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Infiltrating growing pattern xenografts induced by glioblastoma and anaplastic astrocytoma derived tumor stem cells

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Rezumat

Xenogrefe tumorale infiltrative induse de către celule stem tumorale izolate din glioblastomul și astrocitomul anaplazic

Obiectiv: Studiile publicate în literatura de specialitate despre rolul celulelor stem tumorale în inițierea și progresia astrocitoamelor de grad înalt au devenit din ce în ce mai numeroase în cursul ultimilor ani. Acest fenomen a fost intens studiat în cazul glioblastomului, în contrast cu astrocitomul anaplazic despre care există puține studii până în momentul de față. Principalul obiectiv al acestei lucrări este studierea caracteristicilor morfologice și imunohistochimice a xenogrefelor dezvoltate din celulele stem tumorale izolate din glioblastom și astrocitomul anaplazic.
Metoda: Autorii acestui articol au izolat și au caracterizat culturi primare de celule stem tumorale provenite din glioblastom și astrocitom anaplazic. Ulterior, celulele stem tumorale au fost inoculate stereotactic în creierul șoarecilor nuzi iar xenogrefele dezvoltate au fost studiate din punct de vedere morfologic și imunohistochimic.
Rezultate: Xenogrefele tumorale dezvoltate au avut modele de creștere diferite comparativ cu xenogrefele de U87 dezvoltate anterior de colectivul nostru și au depins de tumora de origine (glioblastom versus astrocitom anaplazic). Modelul de creștere difuz și infiltrarea celulelor tumorale au fost mult mai accentuate față de xenogrefele dezvoltate din linia U87.
Concluzii: Rezultatele prezentate în acest articol confirmă rolul celulelor stem tumorale în fenomenul de infiltrare al astrocitoamelor maligne (glioblastom și astrocitom anaplazic). De asemenea, datorită tipului de creștere difuz, aceste xenogrefe pot deveni modele utile de studiu al mecanismelor de invazie și infiltrare al astrocitoamelor maligne.

Cuvinte cheie: glioblastom, astrocitom anaplazic, celule stem tumorale, nestina, xenogrefe

Abstract
Objective: The number of evidences regarding the role of tumor stem cells (TSC) in the initiation and progression of high-grade astrocytomas became more and more numerous in the last years. This issue has been intensively tested in glioblastoma, but little attention has been paid for anaplastic astrocytoma. The main objective of this paper was to study the morphological characteristics of the xenografts developed from glioblastoma and anaplastic astrocytoma derived cancer stem cells.
Methods: The authors of this study successfully isolated and partial characterized primary cultures of glioblastoma and anaplastic astrocytoma derived TSC. Tumors stem cells have been stereotactically inoculated in nude mice brains and the xenografts have been studied using morphological and imunohistochemistry techniques.
Results: The tumor xenografts which have been established in nude mice using TSC had different characteristics when compared with U87 xenografts previously developed by our group, and depend of the origin type of the tumors (glioblastoma versus anaplastic astrocytoma). The diffuse growing pattern and cells infiltration have been more pronounced in both anaplastic astrocytoma and glioblastoma derived TSC xenografts compared with U87 line xenografts.

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Conclusion: Our results support the hypothesis regarding the role of TSC in the infiltration process of glioblastoma and anaplastic astrocytoma. The extensive infiltration growing patterns of these types of xenografts make them useful models for studying the invasion mechanisms in gliomas.
Abbreviations: TSC - tumor stem cells.

Key words: glioblastoma, anaplastic astrocytoma, tumor stem cells, nestin, xenografts

Introduction

The lack of efficiency of actual therapies for glioblastoma multiformis stimulates the researchers to find new and innovative therapies. One reason of therapeutic failure could be the incomplete insight into the origins of this tumor and the absence of an adequate experimental model. The wide used standardized glioblastoma lines (like U87, U251, etc.), develop a uniform phenotypic xenograft model, which are very useful for obtaining reproducible experimental data. Unfortunately, these xenografts are not superposable with the heterogeneous populations from the malign cerebral tumors that are usually occurring in the clinic (1). Therefore, researchers tried to developed xenografts using primary cells cultures developed from gliomas samples (2). Isolation and characterization of TSC from human glioblastoma opened new perspectives in primary brain tumors research and offered an alternative approach for this severe disease (3). On the other hand, this type of cells has a high tumorigenity feature, a small number of CD133 positive cells being able to induce tumor xenografts in nude mice (4). Moreover the xenografts developed from glioblatoma derived TSC resemble the characteristic of original tumor, in contrast with standardized glioblastoma lines xenografts (1). Thus, the glioblastoma TSC became a very useful model for studying in vitro and in vivo malignant gliomas.

Initially the TSC have been isolated only from glioblastomas samples. Moreover, one report underlined the fact that only one type of glioblastoma (primary glioblastoma) could be a source for TSC, and the other type (secondary glioblastoma, developed from lower grade gliomas progression) has no TSC population (5). Latter reports suggested that lower grade gliomas (like anaplastic astrocytoma and fibrilarry astrocytoma) could be also a source for TSC isolation and demonstrated a direct relation between the expression of stem cells markers like CD133, Nestin and the World Health Organization's (WHO) grade of gliomas samples (6). However, a detailed comparative description of morphologic features between glioblastoma and anaplastic astrocytoma derived tumors stem cells xenografts has not been done yet.

In the present study, the authors isolated and cultivated primary cultures obtained from glioblastomas and anaplastic astrocytomas samples, using special stem cells media. In vitro characterization of these cultures has been performed in order to asses the presence of TSC (neurosphere formation, nestin and CD133 expression). Using these primary cultures, tumor xenografts have been develop in nude mice and specific growing patterns and in vivo cells behaviors have been described.

Materials and Methods

Cell cultures establishment

Tumor samples have been obtained with the preoperative written consent of the patients. The histopathological evaluation of the samples showed a grade IV glioma (glioblastoma) and a grade III glioma (anaplastic astrocytoma), according with WHO classification. The samples were prepared by manual fragmentation into pieces up to few millimeters each, followed by enzymatic digestions using 0.125% trypsin and 20 mM EDTA. Then, the cells have been cultivated using specific medium for neural stem cells: DMEM: F12 medium (Sigma-Aldrich, USA) supplemented with 10 ng /ml EGF; 1xB27; 0.5xN2. The cells used for in vivo control group were cultivated in standard medium: Dulbecco Modified Eagle's medium (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100μg/ml streptomycin and 50 μg/ml neomycin.

RT PCR

Isolation of RNA was performed using TRIzol kit (Invitrogen, USA) according to the manufacturer recommendations. The quality of the extracted RNA samples was assessed by the 260/280 nm ratios. Reverse transcription (RT) started from 2 µg RNA, using the kit Access Quick RT-PCR System (Promega, USA). cDNA was used as a template in TaqMan gene expression assays for GFAP, Nestin, hTERT (Applied Biosystems, USA) according with manufacturer protocol.

Flow cytometry

5 X 10^5 cells were collected, washed twice in PBS, 0.1% BSA, and then cells were incubated for 1 h with monoclonal antibody CD133/2-PE (Miltenyi Biotec, Germany). The labeled cells were analyzed using a Beckman Coulter EPICS XL flow cytometer. Ten thousand events were acquired and data were analyzed with FlowJo software. Positive cells were determined as percentages of gated cells.

In vivo tumor model

A total of 16 nude mice, 8–10-weeks-old (Crl: CD-1-Foxn1^{nu}; Charles River Breeding Laboratories, Germany) were used in experiments. Animals were anesthetized by intraperitoneal injection of xylazine 10 mg/kg and ketamine 80 mg/kg. Mice were held in a stereotactic frame with ear bars (TAXIC-600, World Precision Instruments) and received stereotactically guided injections over 3 min into forebrain (2 mm lateral and 1 mm anterior from bregma; depth 3.5 mm from dura mater). Eight animals received 2 x 10^5 cells of glioblastoma derived TSC (Ston - passage number 3) culture in a volume of 3 μl PBS and other eight animals received 2 x 10^5 cells of anaplastic astrocytoma derived TSC (Necu - passage number 6) culture in a volume of 3 μl PBS. As control group, 16 nude mice, 8–10-weeks-old, were stereotactically inoculated using

the previously described coordinates: 8 mice with 2×10^5 cells of serum-conditioned glioblastoma cultures (Ston) and 8 mice with 2×10^5 cells of serum-conditioned anaplastic astrocytoma cultures (Necu). All the surgical and experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee, in accordance with Romanian governmental guidelines for ethics in animal experiments.

Histology and immunohistochemistry

Mice inoculated with glioblastoma and anaplastic astrocytoma derived TSC were sacrificed at two months (four mice bearing Ston xenografts and four mice bearing Necu xenografts) and three months (four mice bearing Ston xenografts and four mice bearing Necu xenografts) after cells implantation. The same partition was used for the control group. Brains were removed and were fixed in Bouin solution or 4% paraformaldehide in PBS, dehydrated in ethanol, cleared in toluene and embedded in paraffin. About 6 Om-thick horizontal sections were used for hematoxylin and eosin (H&E) staining and immunofluorescence staining. Immunohistochemistry was performed using paraffin sections and the following primary antibodies: nestin (5C93) mouse monoclonal antibody (MAb) raised against a 150 amino acid epitope mapping near the C-terminus of human nestin (Santa Cruz, Biotechnology, Inc) diluted 1:100, and mouse anti-human mitochondria MAb (undiluted, USBIO). As secondary antibodies were used Alexa Fluor® 488 F(ab')2 fragment of goat anti-mouse IgG antibody (Invitrogen), diluted 1:200. Sections were incubated with normal goat serum (Santa Cruz, Biotechnology, Inc) in 2% bovine serum albumin (BSA; Fraction V Powder, Sigma A4503) to remove non-specific background staining (1 h). The tissue sections were subsequently incubated overnight, at 4°C, with primary antibodies, followed by rinses in PBS (see below) by the incubation with the secondary antibodies, for 1 h, at room temperature. Each incubation step was followed by four 5-min rinses in PBS. Nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI) solution, mounted in PBS and analyzed by epi-fluorescence microscopy using a DAPI/FITC/Texas red triple band filter set (Zeiss). As a negative control of immunohistochemical reaction, sections were processed as described above except that the primary antibody was omitted. The photomicrographs were taken by digital camera (AxioCam MRc 5, Carl Zeiss) driven by software Axio-Vision 4.6 (Carl Zeiss).

Results

In vitro results
The ability to form neurospheres of brain tumor cells

We initiated two primary cultures from brain tumors: one glioblastoma derived TSC culture (Ston) and one anaplastic astrocytoma derived TSC culture (Necu) using special medium for neural stem cells cultures. The medium was changed every 3 days. Cells survived in cultures but the proliferation rate was low. We observed formation of cells clusters similar to neurospheres at approximately 14-21 days after initiation *(Fig. 1A-B)*. Neurospheres could be passaged multiple times by mechanical dissociation of large spheres and reseeding in fresh proliferative medium every 2–3 weeks.

In order to observe if tumour neurospheres maintain stem cell characters, we evaluated the expressions of two stem cells markers: nestin and CD133. The nestin expression was slightly increased in Ston comparing with Necu as observed by real time PCR *(Fig. 2)*. Contrary, the mRNA CD133 expression was decreased in Ston and increased in Necu cells, and the results were confirmed by protein expression quantified by flow cytometry (Ston 28.86% respective Necu 71.91%) *(Fig. 3)*.

The glioblastoma cells and anaplastic astrocytoma cells cultivated in serum-conditioned medium (Dulbecco Modified Eagle's medium (Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin and 50 μg/ml neomycin) expressed a fibroblastic phenotype, similar with the U87 line cells *(Fig. 4)*. These cells were used for inoculation in the control group of nude mice.

In vivo results
Morphological evaluation of xenografts

Because some studies (7) have shown that human tumor xenografts in nude mice are not always of human origin we used an anti-human mitochondria antibody as immuno-

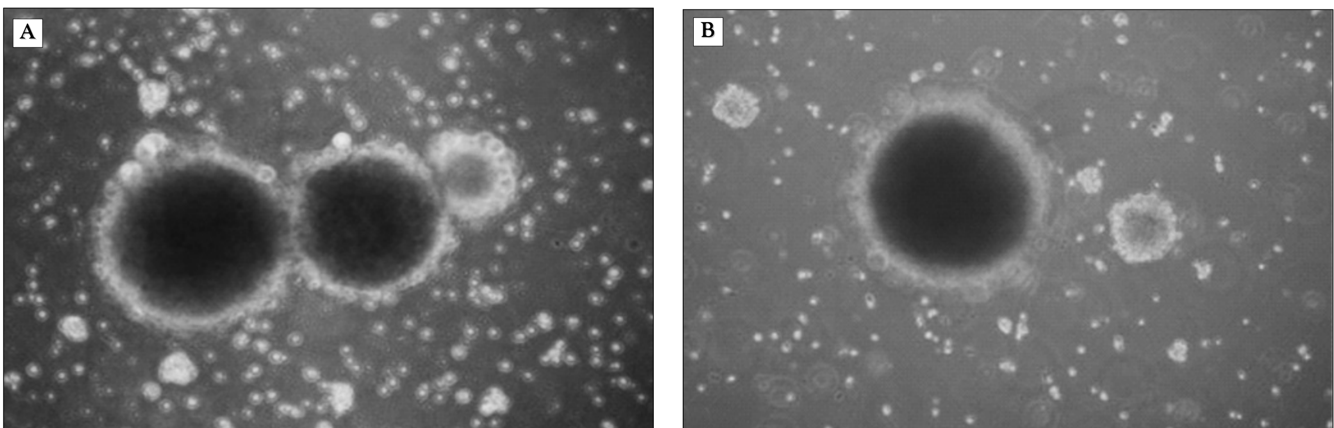

Figure 1. ***A.*** *Tumor neurospheres initiated from glioblastoma sample, Ston (Ob. 100X).* ***B.*** *Tumor neurospheres initiated from anaplastic astrocytoma sample, Necu (Ob. 100X)*

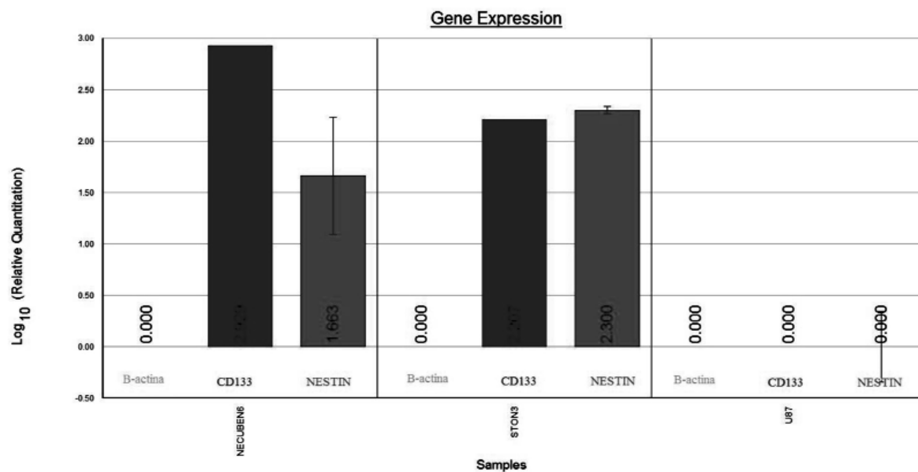

Figure 2. *Neurospheres derived from glioblastoma (Ston – written as STON3) and anaplasticastrocytoma (Necu – written as NECUBEN6) give rise to cells expressing neuronal stem cell markers in various proportions. Gene expression was normalized based on the levels of mRNA for human beta actin. The gene expression levels were plotted as log10 values. The expression level of the calibrator sample (U87- glioblastoma line) appears as 0 in the graph*

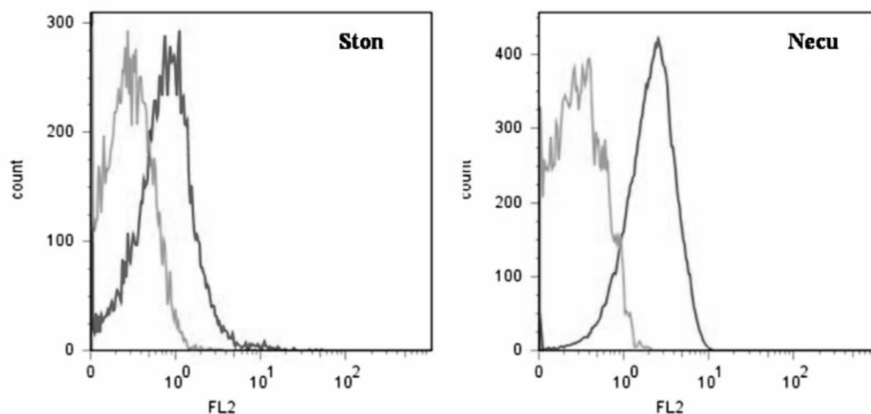

Figure 3. *CD133 expression of Ston and Necu tumor cell lines is represented by black line. The isotype control is represented by grey line*

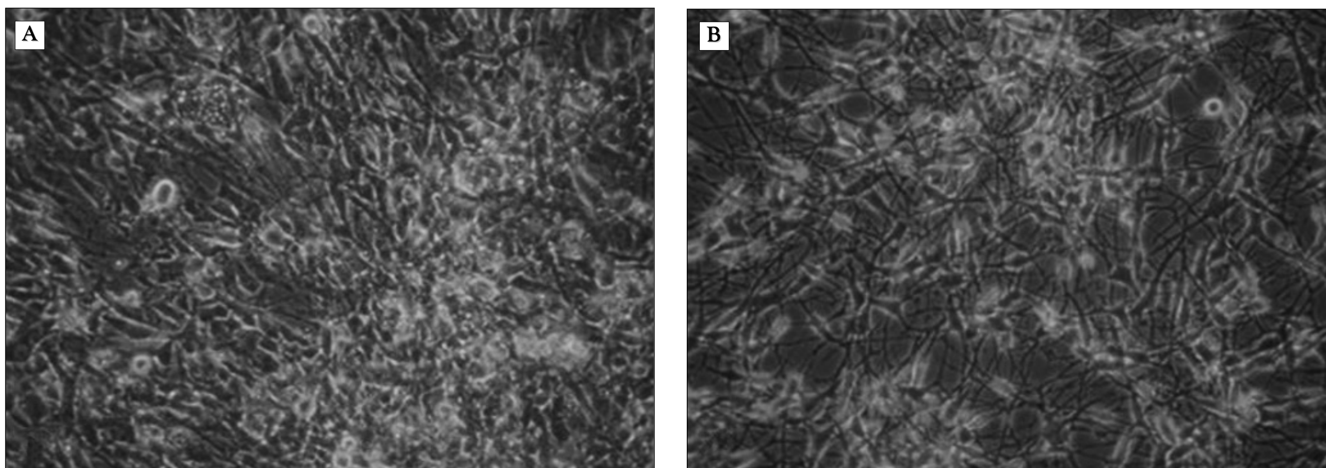

Figure 4. *Glioblastoma cells (a) and anaplastic astrocytoma (b) cells cultivated in serum-conditioned medium express a fibroblastic phenotype (Ob. 40X)*

histochemical marker for cells of human origin. Immunohistochemical observations showed that both Ston and Necu tumor cells are strongly positive, cytoplasm of tumor cells being rich in mitochondria *(Fig. 5A-B)*.

All four mice inoculated with glioblastoma-derived stem-like cells (Ston) developed xenografts at two months after inoculation. Tumor mass has been found either in fimbria hippocampus, near lateral ventricle, beyond lateral ventricle in striatum *(Fig. 6A)* or under hippocampus *(Fig. 6B)*. In one mouse, tumor cells exhibited migration, especially along the white matter tracts to the contralateral fimbria hippocampus *(Fig. 6C)*. Tumor mass was well demarcated from surrounding

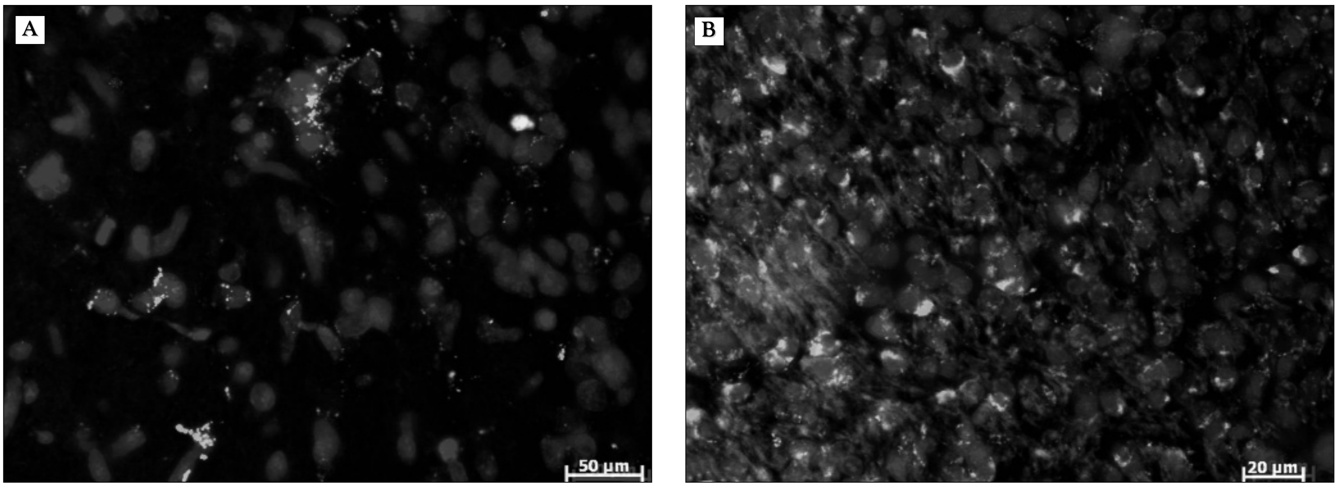

Figure 5. *Immunofluorescence staining for anti-human mitochondria showed diffuse, infiltrative pattern of Necu xenograft (**A**) and more compact tumor growth of Ston xenograft (**B**)*

Figure 6. *Histologic analysis of Ston (glioblastoma) xenografts at two months after inoculation showed presence of tumor cells in fimbria hippocampus (arrow), near lateral ventricle and beyond lateral ventricle in striatum (**A**), under hippocampus (asterisk in **B**) and in the contralateral fimbria hippocampus (asterisk in **C**). Infiltrating tumor cells were detected as single cells (arrows) near or around a blood vessel (**D**). H&E staining*

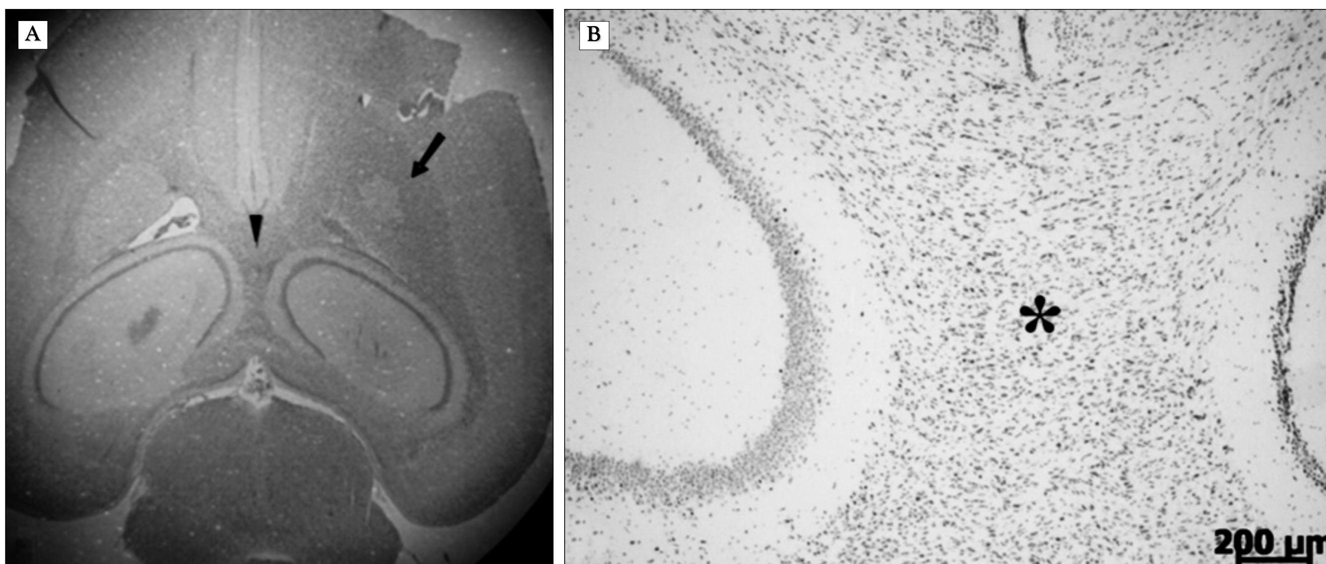

Figure 7. ***A.*** *Horizontal section of the mouse brain at two months after Ston cells inoculation. The main tumor mass is located under ipsilateral hippocampus (arrow) and infiltrated the dorsal hippocampal commissure (arrow head).* ***B.*** *Tumor cells infiltrating dorsal hippocampal commissure. H&E staining*

host brain tissue but infiltrating cells were detected as single cells and as cell clusters located near or around a blood vessel *(Fig. 6D)*.

In one mouse the main tumor mass was located under hippocampus and migrated along the dorsal hippocampal commissure to the contralateral hemisphere *(Fig. 7A-B)*. The shape and size of the tumor mass varied among individual mice of the same experimental group.

Of the other four mice inoculated with Ston cells, only one survived at three months. The sample of the brain obtained from one death mouse showed a voluminous tumor. In this case, as in the previous samples, the infiltration pattern is obvious. The tumor infiltrated the corpus callosum and migrated into the contralateral hemisphere *(Fig. 8)*.

When compares with Ston xenografts at two months, Necu xenografts have a low density cells population which resembles the lower density cells of original type of tumor – anaplastic astrocytoma- compared with glioblastoma cells population. In addition, in this case only three of four mice developed tumor.

Tumor cells were identified either associated with the white matter tracts of alveus hippocampus *(Fig. 9A-B)* or spread in the hippocampus *(Fig. 9C)*. As in the case of Ston xenografts, the shape and size of Necu tumor varied depending on the mouse. One mouse shows tumor cells in the contralateral hippocampus.

One mouse developed a voluminous tumor at two months which infiltrated the contralateral hemisphere *(Fig. 10)*.

After three months all four mice inoculated with Necu cells survived, but only three mice developed tumors, indicating a lower rate of growing of these xenografts. Moreover the xenografts developed in these cases are considerable smaller compared with Ston xenograft at three months. However, as in the case of Ston xenografts, the final behaviors of Necu xenografts were to infiltrate the commissural white tracts through the contralateral hemisphere *(Fig. 11)*.

None of the 16 mice used for the control group injected with serum-conditioned glioblastoma cultures (Ston) and serum-conditioned anaplastic astrocytoma cultures (Necu) primary cultures which expressed a fibroblastic phenotype, have developed tumor xenografts, neither at two months nor at three months after inoculation.

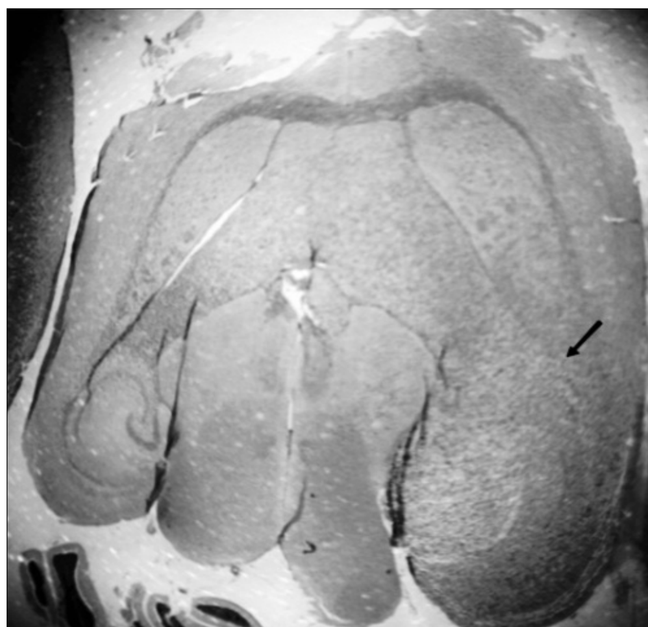

Figure 8. *Voluminous tumor mass (arrow) in a prematurely death mouse inoculated with Ston cells. Notice the infiltration of xenograft into the contralateral hemisphere through the corpus callosum. H&E staining*

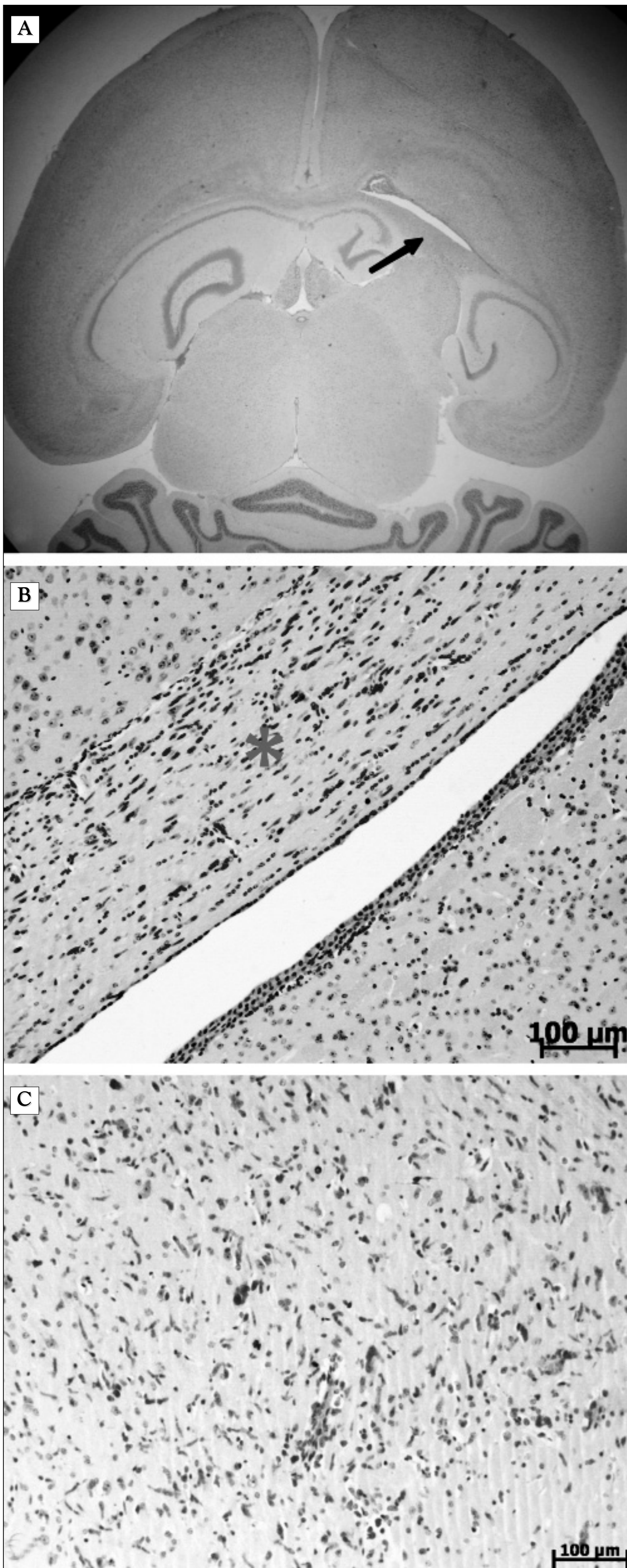

Figure 9. ***A.*** *Histologic analysis of Necu (anaplastic astrocytoma) xenografts at two months after inoculation, showed presence of tumor cells in the white matter of alveus hippocampus (arrow in a and asterisk in **B**) or spread in the hippocampus (**C**). H&E staining*

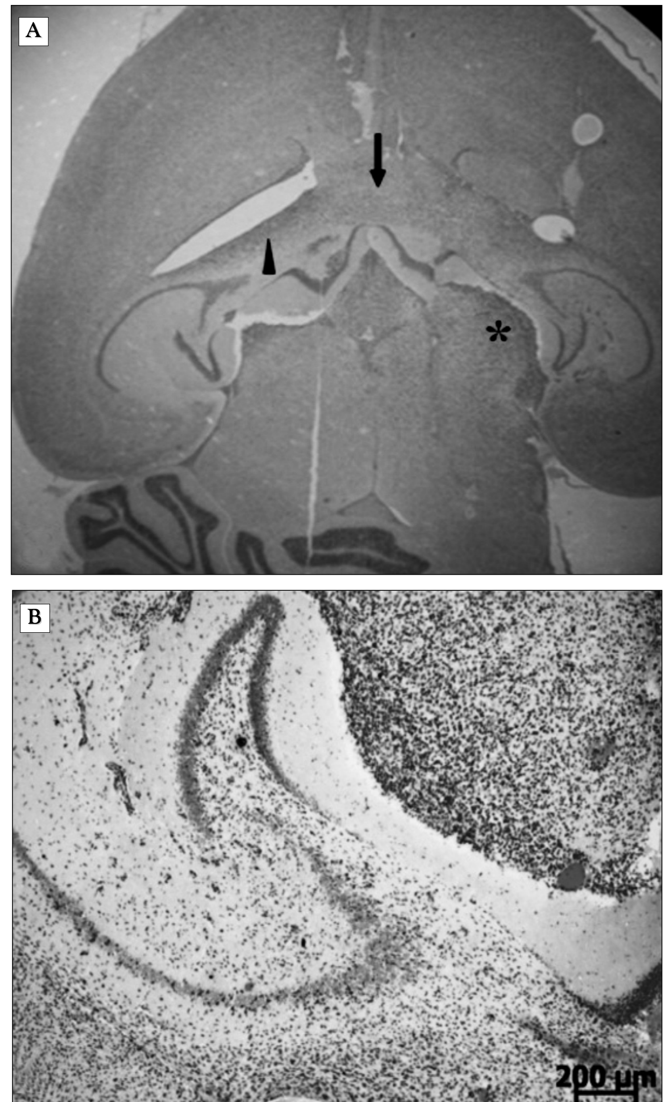

Figure 10. ***A.*** *Horizontal section of one mouse brain at two months after Necu cells inoculation. Tumor cells spread from the hippocampus (asterics), through the dorsal hippocampal commissure (arrow) in the contralateral hippocampus (arrow head).* ***B.*** *Tumor cells showing both a compact and diffuse pattern. H&E staining*

Nestin immunohistochemistry

Nestin is an intermediate filament protein typical for neural precursor cells that has been used as a marker for neural stem cells (8,9). Moreover, nestin is also used as a marker for identifying TSC from CNS tumors (1,10,11). In both Ston and Necu xenografts, the cytoplasm of most tumor cells stained with nestin antibody. Distribution of cells positive for human nestin showed diffuse, infiltrative pattern in Necu xenograft *(Fig. 12A)* and more compact tumor growth in Ston xenograft *(Fig. 12B)*.

Discussions

CD133 and nestin are specific markers for glioblastoma

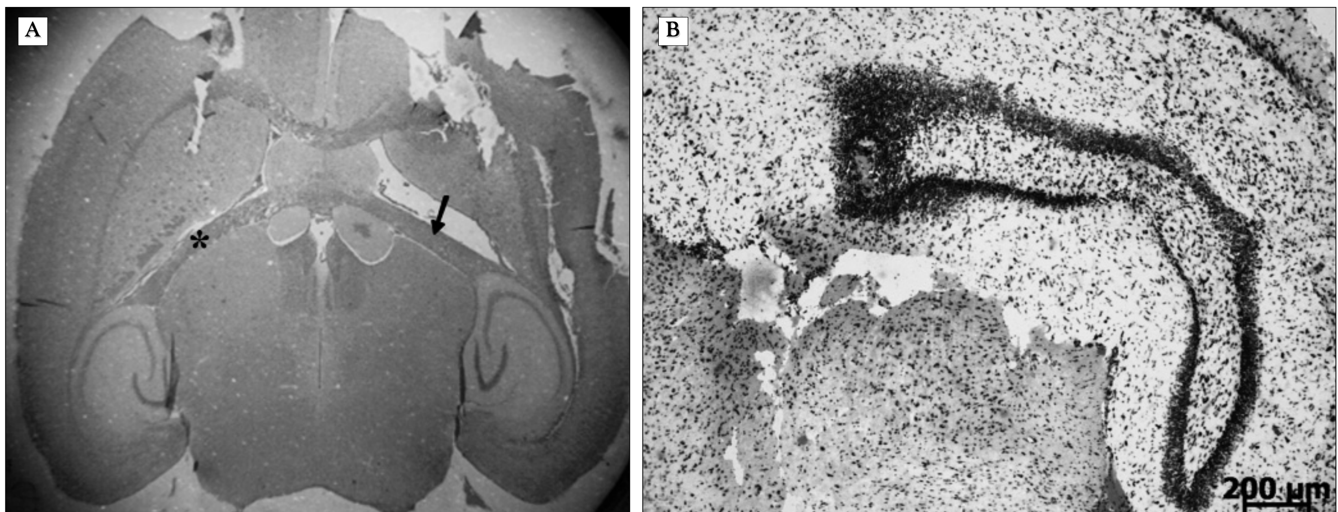

Figure 11. ***A.*** *Horizontal section of one mouse brain at three months after Necu cells inoculation. Tumor cells migrated through the internal capsulae (arrow) into the contralateral hemisphere (asterix).* ***B****. Tumor cells spread through the hippocampus. H&E staining*

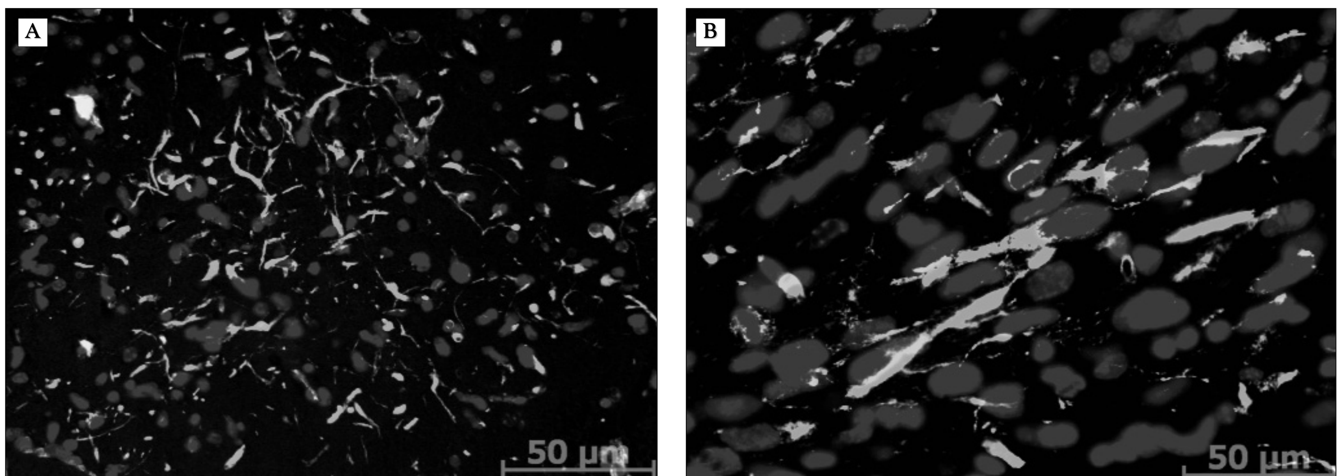

Figure 12. *Distribution of human nestin positive cells in the Necu xenograft (****A****) and Ston xenograft (****B****)*

derived TSC (11). The presence of CD 133 and nestin supports the theory of transformation of neural stem cells as the origin of glioblastoma. Both CD133 and nestin were strongly expressed in glioblastoma derived TSC (Ston) and anaplastic astrocytoma derived TSC (Necu) cultures compared with U87 culture. We found a stronger expression of nestin in Ston culture compared with Necu culture. A reverse situation was found for CD133; the expression of CD133 was stronger in Necu culture compared with Ston culture.

Other researchers isolated TSC from gliomas samples and report a direct correlation between the level of stem cells markers (CD133 and nestin) expression and the degree of glioma malignancy (6). Thus, according with these studies the glioblastoma samples expressed a higher level of stem cells markers compared with anaplastic astrocytoma samples. The small number of our samples doesn't allow us to draw a significant statistical conclusion in this direction.

Compared with U87 xenografts previously developed and described by our group (12), these types of xenografts manifest a slower rate of growing (small tumors developed at two months after inoculation compared with U87 voluminous bulk xenografts developed at 28 days after inoculation), but show a more extensive infiltration pattern. Interestingly, in the case of anaplastic astrocytoma-derived stem-like cells xenograft (Necu) the infiltration pattern is more pronounced. The presence of TSC among tumor population is supported by nestin imunofluorescence staining for both Ston and Necu xenografts. According to our results, the infiltrative pattern seems to correlate with in vitro CD133 expression. Therefore, the anaplastic astrocytoma sample (Necu), which expresses a higher level of CD133 in vitro (71.91% compared with 28.86% for Ston), manifests a more infiltrative growing pattern, compared with the glioblastoma sample (Ston). However, more gliomas samples should be tested in order to draw a statistical significant conclusion. One report demonstrated the presence of nestin mainly in the invasive

cells located at the xenograft periphery (13). On the contrary, in our xenografts, there is no specific pattern of distribution of nestin between migratory and non-migratory cells, and other specific markers for TSC migratory cells should be identified in further experiments.

None of the 16 mice from the control group, inoculated with serum conditioned tumor cultures, have developed tumor xenografts. This result underlines the importance of stem cells phenotype, which confers to the tumor cells tumorigenic features.

Many other researchers underlined the differences of the growth pattern between glioblastoma derived TSC xenografts and standard glioblastoma lines (like U87) xenografts, but little information is available about the growth pattern of anaplastic astrocytoma derived TSC xenografts. In our experiments the glioblastoma-derived TSC (Ston) induced tumors with slower rate of growing then U87 xenografts, but with a faster rate of growing then anaplastic astrocytoma-derived TSC xenografts (Necu). At two months after inoculation Necu xenografts were smaller then Ston xenografts, but showed a more infiltrative pattern. At three months after inoculation of Ston cells, three from four mice died. The samples obtained from one prematurely died mouse showed a voluminous tumor, which was the cause of death. Contrary, all four mice inoculated with Necu cells survived at three months after inoculation and the xenografts were considerable smaller compared with Ston. However, the most important difference between U87 xenografts and TSC xenografts is the growing pattern. As our group previously showed, U87 line developed a solid tumor mass with little sign of brain infiltration (12). Opposite to this situation, both anaplastic astrocytoma-derived TSC (Necu) and glioblastoma-derived TSC (Ston) induced xenografts with a very extensive tumor cells infiltration, not only into the surrounding areas of brain but also in the remote areas of brain parenchyma. The final behavior for both Necu and Ston xenografts is the infiltration through the commissural white matter tracts into the contralateral hemisphere which proves the extensive propensity for infiltration and invasion of these TSC xenografts. Therefore, these evidences support the hypothesis that cancer stem-like cells could be responsible for the infiltrating nature of glioblastoma and anaplastic astrocytoma. The reverse correlation between the rate of xenograft growing and infiltration pattern for U87 and TSC xenografts fits with the older "Go or grow" gliomas hypothesis. This hypothesis proposed that proliferation and migration of gliomas cells are mutually exclusive features (14).

It is well known that in gliomas, like in many other solid cancers, there is a hierarchical organization that includes slowly dividing stem cells, rapidly dividing transit amplifying cells (precursor cells), and differentiated cells (15). One can assume that TSC, during infiltration process, can activate the same mechanisms which neural stem cells use during brain development. Instead of using glial cells as scaffolds (as neural stem cells do (16)), these cells use the white matter tract as migration pathways. This hypothesis is supported by recent reports which suggested that glioma cells migrate like non-transformed, neural progenitor cell (17). There is another interesting report which established a statistical correlation between the location of glioblastoma and the noncontiguous infiltrative recurrences of the tumor. Thus, all contrast-enhancing lesion located in contact with subventricular zone of the brain, will always had tumor recurrences noncontiguous with the initial lesion. On the contrary, if the primary tumor is located at distance from the subventricular zone, it will have recurrences bordering the primary lesion (18).

Therefore, we suggest that the infiltrating gliomas cells are actually a subpopulation of TSC with pronounced migratory features but with low proliferation rate. These cells migrate throughout brain parenchyma contributing to the infiltration nature of the tumor. The majority of TSC population probably differentiates into glial precursor tumor cells, without migratory features but with extensive proliferation rate, which contribute manly to the growth of tumor mass. Recently it has been showed that gliotypic neural stem cells actually appear during normal differentiation process of neural stem cells and display a tumorigenic feature which involved an extensively proliferate capacities (19). This hypothesis may explain some unexpected effects of anti-VEGF therapy in glioblastoma patients who show multifocal reccurences and development of a more infiltrative pattern (20). The proposed explanation for these results is the increasing capability of tumor to develop an invasive phenotype without promoting angiogenesis (21). According with our hypothesis, the anti VEGF therapy will affect manly the proliferating population of glial precursor tumor cells, selecting the infiltrating TSC which are less dependent of the tumor neovascularization.

Several recent studies support the hypothesis described above, regarding the role of cancer stem-like cells in gliomas development and invasion. Thus the infiltrative growing of glioblastoma can be stopped on in vivo models if the differentiation therapy targets the TSC (22). Other authors had used as targets the molecules involved in the migration of neural stem cells and successfully succeeded to block the progression of glioblastoma (23,24).

However, all these experiments have been addressed mainly to glioblastoma and not to other lower grade astrocytomas. Therefore, taking into consideration our results, we suggest that these experimental therapies should be tested also on anaplastic astrocytomas and fibrillary astrocytomas, as the diffuse invasion growing pattern, driven by tumor stem cells, appears to be specific also for grade II and III astrocytomas and not only for glioblastomas.

Conclusions

The majority of studies have been focused on glioblastoma derived TSC xenografts characterization with little attention on anaplastic astrocytoma. In this paper we developed in vitro and in vivo models for both glioblastoma and anaplastic astrocytoma derived TSC. Our results support the hypothesis regarding the role of TSC in the infiltration growing pattern of glioblastoma and, moreover, apply this idea also to anaplastic astrocytoma. Because of the limited number of samples, further studies should be conducted in order to draw a statisti-

cal significant conclusion. However, the extensive infiltration growing pattern of these types of xenografts makes them useful models for studying the invasion mechanism in gliomas and for testing specific molecular therapies targeting tumor stem cells.

Acknowledgements

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