term cultures*. In short-term cultures, the fetal rat brain supernatants of investigated gestation terms (E8-11, E12-16) exhibited a cytotoxic effect of varying degree on the glioma 101.8 cells. The summarized evaluation of results of RPNS influence for CI indices is presented in Table 1. The level of cytotoxic influence was dose-dependent and intensified with increasing duration of the cell incubation with supernatants. RPNS (E12-16) showed a more pronounced cytotoxic effect on the glioma 101.8 cells compared with RPNS (E8-11) ($p < 0.033$).

The RPNS (E8-11) cytotoxic effect was recorded only in one third of the samples of normal rat brain cells suspensions after 24 h incubation and had a tendency to enhancement with increasing concentration and duration of incubation. RPNS (E12-16) exhibited a similar but more pronounced effect.

The RPNS (E12-16) cytotoxic action on the glioma 101.8 cells was significantly higher than CI in the normal rat brain cells suspensions ($p < 0,077$).

II. *The study of RPNS influence on the glioma 101.8 primary cultures*.

The histological examination showed that in the glioma 101.8 intact tissue the dense-cell structure dominated with the presence of «honeycomb-like» construction areas inherent to oligodendroglioma. However, in the overall structure of the tumor the rarefied netting-like structures typical for anaplastic astrocytoma were also determined. Most tumor cells contained rounded-oval nuclei with enhanced density of chromatin substance, whereas the atypical cells with atypical forms and enlarged volume of nuclei of intense color and some multinuclear cells were detected, showing the cytological features of cell polymorphism.

*Table 1.* **Rat progenitor neurogenic cells supernatant (RPNS) impact indicators in short-term cultures**

| Cell type | RPNS | Concentration of preparation, mg/ml | CI, % | | | |
|---|---|---|---|---|---|---|
| | | | 24 h | | 48 h | |
| | | | The average index (M ± m) | samples with cytotoxic effect (%) | The average index (M ± m) | samples with cytotoxic effect (%) |
| Rat glioma 101.8 (n = 12) | E 8-11 | 0.01 | 25.40 ± 4.58 $\&_1$ | 100.0 | 34.57 ± 4.09 $\&_3$ | 100.0 |
| | | 0.10 | 41.84±2.08 $\&_2\wedge_1$ | 100.0 | 48.38 ± 2.86 $\&_4\wedge_1$ | 100.0 |
| | E 12-16 | 0.01 | 68.01 ± 5.63 $\&_1*_1$ | 100.0 | 71.85 ± 0.21 $*_3\&_3\#_1$ | 100.0 |
| | | 0.10 | 86.30 ± 0.65 $\&_2*_2$ | 100.0 | 91.99 ± 2.37 $*_4\&_4\#_1$ | 100.0 |
| Intact rat brain (n = 9) | E 8-11 | 0.01 | 2.25 ± 1.98 | 33.3 | 6.20 ± 3.73 $\&_5$ | 66.7 |
| | | 0.10 | 8.79 ± 3.11 | 33.3 | 16.13 ± 4.36 $\&_6$ | 100.0 |
| | E 12-16 | 0.01 | 6.95 ± 3.78 $*_1\#_2$ | 66.7 | 20.57 ± 5.66 $\&_5*_3$ | 100.0 |
| | | 0.10 | 17.24 ± 8.31 $*_2\#_2$ | 100.0 | 22.90 ± 4.97 $\&_6*_4$ | 100.0 |

Note: * – reliability of the difference between the groups **under** the influence on different cell types: $*_1$ ($p < 0.0077$); $*_2$ ($p < 0.0056$); $*_3$ ($p < 0.0057$); $*_4$ ($p < 0.0019$); # – reliability of the difference between the groups **under** the influence of different concentrations of RPNS: $\#_1$ ($p < 0.0063$); $\#_2$ ($p < 0.0056$); & – reliability of the difference between the groups **under** the influence of different RPNS gestational age: $\&_1$ ($p < 0.0077$); $\&_2$ ($p < 0.006$); $\&_3$ ($p < 0.033$); $\&_4$ ($p < 0.0002$); $\&_5$ ($p < 0.01$); $\&_6$ ($p < 0.0064$); ^ – reliability of the difference between the groups **under** conditions of varying duration of RPNS exposure : $\wedge_1$ ($p < 0.036$).

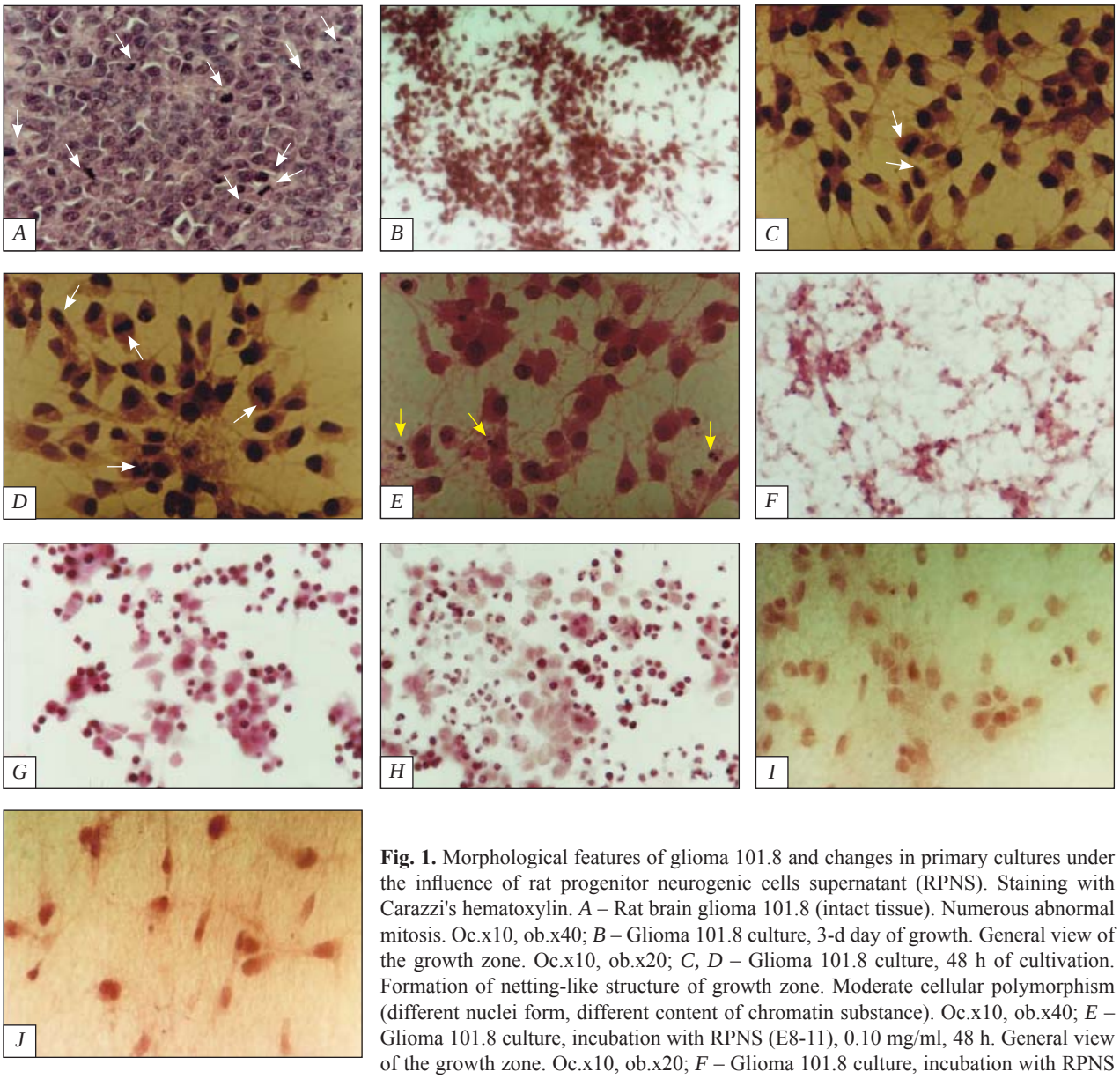


**Fig. 1.** Morphological features of glioma 101.8 and changes in primary cultures under the influence of rat progenitor neurogenic cells supernatant (RPNS). Staining with Carazzi's hematoxylin. *A* – Rat brain glioma 101.8 (intact tissue). Numerous abnormal mitosis. Oc.x10, ob.x40; *B* – Glioma 101.8 culture, 3-d day of growth. General view of the growth zone. Oc.x10, ob.x20; *C, D* – Glioma 101.8 culture, 48 h of cultivation. Formation of netting-like structure of growth zone. Moderate cellular polymorphism (different nuclei form, different content of chromatin substance). Oc.x10, ob.x40; *E* – Glioma 101.8 culture, incubation with RPNS (E8-11), 0.10 mg/ml, 48 h. General view of the growth zone. Oc.x10, ob.x20; *F* – Glioma 101.8 culture, incubation with RPNS (E12-16), 0.01 mg/ml, 48 h. General view of the growth zone. Oc.x10, ob.x40; *G* – Glioma 101.8 culture, incubation with RPNS (E12-16), 0.10 mg/ml, 48 h. General view of the growth zone. Oc.x10, ob.x40; *H* – intact newborn rats brain cells culture on the 6-th day of growth. Oc.x10, ob.x40; *I* – intact newborn rats brain cells culture, incubation with RPNS (E8-11), 0.10 mg/ml, 48 h. Oc.x10, ob.x40. White arrows – figures of mitotic division; yellow arrows – apoptotic cells

The mitotic cells were identified with a frequency of one to nine (usually three-six) in the field of view (Fig. 1, *A*). Among them the pathological forms were detected: stick-like and subtle forms, colchicine-like forms (C-mitosis), mitosis with chromosomes scattering in metaphase, empty metaphase. The content of mitotic cells at the final stage was 14 %. There were also tripolar, multicenter, asymmetric and other types of abnormal mitosis. The average mitotic index in the intact glioma tissue was (4.17 ± 0.26) %.

In perifocal zones, a tumor mainly was clearly distinguished from surrounding edema brain substance, but in some areas there were the signs of infiltrative growing of tumor into the brain tissue in the form of cell complexes and bands of different distribution.

*Features of glioma 101.8 growth in cultivation conditions (control observations)*. In the primary glioma 101.8 cultures, the tumor cells formed a widespread growth area (Fig. 1, *B*), which may indirectly indicate the growth migration potential of cells. After 24 h the rarefaction of cell microaggregates with the formation of monolayer friable areas was observed. In the dense-cell conglomerates, the undifferentiated cells with rounded cytoplasmic bodies dominated. Within 48–72 h after explantation the tumour cells formed around the cell microaggregates the typical netting-like structures of the growth zone (Fig. 1, *C, D, E*), similar to cytoarchitectonics of human anaplastic gliomas. In these areas of the cultures growth zone, the dominated tumour cells were of triangular, rhomboid, rarely polygonal shape with processes of different lengths and signs of terminal branching, forming intercellular contacts, mimicking netting-like structures. Nuclei of tumour cells were hyperchromic, mostly round-oval. Some cells contained nuclei of abnormally increased volume, which is a sign of nuclear polymorphism (Fig. 1, *C, D, E*).

In the regions of monolayer arrangement of tumour cells in the culture growth area, the individual figures of spontaneous death, apoptosis as nuclear fragmentation, were identified (Fig. 1, *E*). Additionally, different types of mitotic division of tumour cells with varying frequency were identified (Fig. 1, *C, D, E*). During the period of the most active proliferation of control cultures (6–8$^{th}$ day) MI was (4.00 ± ± 0.09) % (Table 2). Among the figures of mitotic division of tumour cells, the pathological forms of mitosis (lagging of the chromosomes in metaphase, empty metaphase lamellas, metaphase with chromosomes scattering, C-mitosis) predominated, which confirms the malignant nature of these gliomas. In the peripheral sections of the cultures growth zone, the destructively modified tumour cells dominated.

Thus, the experimental rat brain glioma 101.8 under cultivation generates a widespread growth zone with the signs of cellular polymorphism and significant mitotic activity.

*Morphosructural features of glioma 101.8 under the RPNS influence*. After incubation of the cultures with RPNS (E8-11, 0.01 mg/ml) for 24–48 h, the thinning of growth zone, the emergence of dystrophic and necrobiotic modified tumour cells with the formation of cell- shades were observed. MI reduced to the level of (1.3–1.1) % (Table 2). These changes strengthened with increasing concentrations of RPNS (E8-11) to 0.10 mg/ml, under the action of which the level of MI reduced to 1.0 % (Fig. 1, *F*).

When testing the impact of RPNS obtained from the fetal neurogenic cells of a later term of gestation (E12-16) on the primary glioma 101.8 cultures, the similar but more expressive dynamics of structural changes in the cellular composition of the cultures was detected. With increasing the tested concentrations of preparation from 0.01 to 0.10 mg/ml and

*Table 2.* **Dynamics of changes in the mitotic index (MI) in the primary cultures of 101.8 glioma under the influence of rat progenitor neurogenic cells supernatant (RPNS)**

| Cell type | RPNS | Control | Concentration of preparation, mg/ml | | | |
|---|---|---|---|---|---|---|
| | | | 0.01 | | 0.10 | |
| | | | 24 h | 48 h | 24 h | 48 h |
| Rat glioma 101.8A (n = 12) | E 8-11<br>E 12-16 | 4.00 ± 0.09 | 1.30 ± 0.13*^$_1$<br>1.10 ± 0.11* | 1.10 ± 0.06*^$_1$#$_1$<br>0.90 ± 0.10*#$_1$ | 1.20 ± 0.08*^$_2$<br>1.00 ± 0.12* | 1.00 ± 0.09*^$_2$#$_2$<br>0.85 ± 0.04*#$_2$ |

Note: * – reliability of the difference compared to the control ($p < 0.01$); ^ – reliability of the difference between the groups under conditions of varying duration of RPNS exposure ($p < 0.05$); & – reliability of the difference between the groups under the influence of different RPNS gestational age ($p < 0.05$).

prolongation of the culture incubation with the preparation up to 48 h, the strengthening in cytological signs of damage of tumour cells was observed, It was accompanied by rarefaction of cell arrays due to partial desquamation of degenerated cells (Fig. 1, *G, H*) and a decrease in mitotic activity in the stored areas of growth zones of studied cultures (MI was 0.9 % and 0.85 %, respectively).

Thus, the study on the glioma 101.8 cultures revealed that a direct RPNS impact of the fetal NPC of different gestation terms had dose-dependent cytotoxic and cytostatic effects, which enhanced with the increasing of incubation time of the cultures with the preparation. It is important to emphasize that a higher cytotoxic impact is typical for RPNS obtained from the NPC of a later gestational age.

To determine the specificity of RPNS antiproliferative influence on tumour cells, the peculiarities of preparation action on the primary cultures of intact nerve cells of the newborn rats brain were studied. In control cultures at 6–8$^{th}$ days of cultivation, the netting-like growth area of glial phenotype cells with processes was formed around microexplants of nervous tissue including cells with the phenotype of neuroblasts (Fig. 1, *I*). The mitotically active cells were absent. After exposure of RPNS (E8-11, E12-16) within the area of growth and cellular composition of cultures no significant differences were found (Fig. 1, *J*) compared with control observations, which may indicate a lack of significant cytotoxic impact of RPNS on the intact nerve cells of the brain.

Since RPNS (E12-16) showed a distinct cytotoxic effect in the primary glioma 101.8 cultures, its composition was examined. According to ELISA, RPNS (E12-16) contained 115 pg/ml BDNF, 12 pg/ml TGF-β1 and very low levels of IL-1β and IL-4 (Table 3), which is consistent with the data [5, 6]. Probably, the detected small amounts of these cytokines are related to the minor fractions of proteins, which account for 1 % of the RPNS content.

It is known that TGF-β1 is a homodimer with a molecular weight of 25 kDa and consists of two subunits of 12.5 kDa, linked by disulfide bonds. The TGF-β1 expression increases significantly in gliomas with a high degree of malignancy [13, 14]. TGF-β – signaling is involved in the regulation of proliferation, differentiation and cell survival or apoptosis, its inhibition reduces the viability and invasive properties of gliomas modeled in animals [13]. Considering that the cytotoxic and antiproliferative RPNS effects on the rat glioma 101.8 cells is exactly due to TGF-β1, it must be assumed that the tumour cells express the receptors for TGF-β1 (TβRI or TβRII), that by binding TGF-β1 launch Smad-dependent (involving Smad-proteins) and Smad-independent signaling pathways (Ras / MAPK, p38, ERK, JNK) [15]. It is known that the role of TGF-β is twofold: TGF-β has antiproliferative effects on a number of epithelial cells at the early stages and a promoter effect at the later stages of tumour growth [15].

At the same time it is known that a mature BDNF molecule has the molecular weight of 13–14 kDa and its amino acid sequence is 50 % similar to the nerve growth factor (NGF) and neurotrophins (NT-3, NT-4/5) [16]. BDNF binds to two types of receptors – tropomyosin kinase (trkB) and NGF with the molecular weight of 75 kDa. These receptors are related to the protein superfamily of tumour necrosis factor and their binding leads to the activation of intracellular signaling cascades (NF-κB, Jun-kinase) that mediate the initiation of programmed cell death (apoptosis). Thus, it is possible that the cytotoxic and antiproliferative effect of RPNS on the rat glioma 101.8 cells can be explained by the influence of BDNF.

A possible explanation could also be the synergistic combination of the effects of TGF-β1 and BDNF, but this hypothesis requires subsequent in-depth research.

*Table 3.* **Content of cytokines in rat progenitor neurogenic cells supernatant (RPNS) (E12-16)**

| Cytokines | TNF-a | IL-1β | IL-4 | IL-10 | TGF-β1 | BDNF |
|---|---|---|---|---|---|---|
| concentration, pg/ml | 4.00 ± 0.09 | 0.90 | 1.63 | 0.00 | 12.00 | 115.00 |

## Conclusions

The rat progenitor neurogenic cells supernatants of different terms of gestation revealed the dose-dependent cytotoxic and antiproliferative effects on the cultured glioma 101.8 cells *in vitro*, which intensified with increasing gestational age of rat brain and lengthening the incubation period.

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**Вплив супернатанту прогеніторних нейрогенних клітин щура на клітини гліоми 101.8** *in vitro*

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**Мета**. Оцінити вплив супернатанту прогеніторних нейрогенних клітин (СНК) головного мозку щура на клітини перещеплюваної злоякісної гліоми головного мозку щура (штам 101.8) в умовах культивування. **Методи**. Первинні культури отримували з фрагментів гліоми 101.8 (n = 12) та головного мозку інтактних новонароджених щурів (n = 9). СНК отримували з суспензії нейрогенних клітин фетального мозку щурів на 8-11-у (Е8-11) та 12-16-у (Е12-16) добу гестації. **Результати**. СНК, отримані з фетального мозку як раннього (Е8-11), так і більш пізнього (Е12-16) терміну гестації, виявили цитотоксичну дію на короткострокові культури клітин гліоми 101.8, ступінь якої був дозозалежним та посилювався із збільшенням тривалості інкубації культур з СНК. СНК (Е12-16) виявив більш виражену цитотоксичну дію на культивовані клітини гліоми 101.8, порівняно з СНК (Е8-11). Показник цитотоксичного індексу (ЦІ) СНК (Е12-16) стосовно клітин гліоми 101.8 достовірно перевищував ЦІ, визначений у суспензіях клітин нормального мозку щура (ЦІ становив (91,99 ± 2,37) % та (22,9 ± 4,97) % відповідно через 48 год інкубації культур з СНК). Після впливу СНК (Е8-11) на первинні культури гліоми 101.8 спостерігались ознаки дозозалежної цитотоксичності: розрідження зони росту, поява дистрофічних та некробіотичних змін у пухлинних клітинах, зниження мітотичного індексу. Ці ознаки посилювались за умов впливу СНК(Е12-16). **Висновки**. СНК фетального мозку щура виявляє дозозалежний цитотоксичний та антипроліферативний вплив на культивовані клітини гліоми 101.8, який посилюється при збільшенні терміну гестації щура та тривалості інкубації культур з СНК. Передумовою такого впливу, ймовірно, є здатність НПК до продукції речовин з протипухлинною дією.

**Ключові слова:** прогеніторні нейрогенні клітини, фетальний мозок щура, супернатант, гліома 101.8, цитотоксичний індекс, мітотичний індекс.

**Влияние супернатанта прогениторных нейрогенных клеток крысы на клетки глиомы 101.8** *in vitro*

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**Цель**. Оценить влияние супернатанта прогениторных нейрогенных клеток (СНК) головного мозга крысы на клетки перевивной злокачественной глиомы головного мозга крысы (штамм 101.8) в условиях культивирования. **Методы**. Первичные культуры получали из фрагментов перевивной злокачественной глиомы головного мозга крысы (штамм 101.8) (n = 12) и головного мозга интактных новорожденных крыс (n = 9). СНК получали из суспензии нейрогенных клеток фетального мозга крысы на 8-11-е (Е8-11) и 12-16-е (Е12-16) сут гестации. **Результаты**. СНК, полученные из фетального мозга как раннего (Е8-11), так и более позднего срока гестации (Е12-16), проявили цитотоксическое воздействие на краткосрочные культуры клеток глиомы 101.8, уровень которого был дозозависимым и усиливался с увеличением длительности инкубации культур с СНК. СНК (Е12-16) выявлял более выраженное цитотоксическое воздействие на культивируемые клетки глиомы 101.8, по сравнению с СНК (Е8-11). Показатель цитотоксического индекса (ЦИ) СНК (Е12-16) по отношению к клеткам глиомы 101.8 достоверно превышал ЦИ по отношению к суспензиям клеток нормального мозга крысы (ЦИ составлял (91,99 ± 2,37) % и (22,9 ± 4,97) % соответственно через 48 час инкубации культур с СНК). Под влиянием СНК (Е8-11) на первичные культуры глиомы 101.8 наблюдались признаки дозозависимой цитотоксичности: разрежение зоны роста, дистрофические и некробиотические изменения в опухолевых клетках, снижение митотического индекса. Эти признаки усиливались после влияния СНК(Е12-16). **Выводы**. СНК фетального мозга крысы выявляет дозозависимое цитотоксическое и антипролиферативное воздействие на культивируемые клетки глиомы 101.8, усиливающееся по мере увеличения срока гестации крысы и длительности инкубации культур с СНК. Предпосылкой такого влияния, возможно, является способность НПК к продукции веществ с противоопухолевым действием.

**Ключевые слова:** прогениторные нейрогенные клетки, фетальный мозг крысы, супернатант, глиома 101.8, цитотоксический индекс, митотический индекс.

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