Bioluminescence imaging in brain tumour – a powerful tool in drug discovery

Poster n. MO-05

siena biotech

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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). Despite new therapeutic approaches have been performed in the last decade the average survival of GBM patients is around 1 year. The identification of new relevant therapeutic targets in brain tumors can provide new ways to approach and treat brain tumors. 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, bioluminescence imaging and histology) 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 RE-luciferase and orthotopic implantated onto immunodeficient mice. Bioluminescence technology was used to follow tumour growth in parallel with classical Magnetic Resonance Imaging on the same animals. Complete characterization, histology, gene expression and phenotype of the glioblastoma model have been performed.

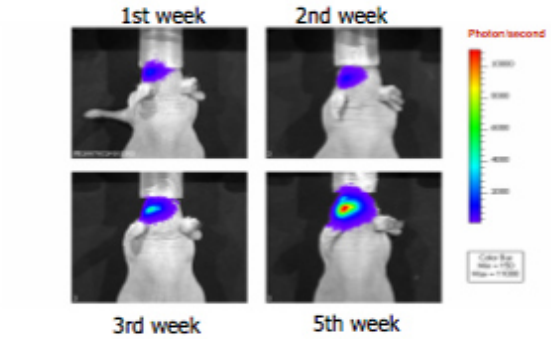

Figure 1. Bioluminescence images of tumors in the brain of nude mice at different time after cell injection.

Material and Methods

1. Cell and surgery details

DBTRG cells were stably transfected with RE-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 stereotactic guidance (coordinates from bregma: + 0.5 mm anterior, + 2.2 mm lateral and - 3.0 mm depth).

2. Tumour visualization:

Optical Imaging

A VivoVision Systems, IVIS® 200 Series, Imaging System for small laboratory animals (Xenogen Corporation, Alameda USA) was used for all experiments. Images were acquired and analyzed with Living Image 2.6 software and Living Image 3D (Xenogen Corporation, Alameda USA).

Acquisition details: field of view = 6.6cm x 6.6cm; opening of diaphragm (f/stop) = 1. Binning factor = 8-16, exposition time = 5 min; emission filters for 3D reconstruction = 560-580-600-620-640-660 nm. Immediately after a PRE acquisition D-Luciferin firefly, potassium salt (Xenogen), 150mg/kg/10ml in DPBS was injected intraperitoneally; then a sequence of images was acquired for 40 minutes. 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-weigmted 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=1 mm, 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.

Immunohistochemistry

Six weeks after the injection, brains from all the injected animals were rapidly excised and fixed in 4% paraformaldehyde. Sections from each mouse brain were stained with the following antibodies: Vimentin (1:200, Abcam), CXCR4 (1:1500, Abcam), Ki67 (1:1500, Abcam), CD31 (1:25, kindly provided by Prof. Dejana, IEO, Milan, Italy), CD34 (1:25, kindly provided by Prof. Dejana, IEO, Milan, Italy).

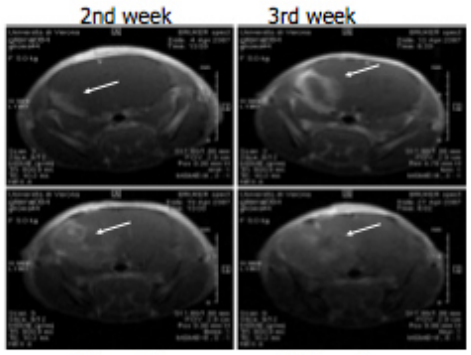

Figure 2. Magnetic Resonance Imaging of the same animal as depicted in Figure1 (white arrow indicate tumour area) at the indicated times. In the figure contrast enhanced T1-weighted images were acquired using Gadolinium-DTPA (Magnevist®) injected i.v. trough the tail vein at 100-300 mmol/kg.

Results

After injection of glioma cells stably transfected with luciferase we followed the development of the tumours using Optical Imaging and MRI in parallel for a total of 6 weeks. Using Optical Imaging, one week after cell engraftment glioma cells were visible in almost all animals (**Figure 1**). MRI detected tumour development only starting from two 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 animal body showed the distribution of light emitted by tumour cells in the mouse brain (**Figure 4**).

Six week after injection, animals were sacrificed and immunohistochemistry performed in brain section. H&E staining showed that tumours formed in the mouse brain were highly heterogeneous and infiltrating mimicking very well the patient tumours (**Figure 5A**). Staining with vimentin, Mib-1 and CXCR4 corroborate the invasiveness and aggressiveness characteristics of this glioma cell line (**Figure 5B-D**).

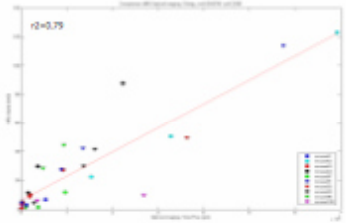

Figure 3. Correlation between Optical Imaging (flux) and MRI (volume) in all animals examined

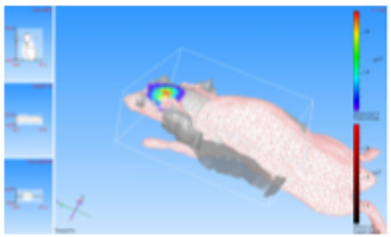

Figure 4. 3D-reconstruction of the animal body, 2 weeks after the injection of DBTRG cells in the brain.

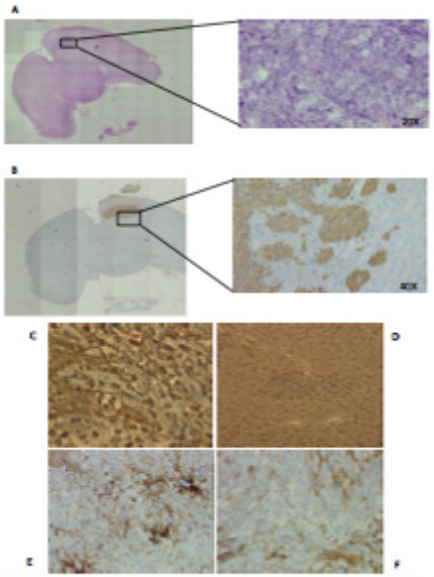

Figure 5. Hematossilin & Eosin staining and immunohistochemistry analysis of sections from a representative tumour injected mouse. A= H&E staining in one section of mouse brain. A particular is magnificated on the right. B= Vimentin staining showing the human tumor in mouse brain and magnification of infiltrating tumor cells. C= Mib-1 staining (40x). D= CXCR4 staining (20X). E= CD31 staining (20x). F= CD34 staining (20x).

Conclusions

Data presented here suggested that:

1. Optical Imaging can be used to monitor *in vivo* intracranial tumor growth;
2. DBTRG-05MG cells mimic better than other cell lines the clinical features of a human GBM;
3. The potentiality of Optical Imaging and MRI imaging may open new perspectives also to use these tools for drug discovery by directly monitoring the efficacy of the therapy.

References

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