Comparison of 6 experimental models of brain tumors : morphological and vascular characteristics

4372

M. Gandit[2], N. Just[1], B. van der Sanden[2], O. Duchamp[1], H. Lahrech[2], N. Guilbaud[1], P. Genne[1], C. Rémy[2]

[1] Oncodesign Biotechnology, Dijon, France; [2] INSERM U594, Université Joseph Fourier, Grenoble, France

Introduction

Treatment of glioblastoma multiforme (GBM) often fails due to tumor resistance to conventional cytotoxic chemotherapy and radiotherapy.

The effectiveness of the various antitumor therapies is conditioned by the cancer cells themselves but also by the degree of oxygenation and vascularization, the blood flow and the vessel permeability. These physiological parameters vary according to the type of tumor and also from one patient to another, making very difficult the prediction of response to a therapy. In addition, a treatment efficacy is currently evaluated by the tumor volume reduction which occurs often lately compared to physiological or morphometric parameters changes of vascularization. Non-invasive techniques, like MRI, make it possible to characterize in vivo the tumoral micro-environment, more particularly the vascularization. They are of interest for therapeutic orientation and follow-up of the patients. Some of these methods start to be used in clinic but their validation and their robustness yet are not completely evaluated, in particular in the case of brain tumors. To be robust, the evaluation of new therapies and predictive indicators of therapeutic response requires studies on several experimental models presenting different responses to treatments and different tumor micro-environment (oxygenation, vascularization...) to mimic the human variability.

Objectives

- To characterize the morphological and vascular characteristics of 6 experimental orthotopic models (3 syngeneic and 3 xenogeneic) of brain tumors in rats
  - *in vivo MRI using blood volume and vessel size imaging
  - *in vitro using histology (tumor morphology) and immunohistology (vessel density, vessel size and permeability)
- To compare the in vivo and in vitro data

Material and methods

Experimental brain tumor models

Glioma cells were inoculated by stereotactic injection in the right striatum of 10 rats per model:

Murine tumors	Human tumors
- C6 : astrocytoma (Wistar)	- CGL9: glioblastoma (Nude)
- GV1A-1: mixt glioma (BDIX)	- CGL3: glioblastoma (Nude)
- 9L: gliosarcoma (Fischer 344)	- U87-MG: grade III astrocytoma (Nude)

These 6 glioma models were previously screened for their *in-vitro* and *in-vivo* sensitivity to BCNU treatment

MRI

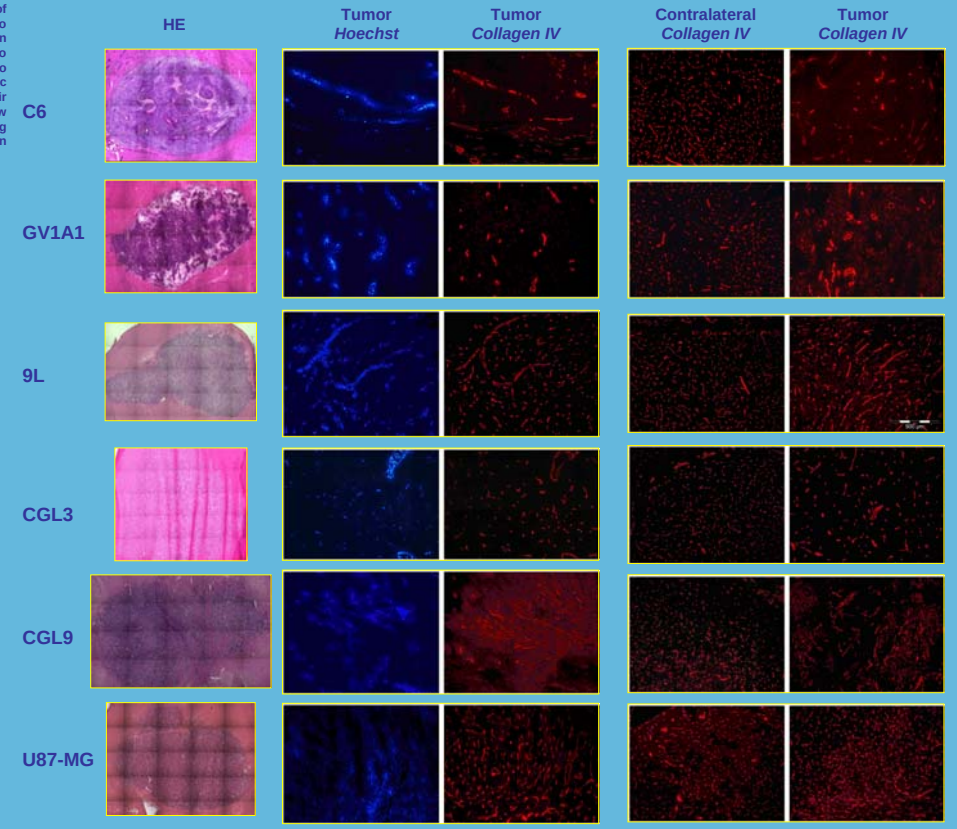
- Morphological T_2-weighted MRI (2.35T) to compose groups of 5 to 8 rats bearing gliomas of identical volumes (50-75 mm³).
- One day after, diffusion imaging followed by acquisition of Multiple Gradient Echoes - Spin Echo images before and 4 min after intravenous injection of an intravascular contrast agent (Sinerem®, Guerbet). Vessel size index (VSI) and Blood Volume (BV) maps were computed from the ΔR_2^* (gradient echo images), ΔR_2 (spin echo images) and water diffusion coefficient maps (Tropres et al., 2001).

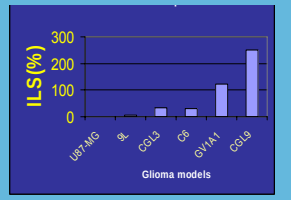
Histology

Rats were sacrificed at the end of MRI and 1 minute after intravenous injection of a Hoechst 33342 solution, for analysis of the perfused vessels and assess their permeability on 10 µm thick cryo-sections . Collagen IV immunostaining on the same sections allows detection of all vessels. Hematoxylin Erythrosin staining (HE) was performed on adjacent section.

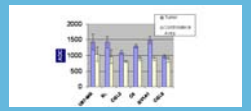
Results

	C6	GV1A1	9L	CGL3	CGL9	U87-MG
Edema: Intratumoral / Periphery	+ / +	+ / +	+ / ++	+ / +	+ / ++	++ / ++
Cell density	++	++	++	+	++	++
Limits	sharp	sharp	sharp	fuzzy	infiltration	sharp
Necrosis	yes	no	no	no	no	no
Pseudo-cysts	no	yes	no	no	no	no
Nuclei aspects	small-round	small-round large-round	small-round small-oblong	small-irregular	small-round small-oblong	small-round
Vessel density /normal tissue	<	<	≤ ?	≤ ?	<	≥ ?
Vessel size /normal tissue	>	>	>	≈	>	≈
Collagen IV over-expression	± ?	-	+	-	++	+
Vessel - perfusion -permeability	most yes	all yes	most yes	all no	not all yes	all yes

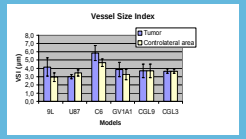


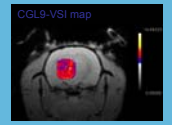


Increased Life Span after BCNU treatment of Nude rats bearing a glioma. Rats bearing CGL-9 presented no glioma on the day of sacrifice



Apparent Diffusion Coefficient (averaged over 6 rats) for each glioma model and the contralateral part of the brain





Vessel Size index (VSI) determined by MRI in each glioma model and the controlateral rat brain. Example of vessel size index map in a CGL9 glioma

Results obtained by MRI and histology were coherent regarding vessel size which was higher in C6, 9L, GV1A1 than in contralateral tissue, similar for CGL3 and U87-MG. The discrepancy observed for CGL9 (vessels appeared thicker in the tumor than in the contralateral tissue, while VSIs were similar) might arise from only a small portion of perfused vessels and/or CGL9 tumor cells around the vessels over-expressing collagen IV.

More discrepancies were observed between BV and vessel density. However when using MRI, BV depends on both vessel density and vessel size of perfused vessels. The vessel density was obviously lower in the C6, GV1A1 and CGL9 tumors than in the contralateral tissues, while BV was lower only in the C6 tumors.

Conclusions

- The 6 brain tumor models exhibit different morphological and vascular characteristics which may explain the differences of sensitivity to BCNU treatment
- MRI and histology demonstrated their complementary potentials and their usefulness in the assessment of the characteristics of glioma models
- This thorough investigation of 6 glioma models warrants their use to explore new antiangiogenic and cytotoxic therapies and their rapid transfer to the clinics