Slov Vet Res 2011; 48 (1): 5-14
UDC 577.2:616.8:616.714.1-006:599.323.4

Review Article

A RODENT BRAIN ORTHOTOPIC MODEL TO STUDY HUMAN MALIGNANT GLIOMA

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Summary: Glioblastomas are fatal brain tumors. They have a relatively low incidence, but the fact that regardless of the advances in therapy there hasn't been a major progress in survival over several decades urges the scientists to search for novel diagnostic tools, anti-tumor drug targets and therapies. It appears that our inability to successfully treat brain cancer mostly stems from the lack of understanding of the underlying brain tumor biology. Many rodent orthotopic models have been developed to address issues in drug development as well as biological origins of malignant gliomas. Establishing clinically relevant animal models of glioblastoma multiforme (GBM) remains a challenge, and many commonly used cell line-based models do not recapitulate the invasive growth patterns of patient GBMs. A novel orthotopic rat model of glioblastoma-the most malignant glioma in human-was recently developed, showing some stem-cell properties. The model is based on xenotransplantation of biopsy spheroids from human tumor tissue, into the brain of immunodeficient rats, where they initiated the growth of primary in most cases invasive and angiogenesis-independent glioblastomas. After serial passaging of tumors via spheroids in the subsequent generation of animals, the phenotype of the tumor changed. The most dramatic change was observed in approximately 1/3 of initially invasive tumors that changed into highly angiogenic and very aggressive. Some tumors though, remained invasive even after serial passages, while some were angiogenic from the start. The model thus provides combinations of angiogenic and invasive phenotypes and represents a good alternative to *in vitro* propagated cell lines for dissecting mechanisms of brain tumor progression. *In vivo* passaging of patient GBM biopsies produced tumors representative of the patient tumors, with high take rates and a reproducible disease course. The model has also been adapted to eGFP expressing immunodeficient mouse in which fluorescently marked tumors can be established *in vivo*.

Key words: malignant brain tumors; glioblastoma; nude rat; e-GFP NOD/SCID mice; xenograft; orthotopic transplantation; biopsy spheroids; translational medicine; molecular neuro-oncology

Introduction

Gliomas

Gliomas are brain tumors with some characteristics of brain support tissue – the glia. Malignant gliomas (anaplastic astrocytoma and glioblastoma multiforme–grade III and IV astrocytomas on WHO scale respectively) are predominant malignant brain tumors in human and have a poor prognosis with an average patient survival under current treatment regimens ranging between 12-14 months. Tumors are characterized by rapid cell growth, extensive neovascularization and diffuse cellular infiltration of normal brain structures (1). They have a relatively low overall incidence, but the fact that in spite of major progress in neurosurgery and oncology (including chemo-, radio- and, more recently biological-therapy) there hasn't been a major progress in malignant gliomas survival over several decades (2-7), urges the scientists to search for novel diagnostic tools, anti-tumor drug targets and therapies.

Received: 25 October 2010
Accepted for publication: 17 November 2010

Animal Tumor Models

In vivo animal modeling provides essential tumor-host interactions and is a more accurate way of mode-

ling human cancer than *in vitro*. We subdivide rodent models into xenograft tumor models – addressed in particular in this review - and models of spontaneous tumor formation in genetically engineered rodents. These models help us address important issues in drug development: toxicity and *in vivo* antitumor effectiveness (8, 9) (where the models are a matter of highly standardized procedures) as well as other basic phenomena in tumor origin and function such as angiogenesis, invasion and many others (3, 4, 10, 11) in the field of cancer research. *In vivo* modeling of drug efficacy is a gold standard required by a majority of pharmaceutical companies (12).

Xenograft tumors are initiated by implantation or injection of primary tumor cells or cell lines s.c. (under the skin) or orthotopically (into native tumor site) of syngeneic (genetically identical or closely related, so as to allow tissue transplant; immunologically compatible.), xenogeneic (derived or obtained from an organism of a different species, as a tissue graft), allogeneic (genetically different although belonging to or obtained from the same species, as in tissue grafts) or, immunosuppressed, immunodeficient, or newborn immunonaive animals (8, 9, 13, 14).

Rodent glioblastoma (GBM) models have been used for over 40 years but the extent to which they recapitulate the characteristics encountered in human GBMs remains controversial (8, 9). The advantages of xenograft glioma models are their highly efficient gliomagenesis, reproducible growth rates, and an accurate knowledge of the location of the tumor (6, 15, 23). However, in xenograft models stepwise genetic changes thought to occur during tumor progression are often missing; injected or implanted cells or cell lines have often been altered by culture or isolation conditions and lack their native tumor stroma. Murine models (e.g. (18) of GBM appear to recapitulate several of the human GBM histopathological features and, considering their reproducibility and availability, they constitute a valuable *in vivo* system for preclinical studies (8). On the other hand, many xenograft tumors lack histologicaly accurate vascularization, and rarely recapitulate tumor-of-origin phenotype (17). Immunodeficient rodents do not show antitumor immune effects and can produce false positives during drug trials (9). Syngeneic murine models, such as GL26 mouse glioma cells in C57BL6 mice (18) and CNS1 rat glioma cells in Lewis rats (19, 20) are non-immunogenic. Thus, syngeneic glioma models are excellent for studying the response of brain tumors to immunotherapy (19, 20). Establishing clinically relevant animal models of gliomas that would fully reflect the situation in human malignant glioma remains a challenge.

Two novel rat and mouse models to study glioblastoma

An orthotopic rat xenograft model of glioblastoma was developed (21) to address, among others, the tumor-host interaction issues leading to tumor circumscription, histologically accurate vascularization and to recapitulate accurately the tumor-of-origin phenotype. The model is based on a serial xenotransplantation of glioma biopsy spheroids, generated from glioma tissue (22) into the brain of nude rats. There the glioma spheroids induce the growth of primary, in most cases very invasive glioblastomas in 4-6 months time. These tumors co-opted the host vasculature and presented as an aggressive disease without signs of angiogenesis. The malignant cells expressed neural stem cell markers and showed a migratory behavior similar to normal human neural stem cells. When the rats became ill, they were sacrificed and spheroids were generated from their (human) tumors and implanted in the next generation of nude rats. This way, the tumors were passed in a total of 4-6 consecutive generations of nude rats (Figure 1). The most dramatic change was observed in approximately 1/3 of initially invasive tumors that changed into highly angiogenic and very aggressive, less invasive (more circumscribed), but which grew much faster due to good blood supply, killing a rat in 2-3 months. This switch to angiogenic phenotype was characterized by a reduction in stem cells markers (21, 23). Some tumors though, remained invasive even after serial passages, while some were angiogenic from the start. At the level of gene expression and immunoblotting proinvasive genes were up-regulated and angiogenesis signaling genes were down-regulated in invasive tumors. In contrast, proinvasive genes were down-regulated in the angiogenesis-dependent tumors derived from the invasive tumors (21). Uncoupling of invasion and angiogenesis, represented by the stem-like cancer cells and the cells derived from them respectively, points at two different mechanisms that drive tumor progression. Although the mechanism behind the phenotypic shift is not fully understood, HIF-1 expression seems to be triggered by hypoxia, because it was not constitutively expressed by high-generation tumor spheroids cultured under normoxic conditions (21) .

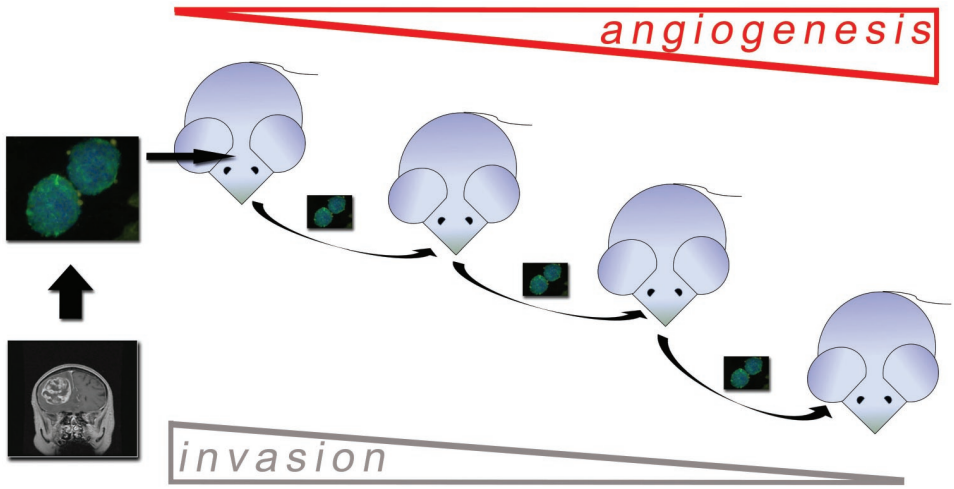

Figure 1: The orthotopic rat glioblastoma model

Thus, by serial passaging, this model uncouples and recapitulates for the first time the two major phenotype characteristics of human glioblastoma which makes it one of the best available glioblastoma models and opens the door to multiple applications in basic, translational and pre-clinical research. The major difference and - possibly – the advantage of this model over other described rodent models of glioblastoma (8, 9) arises from the fact that in the biopsy spheroids, which are structures of heterogenic cellular population, at least part of original glioma microenvironment is conserved, a part essential in inducing the tumor upon transplantation. This microenvironment is absent in xenografts generated from immortalized glioma cell lines (8, 9), but may be conserved to some extent in primary glioma cell lines derived from glioma patients and in glioma tissue grafts. The phenotypes, derived from the same tumor sample, may develop through a selection process where most cells in the biopsy specimens die following implantation in the rat brain. The cells that survive show stem cell-like properties, and are able to adapt to the new microenvironment where they divide and produce new tumor clones that show rapid growth and angiogenesis (21, 24).

The concept of the Rodent Glioblastoma model

Samples of glioblastoma, resected from the patients are finely minced and grown in cell culture medium. There the cells organize in tumor biopsy spheroids of about 300μm in size. Spheroids are orthotopically implanted into the brain of immunodeficient rodents. There, in most cases, they induce the growth of primary glioblastomas of invasive nature, but non-angiogenic in 4-6 months. With serial passaging in the subsequent generations of rodents via spheroids, the tumor phenotype will change in 1/3 of cases to fully angiogenic in the last generation xenograft with an onset of tumor in 2-3 months.

eGFP NOD/Scid mouse model

This glioblastoma model was recently adapted to a NOD/Scid mouse expressing enhanced green fluorescent protein (eGFP) (Figure 2). In this model human and mouse tumors marked with red fluorescent protein can be established *in vivo*, both at subcutaneous and orthotopic locations (25). Using modern microscopy techniques, the intricate co localization of tumor and host cells *in situ* can be visualized in detail. Technology of fluorescence-activated cell sorting (FACS), allows for complete and reliable separation of the host cells from the tumor cells, thus providing a system for detailed cellular and molecular analysis of tumor-host cell interactions. The fact that tumor and host cells can be reliably identified also allows for detection of double-positive cells, possibly arising from cell fusion events or horizontal gene transfer (two possible origins of tumor initiating – tumor stem cells; (26)). Similarly, the model can be applied for the detection of circulating metastatic cells and for detailed studies on the vascular compartments within tumors, including vasculogenic mimicry (25). Thus, the model should provide significant insight into how tumor cells communicate with their microenvironment.

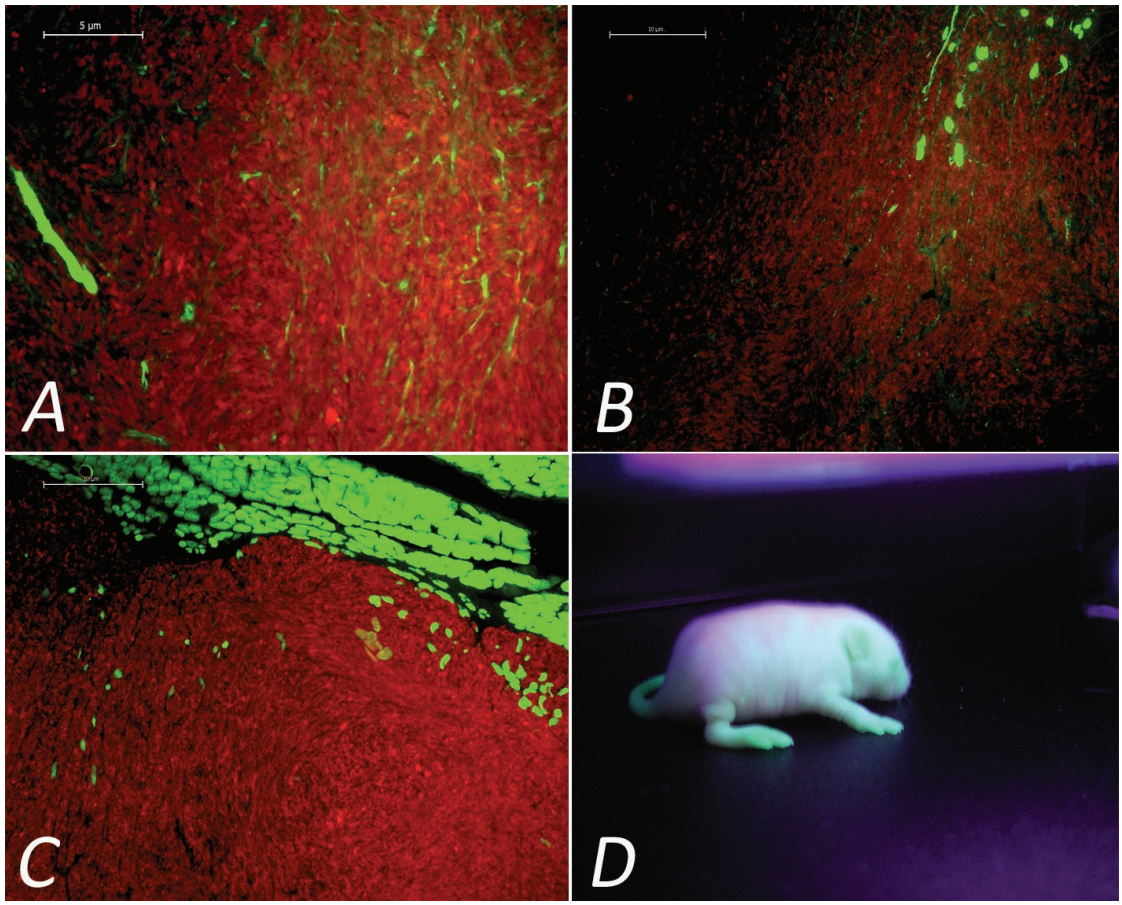

Figure 2: Tumor host interaction studies in an eGFP NOD/Scid mouse model

U87 dsRed expressing human glioblastoma cells were implanted in the flank of eGFP NOD/Scid mouse. (A-C) Endogenous fluorescence on a frozen histological section from a U87 subcutaneous tumor showing eGFP-expressing host cells within the tumor bed (direct eGFP and dsRed fluorescence). Scale bars = 5 µm (A); 10 µm (B, C). (D) a newborn eGFP NOD/Scid mouse glowing in green fluorescence under the UV light

Applications of the rodent glioblastoma model

Since its establishment, the rat model of glioblastoma was characterized in detail on several levels and used in multiple assays ranging from basic research to drug testing.

MR spectroscopy

In this study the metabolic properties of the two experimental phenotypes were analyzed. The early generation (EG) showed an intact blood–brain barrier and normal vascular morphology. In contrast, the high generation (HG) exhibited leaky vessels and necrosis. The rats with HG tumor had raised concentrations of choline and myo-inositol, and decreased concentrations of glutamate and N-acetylaspartate. In the LG tumor group, similar changes in metabolic concentrations were detected, although the alterations were more pronounced. The LG tumors also had higher concentrations of choline, taurine, and lactate. Results of this study showed that metabolic profiles could be used to distinguish between two glioblastoma phenotypes. More pronounced anaerobic metabolism was present in the LG stem-cell-like tumors, suggesting a more malignant phenotype (27).

Table 1: Applications of the rodent glioblastoma model

Applications		Model	Reference	Comments
Metabolite analysis	Determination of metabolic properties of the xenografts by Magnetic Resonance Spectroscopy (MRS)	Nude rat glioma xenograft	Thorsen F., et al., 2008 (27)	Metabolic profiles produced by MRS could be used to distinguish between two distinct glioblastoma phenotypes
Tumor initiation, take and reproducibility	Othotopic, stereotactic glioblastoma xenograft model construction and characterization	Nude rat glioma xenograft	Sakariassen PO et al., 2006 (21)	Separation of early /invasive tumor phenotypes and late/angiogenic tumors by serial passaging in nude rats
	Tumorigenesis; neuropathological and radiological features of rat glioblastoma xenograft model	Nude rat glioma xenograft	Wang J et al., 2009 (23)	In vivo passaging of patient GBM biopsies produced tumors representative of the patient tumors, with high take rates and a reproducible disease course.
	Tumor-host interaction studies	eGFP expressing NOD/SCID mouse	Niclou SP et al., 2008 (25)	Fluorescence-based intricate co localization of tumor and host cells *in situ*.
Anti-tumor drug testing and glioma treatment studies	Radio surgery	glioblastoma spheroids, nude rat glioma xenograft	Thorsen FA et al, 2007 (30)	Radio surgery of malignant gliomas might be effective in controlling tumor progression in selected glioblastoma patients.
	The response of the two phenotypes to doxorubicin	Nude rat glioma xenograft	Johannessen T-CA et al., 2009, (24)	Highly invasive tumors shown to be more chemo resistant than angiogenic tumors derived from the same patients.
	The effect of hyperoxic treatment on BT4C rat glioma xenografts	BT4C rat glioma xenografts	Stuhr LEB et al., 2007 (31)	Increased pO2–levels in experimental gliomas, using normobaric and moderate hyperbaric oxygen therapy, caused a significant reduction in tumor growth, a process characterized by enhanced cell death, reduced vascular density and changes
Gene Therapy	Adenoviral vector (AAV) transduction	Glioma cell lines, glioblastoma spheroids, nude rat glioma xenograft	Thorsen FA et al., 2006 (32)	AAV4 and AAV5 serotypes may be used to transduce biologically diverse glioma cell lines. They also penetrate and transduce solid human tumor tissue derived from patient biopsies.
	Lentiviral vector transduction	human embryonic kidney cell line 293T, TE671 cell line, nude rat glioma xenograft	Huszthy PC et al., 2009 (33)	Lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) and vesicular stomatitis virus glycoprotein (VSVG) pseudotyped lentiviral vectors efficiently transduced human glioblastoma cells and cancer stem-like cells. Pseudotyped gamma retroviral vectors, similar to those evaluated for clinical therapy of glioblastoma, showed inefficient gene transfer.
	Oncolytic HSV-1 based vector G207	Nude rat glioma xenograft	Huszthy PC et al., 2010, (34)	Favorable cellular responses to G207 treatment seen from a clinical viewpoint, such as reduced tumor cell proliferation, more frequent events of tumor cell death and a strongly attenuated tumor vascular compartment.
Biomarker research	The glioma-associated gangliosides 3k-isoLM1, GD3 and GM2	Nude rat glioma xenograft	Hedberg KM et al, 2001, (35)	Different biological roles for individual gangliosides; antibodies or ligands directed against GD3 and 3k-isoLM1 might be complementary when applied in the treatment of human glioblastomas.
	Expression of extracellular matrix components in a highly infiltrative glioma model	Glioma cell lines, nude rat glioma xenograft	Mahesparan R et al., 2003, (16)	Possible biological function of tenascin, vitronectin, laminin, fibronectin and collagen type IV in highly invasive malignant tumors of glial origin.
	PDI protein expression in invasive phenotype tumors	Nude rat glioma xenograft	Goplen D et al., 2006 (36)	PDI was shown to be expressed on migrating and invading glioma cells.
	Tumor initiating cell markers	Nude rat glioma xenograft	Wang J et al., 2007(29)	Cd133- glioma cells are tumorigenic and can produce cd133+ tumors, CD133 expression coincides with the onset of angiogenesis and a shorter survival
	Global membrane proteomics	Nude rat glioma xenograft	Rajcevic U et al., 2009 (37)	Known and novel candidate proteins were identified that characterize the switch from a non-angiogenic to a highly angiogenic phenotype. Enhanced intercellular cross-talk and metabolic activity adopted by tumor cells in the angiogenic compared with the non-angiogenic phenotype.
	Neural cell adhesion molecule (NCAM) as a glioma marker for the biological aggressiveness	Nude rat glioma xenograft	Duenisch P et al., 2010, (38)	The expression of NCAM-140 inversely correlated with the WHO grade of human gliomas

Tumor initiation, take and reproducibility

The tumor take rates for xenografted GBM biopsies were 96% and close to 100% at subsequent passages *in vivo*. Only one of four lower grade tumors engrafted. MRI typically revealed changes related to tumor growth, several months prior to the onset of symptoms (23).

In another study, CD133 expression was analyzed at various passages. CD133 is a cell surface marker identified as a putative marker of brain tumor-initiating cells (28). During passaging, the tumors gradually displayed more contrast enhancement, increased angiogenesis, shorter survival and increased CD133 expression. CD133 negative cells derived from 6 different patients were tumorogenic when implanted into the rat brains. For 3 of these patients, analysis showed that the resulting tumors contained CD133 positive cells. In this assay, the authors showed that CD133 negative glioma cells were tumorogenic in nude rats, and that CD133 positive cells can be obtained from these tumors. Upon passaging of the tumors *in vivo*, CD133 expression is up regulated, coinciding with the onset of angiogenesis and a shorter survival. Authors also suggested that CD133 may not be essential in tumor initiation process (29).

Anti-tumor drug testing and glioma treatment studies

In a study focused on the radiobiological effects of the Gamma knife (Gamma Knife is currently used to boost treatment of malignant gliomas) the growth and invasiveness of human glioblastoma spheroids xenografted into nude rat brains were assessed after radio surgery. A dose-dependent inhibition of tumor growth and invasion, as well as a dose-dependent increase in animal survival was observed. The results indicated that radio surgery of malignant gliomas might be effective in controlling tumor progression in selected glioblastoma patients (30).

In a study aimed at investigating how the two phenotypes responded *in vitro* to doxorubicin, a clinically potent cytotoxic drug for solid tumors, highly invasive tumors shown to be more chemo resistant than angiogenic tumors derived from the same patients. It was suggested that treatment resistance in glioblastomas could be related to PI3K/AKT activity in stem-like tumor cells, and that targeted interference with the PI3K/AKT pathway might differentiate and sensitize this subpopulation to chemotherapy (24).

Another study described the biological effects of hyperoxic treatment on BT4C rat glioma xenografts *in vivo* with special reference to tumor growth, angiogenesis, apoptosis, general morphology and gene expression parameters. Increased pO2–levels in experimental gliomas, using normobaric and moderate hyperbaric oxygen therapy, caused a significant reduction in tumor growth. This process was characterized by enhanced cell death, reduced vascular density and changes in gene expression corresponding to these effects (31).

Gene Therapy

In one of the initial studies on the delivery vehicles for gene transfer strategies directed at the central nervous system (CNS), muscle and liver performed on the featuring model the transduction efficacy of AAV serotypes 4 and 5 were compared to AAV2, both *in vitro* and in intracranial GBM xenografts . While all three AAV serotypes were able to transduce the glioma cell lines when added individually or when they were administered in concert, AAV2 transduced the glioma cells most effectively compared to AAV4 or AAV5. Upon infecting glioblastoma spheroids *in vitro*, all three AAV serotypes efficiently transduced cells located at the surface as well as within deeper layers of the spheroids. In addition, both AAV4 and AAV5 were able to transduce human glioblastoma xenografts implanted intracranially. Authors suggested that beside AAV2 serotype, AAV4 and AAV5 serotypes may also be used to transduce biologically diverse glioma cell lines and may be used in developing treatment vehicles for human malignant gliomas (32).

The rat xenograft model was used to analyze the transduction pattern and therapeutic efficacy of lentiviral pseudotyped vectors. Both, lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) and vesicular stomatitis virus glycoprotein (VSVG) pseudotyped lentiviral vectors efficiently transduced human glioblastoma cells and cancer stem-like cells. In contrast, pseudotyped gamma retroviral vectors, similar to those evaluated for clinical therapy of glioblastoma, showed inefficient gene transfer. In a therapeutic approach using the suicide gene herpes simplex virus thymidine kinase (HSV-1-tk) fused to eGFP, both lentiviral vectors mediated a complete remission. In all recurrent tumors, surviving eGFP-positive tumor cells were found, advocating prodrug application for several cycles to even enhance and prolong the therapeutic effect. The inefficient gene delivery by gamma retroviral vectors is in line with the results obtained in clinical therapy for GBM and thus con-

firms the high reproducibility of the invasive glioma animal model for translational research (33).

In another assay the cellular effects of the oncolytic HSV-1 based vector, G207, on the tumor microenvironment were evaluated. The xenografted tumors were quantitatively evaluated 10-30 days after G207 injection for virus-induced changes in proliferation, apoptosis and vascularity. Vector spread and the infiltration pattern of CD68-positive inflammatory cells were assessed. Proliferation indices were lower, whereas apoptotic counts were elevated in plaques as compared with that in non-infected areas of the same lesions, as well as in corresponding control xenografts. A decline in the number of blood vessels was noticed in the plaques and the vascular area fractions were reduced. CD68-positive inflammatory cells accumulated in the plaques. The study highlighted the favorable cellular responses to G207 treatment seen from a clinical viewpoint, such as reduced tumor cell proliferation, more frequent events of tumor cell death and a strongly attenuated tumor vascular compartment (34).

Biomarker research

A substantial number of biomarker research studies were thus far performed on the model. Initially roles for glioma-associated gangliosides (3k-isoLM1, GD3 and GM2) were assessed in the model, pointing out that different biological roles for individual gangliosides may exist and that the antibodies or ligands directed against GD3 and 3k-isoLM1 might be complementary when applied in the treatment of human glioblastomas (35).

In a different study expression of extracellular matrix (ECM) components in this highly infiltrative *in vivo* glioma model was analyzed. The cellular origin for several of these ECM components was identified using human-specific monoclonal antibodies and polyclonal antibodies detecting epitopes from both species (human and rat). Observed staining patterns clarified the cellular origin and indicated the possible biological function of tenascin, vitronectin, laminin, fibronectin and collagen type IV in these highly invasive malignant tumors of glial origin (16).

In the initial proteomic assays on biomarkers on the featuring model the researchers showed that PDI, one of the most prominently up-regulated proteins in invasive tumors is strongly expressed on invasive glioma cells, in both xenografts and at the invasive front of human glioblastomas. Using *in vitro* assays, PDI was shown to be expressed on migrating glioma cells. Functional significance of PDI in cell migration and invasion was tested *in vitro*, showing an important role of PDI in glioma cell invasion (36).

In a global proteomics comparison of the two xenograft phenotypes we were able to identify several thousand proteins in membrane-enriched fractions of which 1460 were extracted as quantifiable proteins (isoform- and species specific and present in more than one sample). Known and novel candidate proteins were identified that characterize the switch from a non-angiogenic to a highly angiogenic phenotype. The data pointed to enhanced intercellular cross-talk and metabolic activity adopted by tumor cells in the angiogenic compared with the non-angiogenic phenotype. The identified proteins could be further exploited as biomarkers or therapeutic targets for malignant gliomas (37).

In a different assay the relevance of neural cell adhesion molecule (NCAM) as a glioma tissue marker for the biological aggressiveness of these tumors was assessed. The expression of NCAM-140 inversely correlated with the WHO grade of human gliomas. The lost expression of NCAM-140 in human glioblastomas and in brain metastases enabled the investigation of the brain– tumor interface and the definition of glioblastoma invasion patterns and showed that brain metastases are more invasive than ever thought (38).

Conclusions

In vivo passaging of patient GBM biopsies in rats or mice produced tumors representative of the patient tumors, with high take rates and a reproducible disease course. The main advantage of the model provides combinations of angiogenic and invasive phenotypes and represents a good alternative to *in vitro* propagated cell lines for dissecting mechanisms of brain tumor progression (Wang J, BMC Cancer 2009) as well as to other *in vivo* orthotopic rodent GBM models. Thus far, the model has been extensively used in a variety of applications, ranging from basic research of cancer biology and biomarkers including genomics, transcriptomics, proteomics and metabolomics, through translational research of therapeutics and therapeutic modalities to preclinical research of drugs approved for use in other types of cancer. The results of this research provided important novel insights into the mechanisms of tumor initiation, promotion and progression

through transcriptomic markers, through novel protein biomarkers, validated on clinical and pre-clinical material. Results also indicated the metabolic changes linked to phenotype switch in the model. A possibility of separating the tumor-host compartments in the model based on cellular, fluorescence based- or biochemical, protein sequence-based level enables an unprecedented potential in tumor-host interaction studies, the complex of events crucial for the understanding of tumor initiation and function. The research of novel therapeutics and therapeutic modalities has shown a great potential of this model as it recapitulates the main features of the GBM, for *in vivo* studies including radio-, chemo- and gene therapies.

Acknowledgements

Dr Simone P. Niclou, NorLux Neuro-Oncology Laboratory, CRP-Santé, Luxembourg is kindly acknowledged for the critical review of the manuscript and for providing photos of the animal model. The work was funded by Fonds National de la Recherche (FNR), Luxembourg (contract FNR-AFR # PDR-08-007) to U.R. and by Slovene Research Agency (Program Grant # P1—0245).

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GLODAVSKI MOŽGANSKI ORTOTOPIČNI MODEL ZA ŠTUDIJ ČLOVEŠKEGA MALIGNEGA GLIOMA

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Povzetek: Maligni gliomi so neozdravljivi možganski tumorji. Se relativno redko pojavljajo, vendar ne glede na izboljšave pri terapiji večjega napredka pri preživelosti bolnikov ni bilo že več desetletij. Zaradi tega je pomembno raziskovanje novih diagnostičnih orodij, ugotavljanje novih tarč v tumorskem tkivu in razvijanje novih načinov zdravljenja. Zdi se, da naša nezmožnost uspešno pozdraviti raka na možganih izvira iz nepoznavanja bioloških in biokemičnih lastnosti tumorjev. Z leti je nastalo več glodavskih ortotopičnih modelov, ki so lahko uporabni pri razvoju novih zdravil in pri proučevanju bioloških osnov malignih gliomov. Razvoj klinično pomembnih živalskih modelov za multiformni glioblastom (GBM) ostaja izziv in številni, pogosto uporabljeni modeli na osnovi celičnih linij, ne odražajo vzorcev invazivne rasti GBM pri bolnikih. Nedavno je bil razvit nov ortotopični podganji model glioblastoma – najbolj maligne oblike glioma pri človeku, ki kaže nekatere značilnosti, podobne matičnim celicam. Model je nastal na osnovi medvrstne presaditve biopsijskih sferoidov iz vzorcev človeških tumorjev v možgane podgan z oslabljenim imunskim sistemom, kjer so sferoidi povzročili rast primarnih in večinoma invazivnih, od angiogeneze neodvisnih glioblastomov. Po zaporednem prenosu teh tumorjev preko sferoidov v naslednje generacije živali se je fenotip tumorja spremenil. Najbolj dramatična sprememba je bila ugotovljena pri približno tretjini sprva invazivnih tumorjev, ki so se spremenili v visoko angiogene in zelo agresivne. Nekaj tumorjev je ostalo invazivnih kljub zaporednemu prenosu v naslednje generacije podgan, medtem ko so bili nekateri angiogeni že od začetka. Model torej prinaša kombinacijo angiogenih in invazivnih fenotipov in predstavlja dobro alternativo celičnim linijam, gojenim v pogojih *in vitro*, za ugotavljanje mehanizmov napredovanja možganskih tumorjev. Zaporedni prenos bolnikovih biopsij GBM *in vivo* je povzročil nastanek tumorjev, podobnih pacientovim, z visoko ravnijo tumorigeneze in ponovljivim potekom bolezni. Model je bil prilagojen tudi mišim z oslabljenim imunskim odgovorom, ki izražajo zeleno fluorescenčno beljakovino in kjer lahko vzpodbudimo nastanek fluorescenčno označenih tumorjev pri živih živalih.

Ključne besede: maligni možganski tumorji; glioblastom, gole podgane; miši e-GFP NOD/SCID; ksenograft; ortotopična transplantacija; biopsijski sferoidis; translacijska medicina; molekularna nevroonkologija