Bioluminescence imaging in brain tumour -a powerful tool-

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Introduction

Glioblastoma represents the most malignant and lethal among brain tumours because of its highly infiltration capacity and invasion into the normal brain that account for its resistance to treatments (chemotherapy and radiotherapy). Recent advance and development of technologies to non-invasively image brain tumour growth in living animals can open an opportunity to monitor directly the efficacy of the treatment on tumour development. *In vivo* bioluminescence imaging is based on light-emitting enzymes, luciferases, which require specific substrates for light production. When linked to a specific biological process/pathway in an animal model of human disease, the enzyme-substrate interactions become biological indicators that can be studied.

In order to explore and compare different imaging modalities (MRI and bioluminescence imaging) we have validated the use of bioluminescence imaging to monitor glioblastoma progression in vivo.

The human glioma cell line (DBTRG-05MG) derived from an adult patient with glioblastoma multiforme who had been treated with local brain irradiation and multidrug chemotherapy has been used for the experiment. The DBTRG-05MG cell line was stably transfected with TCF-luciferase and orthotopic implantated onto immunodeficient mice. Bioluminescence technology was used to follow tumour growth in parallel with classical MRI on the same animals.

Material and Methods

Cell and surgery details: DBTRG cells were stably transfected With TCF-Luciferase and cultured at limiting clonal dilution. One Clone with high luciferase expression was chosen for the *in vivo* experiments. 5x10^5 cells in 5 µl of PBS 1X were injected in Each animal under sterotactic guidance (coordinates from bregma: + 0.5mm anterior, + 2.2mm
lateral and - 3.0mm depth).

Optical Imaging: A VivoVision Systems, IVIS® 200 Series, (Xenogen Corporation, Alameda USA) was used for all experiments. Images were acquired and analyzed with Living Image 2.6 e and Living Image 3D (Xenogen Corporation, Alameda USA). Acquisition details: FOV= 4x4cm centred on The brain; (f/stop) = 1. Binnig factor, exposition time and use Of filters of emission set for 3D reconstruction was set. After
a pre acquisition it was performed a post D-Luciferin (firefly, Potassium salt, Xenogen), 150mg/kg/10ml IP, injection acquisition. Optical imaging acquisitions will be carried out
after 1-2-3-4-5 weeks from cell inoculation.

Magnetic Resonance Imaging Images were acquired at 4.7 T using a helmet shaped coil optimized for the mouse brain. After a scout acquisition to localize the brain, multislice T2 Weighted RARE images were acquired to measure the tumor volume. Acquisition parameters were: TR=5000 ms TE=60 Ms FOV=2.5x2.5 cm2; slice thickness=1mm, number of slice = 11. MRI examinations were performed 2, 4, 6 weeks after cell implantation. At selected time points contrast enhanced T1 weighted images were acquired using Gadolinium-DTPA (Magnevist®) injected i.v. trough the tail vein at
100-300 mmol/kg.

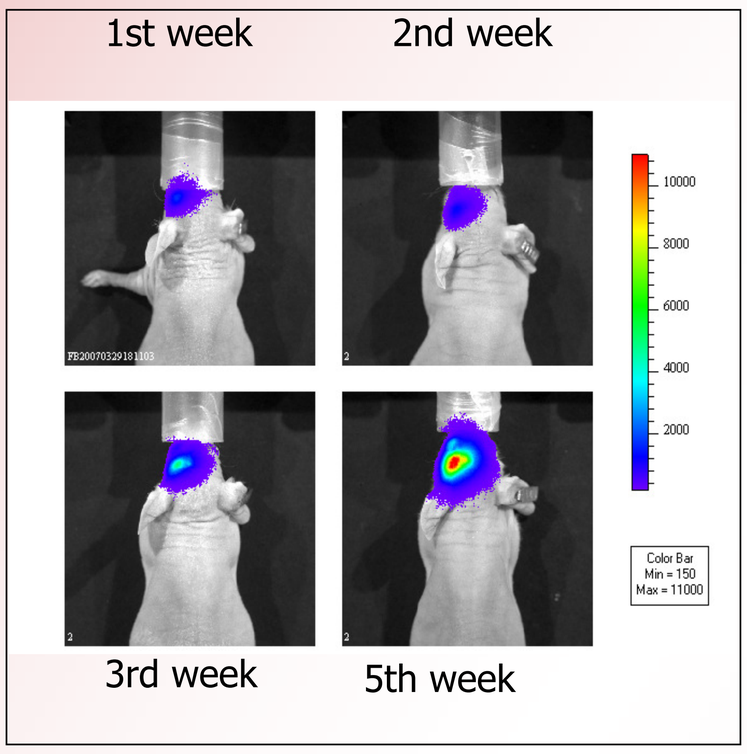

Figure 1. Bioluminescence images of tumours in the brain of nude mice at different time after cell injection.

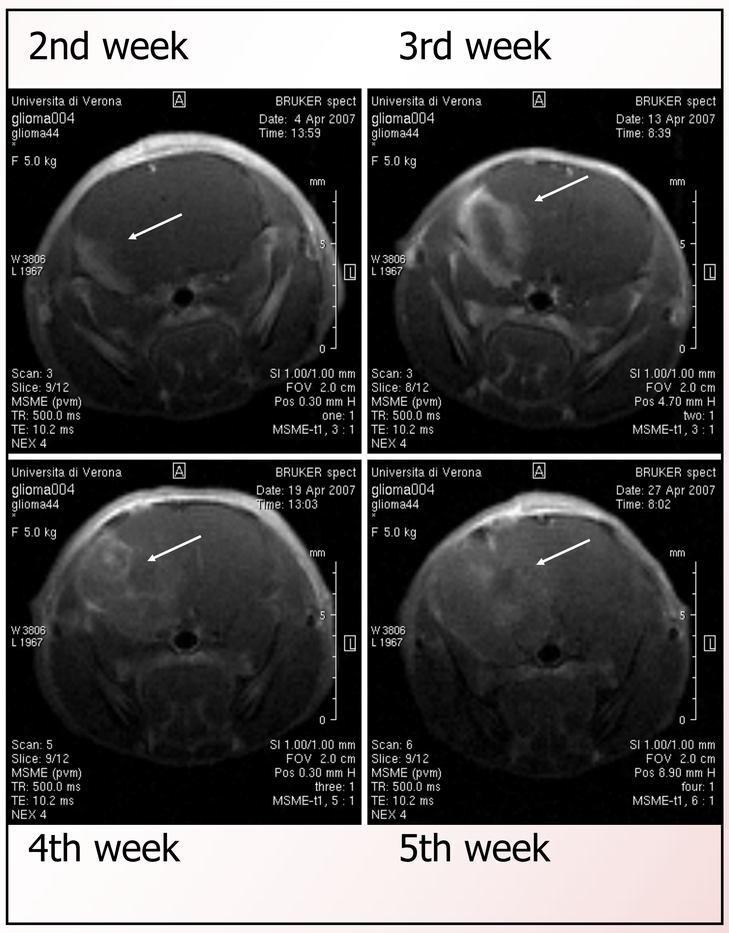

Figure 2. Magnetic Resonance Imaging of the same animal as depicted in Figure1 (white arrow indicate tumour area) at the indicated times. In the figure contrast enhanced T1-weighted images were acquired using Gadolinium-DTPA (Magnevist®) injected i.v. through the tail vein at 100-300 mmol/kg.

Results

After injection of glioma cells stably transfected with luciferase we followed the development of the tumours using bioluminescence and MRI in parallel for a total of 6 weeks.

Using bioluminescence, 1 week after cell engraftment, glioma cells were visible in almost all animals (Figure 1). MRI detected tumour development only starting from 2-3 weeks after cell injection (Figure 2). However, when the two techniques were compared over time we obtained a nice correlation (Figure 3). 3-D reconstruction of luminescence signal shoe distribution of light emitted by tumour cells in the mouse brain (Figure 4). 6 week after injection, animals were sacrificed.

Conclusions

Data presented here suggested that:

Bioluminescence can be used to monitor in vivo intracranial tumor growth

The potentiality of bioluminescence and MRI imaging may open new perspectives also to use these tools for drug discovery by directly monitoring the efficacy of the therapy

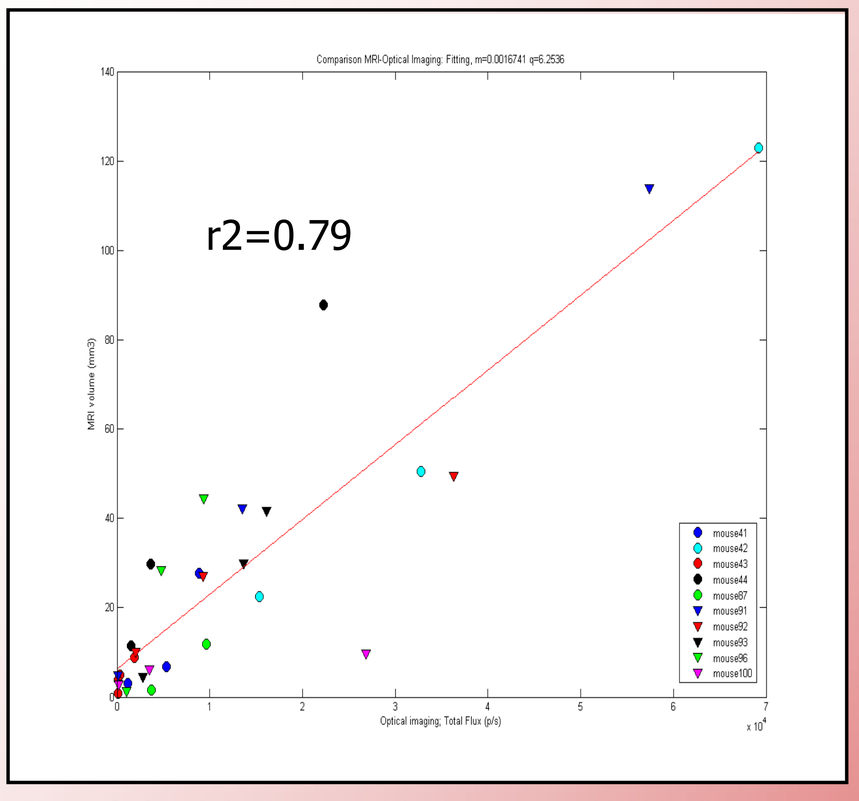

Figure 3. Correlation between bioluminescence and MRI in all animals examined

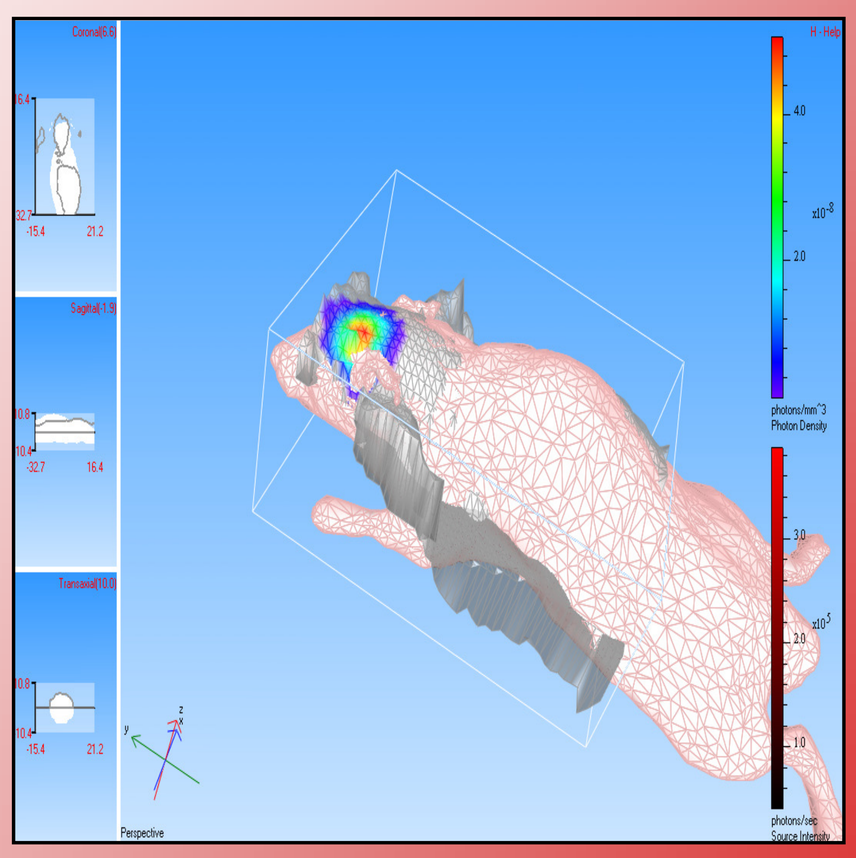

Figure 4. 3D-reconstruction of luminescent tumor in the mouse brain 5 weeks after the injection

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