ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Tumor-Association Inflammation Determines Malignancyat Brain Gliomas.

N.Ya. Gridina*, S.P. Syatkin, A.S. Skorik, I.P. Smirnova, S.M. Chibisov M.L., G.I. Myandina, M.L. Blagonravov, S.A. Shastun, O.M. Kuznetsova, and E.V. Neborak.

[1]The State institution "A.P. Romodanov Institute of Neurosurgery NAMS of Ukraine", Kyiv, 04050, Ukraine
Russian Peoples' Friendship University, Miklukho-Maklaya str., 8, Moscow, 117198, Russia

ABSTRACT

It is known, that brain gliomas may progressing and become more malignant as comparison with in an initial stage. At the same time not take into account the decisive role of tumor – association inflammation (TAI), which accompanies gliomas growth and progression. It is quite possible, that exactly TAI promoting malignancy of tumor process. With the purpose of confirm or disprove this assumption, the sophisticated experiments was carry out with implantation glioma 101.8 cells into embryonic rat brains, in which inflammation is absence, and with combined cultivation in vitro rat normal embryonic cells with high malignant glioma 101.8 cells. It was reveal cytolysis of malignant glioma 101.8 cells to be in touch with embryonic cells in experiments vivo and in vitro. While after combined inoculations into adult brains death of rats was observed as a result of invasive tumor growth. Consequently, one of the main reasons of glioma malignancy is tumor-association inflammation, in the absence thereof high malignant glioma cells losing its malignancy.

Keywords: Rat brain glioma 101.8, Rat embryonic cells, Tumor-association inflammation, Cocultivation *in vitro*, Combined inoculation into embryonic brain, Lost of glioma cells malignancy.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Malignant brain tumors, almost half of which are cases of cerebral glioma, constitute one of the deepest problems in brain surgery sector. They are defined by recurrent pearl that occurs on long date after the surgical aggression in spite of conducted courses of chemotherapy and radiotherapy and other methods of antitumor therapy known currently.

Still, there is one more reason of low efficiency of malignant gliomas treatment. It is widely accepted that a tumor grows from the only one mutated cell, getting through proliferation stages and turning from innocent growth into malignant one. In addition, hardly anybody takes into consideration the implication of necrosis in malignant tumors that serve as stimulator of aseptic tumor associated inflammation (TAI) [1-5]. Subject to the existence of TAI a constant tumor growth forcing takes place, and aggressive factors effused by inflammatory genesis cells may overwhelm the organism. Thus, TAI actual existence in cases of gliomas, as well as in cases of other malignant tumors, puts in question the innocent tumor cells capability to turn into malignant ones. It may be that, this is TAI that is responsible for development of proliferation. In order to prove or deny this hypothesis the following experiments were carried out: co-culture of rat normal primary nerve cells and malignant glioma cells 101.8, plus implantation of glioma cells into rat embryo cerebrum.

MATERIALS AND METHODS

The experiments were carried out on 250 Wistar rats. The experiment models were: a) the interaction between embryonic nervous tissue (ENT) and glioma 101.8 [6] during their co-culture under in vitro conditions; b) their joint transplantation into cerebrum under in vivo conditions; c) inoculation of glioma cells 101.8 straight into embryonic cerebrum (the eleventh day of embryogenesis). Concurrently an assessment of results of glial tumor cells expansion in case of the absence of TAI in tissue culture and embryonic cerebrum was made, and also in cases of TAI promotion in experimental animals' organisms with inoculated glioma strain for the purpose of estimation of TAI during the glioma growth.

During the first series of experiments a co-culture of tumor tissue and embryonic nerve one was conducted. Inoculated glioma was used (a strain 101.8 received from Research Institute of Human Morphology, Moscow, RF). Culture growth (up to 14 days) was studied in one's lifetime and also using histology specimen stained with Carazzi's hematoxylin. The mitotic index in cell monolayers was rated in control cultures and test ones, and also the average number of cells per unit of substrate in monolayer sections of culture growth zone was predetermined. In total 72 cultures were studied, during each experiment the results of cytometry of not less than 50 cells were analyzed. During the second series of tests 150 of white Wistar rats were used to make a joint transplantation of ENT fragments and glioma 101.8 into the cerebrum. Only a tumor tissue was implanted into the cerebrum of the animals of control set (30 rats). During estimation of results the animals' lifetime was taken into account. During the third series of tests the inoculation of glioma 101.8 cells straight into the cerebrum tissues of rat fetus was carried out with the condition of continuation of the further rats' gestation course. The day before birth the fetus was extracted from the womb, the cerebrum tissues of the fetus were examined using histology specimens.

RESULTS AND DISCUSSION

When co-culturing the fragments of tumor and ENT the decreased number of tumor cells migrated from explants, cell sheet discomplexations, and various stages of dystrophia and necrobiotic changes in tumor cells were observed on the 4-5 day near ENT growth zone [Fig.1-2]. The mentioned changes were accompanied by cellular density decrease, mitotic activity decrease and decrease of cytometry indices of visually conserved tumor cells in comparison with test culture (Table). No ENT cells death was detected. No cytolitic effect of ENT cells on normal cells of formed cerebrum was determined during co-culture of tissue fragments of formed cortex and the embryonic one over a period of 48 hours.

ISSN: 0975-8585

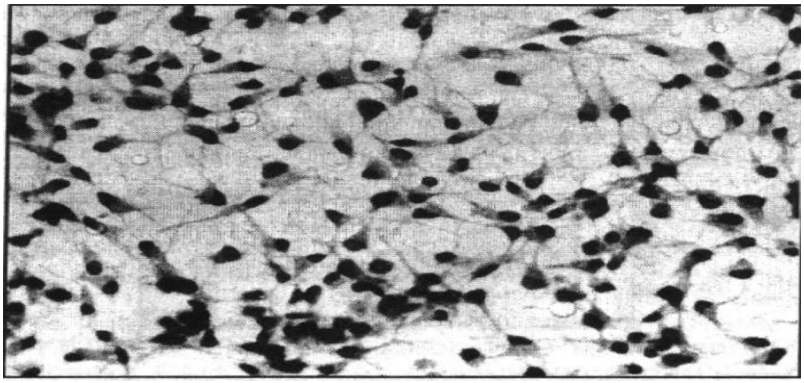

Fig.1. Cellular structure of glioma 101.8 growth zone on the 5-th days of cultivation (x400)

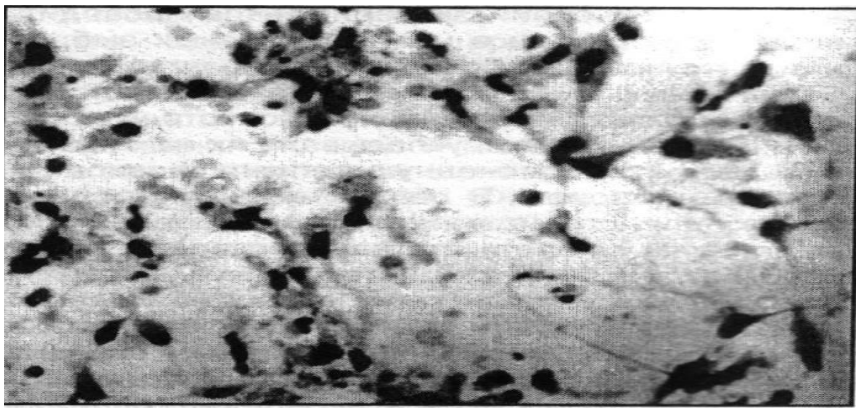

Fig 2. Glioma growth zone, which cultivated in common with embryonic nervous tissue:dystrophic and necrobiotic overpatching of glioma cells on the 5-th days of cultivation (x400)

When embryonic and tumor tissues were jointly transplanted into the rats cerebrum so the lifetime of the experimental animals didn't firmly differ from the test scores. The experiments in terms of tumor cells inoculation straight into the tissue of embryonic cerebrum allowed observing the result of direct contacts between tumor cells and embryonic cells under in vivo conditions. During the histology studies of embryos' brain slice the moderate diffuse tumor cell nests with destroyed cytoplasm were disclosed. Lots of cell nucleuses were hyperchromic or hypochromic ones bearing signs of pyknosis or karyolysis. At the same time the tissue of embryo's cerebrum around puncture path and throughout conserved a normal structure and architectonics similar to targets.

Table. Cytometric indexes of glioma cells at separate (control) and combined (experiment) cultivation with embryonic nervous tissue.

Cytometric index	Control	Experiment M ± m	p
Average area of nuclear, мсм^2	88,17 ± 4,0	51,89 ±2,5	<0,05
Average area of nucleolus, мсм^2	6,94 ±1,0	4,17 ±0,1	<0,05
Average area of cytoplasm, мсм^2	272,53 ± 6,2	203,00 ± 5,4	<0,05
Nuclear-cytoplasm relation	0,39 ± 0,05	0,20 ± 0,04	<0,05
Cells density (cells number in field of vision)	59,00 ± 2,0	39,00 ±1,0	<0,05
Kariokynetic indexes, %	2,0	0,4	

By comparison of growth characteristics of glioma cells that were transplanted into the cerebrum of an adult animal and embryo it was found that in the first case the growth of tumor graft was of progressive nature and in the second case the tumor graft was subjected to involution. Obtained results showed that glioma 101.8 hadn't given it's malignant properties either during co-culture with ENT or during inoculation of glioma cells into the cerebrum of rat embryos. More importantly, for the first time it was found that malignant glioma cells had fallen under the cytolysis during the contact with ENT in experiments that were carried out under in vitro and in vivo conditions. In the meantime, such an effect was absent when joint transplantation of ENT and glioma 101.8 into the animals' cerebrum was conducted, the animals died because of the tumor invasive growth.

CONCLUSION

Glioma cells death certifies that the cells don't have malignant properties when co-cultured with ENT or implanted into the cerebrum of rat fetus. One of the principle factors that contribute to glioma growth is an inflammation which, as is known, is missing during embryogenesis. The research data may speak for significant value of the inflammatory process of glioma cells proliferation and invasion in the organism of an adult. At the same time the deficiency of cytolysis effect of ENT cells on the tissues of formed cerebrum gives evidence of mediated nature of glioma cells cytolysis during the contact with ENT. It is known, that transplantation ENT into adult brain regeneration processes is expressed [7-8] and that embryonic cells inhibited growth of different tumor cells [9-11]. It is very interestingly to investigate the "antitumor activity" of ENT. Why glioma 101.8 cells died in contact with embryonic nervous tissue in the absence thereof inflammation? For the understanding of this process was investigated mechanism «persisting wound syndrome» at tumor growth [12]. Toward this end *in vitro* was added phytohemagglutinin (PHA) in the appointed concentration, which promoted to decreasing of transmembrane potential on cultivated blood cells membranes in patients with spinal cords and malignant brain gliomas. Diamine oxidize (DAO) and polyamine oxidize (PAO) activity, which can oxidize polyamines (PA), was determined after ending of cultivation in supernatant. It is very important factors in regulation proliferation function of constitution cells in the III stage at inflammation and at tumor growth [13]. The results of investigations shown that differences between indexes in chronic inflammation groups (spinal cords) and tumor-association inflammation at gliomas III degree of malignancy against a background increasing of blood cells aggregation (transmembrane potential decreasing) based in activation DAO and PAO at chronic inflammation and decreasing of activation this enzymes at malignant gliomas. The activity decreasing of PA oxidizing enzymes will promote persistent proliferation of cells in inflammation focus, thus preventing healing processes and maintenance of tumor cells proliferation as a result of high level of non-oxidizing PA. There for, at TAI against a background decreasing of transmembrane potential level on the blood cells membrane the level of oxidize enzymes PA greatly decreasing, which lead to constant activation of cells proliferation processes in tumor focus. The absence of inflammation stimulation of glioma cells by compounds, which is synthesizing by embryonic nervous tissues and activating reparation and regeneration processes [14], leading to glioma cytolysis as a result of deficient level of proliferative stimulation in the lack of differential potential. This results is the confirmation of facts, that glioma pathogenesis are too tightly bind with tumor-association inflammation.

ACKNOWLEDGEMENT

Author thanks professor Semyonova V.M. and scientist Stayno L.P. for the carrying out cells cultivation and morphological indices.

REFERENCES

[1] Luchnik A.N. (2000) The common component in the mechanism of self-maintaining the malignant growth: syndrome of a nonhealing wound. Ontogenez, 31, 227-31 (in Russian).

[2] Schwartsburd P.M. (2003) Chronic inflammation as inductor of pro-cancer microenvironment: Pathogenesis of dysregulated feedback control. Cancer & Metastasis Reviews, 22, 95-102.

[3] Schwartsburd P.M. (2004) Age-promoted creation of procancer microenvironment by inflammation: Pathogenesis of disregulated feedback control. Mech. Aging Develop., 125, 581-590.

[4] Witz I.P. (2009) The Tumor Microenvironment: The Making of a Paradigm //5th International Conference on Tumor Microenvironment: Progression, Therapy & Prevention.-Versailles, France, October 20-24, 2 (Suppl. 1): S9-S17.

[5] Gridina N., Maslov V., Ushenin Yu. (2013) Tumor - association inflammation and brain gliomas. Lambert Academic Publishing, Saarbrucken. 196 p. (in Russian).

ISSN: 0975-8585

[6] Khalansky A.S., Kondakova L.I., Avtsyn A.P. (1995) The new interweaving culture of rat brain gliomas. Vopr. Neurokhirurgii, 2, 23-25 (in Russian).
[7] Bjorklund A., Gage F.H. (1985) Neural grafting in animal models of neurodegenerative diseases. Ann. N. Y. Acad. Sci., 457, 53-79.
[8] Polezhaev L.V. Alexandrova M.A., Vitvitsky V.N., Cherkasova L.V. (1993) Transplantation of brain tissue in biology and medicine. Moscow: Nauka, 1239 p. (in Russian).
[9] Zozulia Yu. A., Semyonova V.M., Lisiany N.I., Oleinik G.M., Markova O.V., Staino L.P. (1995) Antitumor activity of murine developing brain cells. Morphological Aspects. J. Exper. Clin. Cancer Res., 14, 32-34.
[10] Kondakova L.I., Levitman M.Kh., Khalansky A.S. (1993) Proliferation inhibition action of normal fibroblast on brain tumor cells. Actual problems of common and particular pathology, RAMS, 179-182 (in Russian).
[11] Niwa N., Matsano T., Mizuno S. (1985) Inhibition of tumor cell growth by protein factors derived from the developing mouse brain. J.Cancer Res. Clin. Oncol., 110, 196 - 202.
[12] Sytkin S.P., Frolov V.A., Gridina N.Ya., Draguntsova N.G., Veselova O.I., Maslov V.P., Ushenin Yu.V. (2014) The mechanism of immune suppression at brain gliomas. Bulletin of RUDN.-Series: Medicine, 1, 11-17 (in Russian).
[13] Syatkin S.P., Berezov T.T. (1982) The role of polyamines in malignant tumors. Bulletin of RAMS. 3, 10-21 (in Russian).
[14] Tsymbalyuk B.I., Nosov A.T., Radchenko M.R. (2003) Influence of embryonic nervous tissue on morphological and functional status of oculus analyzer at methanol intoxication. Ukrainian Journal of Neurosurgery, 1, 1- 8 (in Ukranian).