Image and behavior analysis in C6 glioma model

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Abstract

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Protocol

Tumor Induction

Step 1.

- The animals are weighed and anesthetized with an *i.p.* injection of ketamine (100 mg/kg) and xylazine (20 mg/kg) and place in a stereotaxic apparatus.
- The C6 (10^6 cells/30 mL) cells are implanted. The implantation position is determined at the following coordinates: 2.0 mm anteroposterior, 2.0 mm laterolateral, and a depth of 2.5 mm. The cells are diluted to a concentration of 10^6 cells/30 mL.
- The cells are slowly injected over a 10-min period. For the control group, culture medium is injected without the cells.
- The bone is then reassembled by using bone wax, and the skin is sutured using cotton thread.

Tumor growth monitoring by MRI and bioluminescence images

Step 2.

- The animals are weighed and anesthetized with an *i.p.* injection of ketamine (100 mg/kg) and xylazine (20 mg/kg)
- The MRI and bioluminescence images are acquired at base time and at 7, 14, 21, and 28 days after tumor induction surgery for tumor growth analysis.
- T2-weighted images are acquired by using a rapid acquisition with relaxation enhancement sequence (RARE).
- The total tumor volume is calculated by the sum of tumor area in each slice multiplied by the thickness and gap per slice.
- In each *in vivo* bioluminescence imaging procedure, rats are anesthetized with isoflurane and D-Luciferin is injected i.p. at a dose of 450 mg/kg 10 min before of the BLI acquirement in IVIS.
- Afterward, for *ex vivo* BLI acquiring, one animal per group of the animals that acquired the *in vivo* BLI is euthanized, and the same brain of *ex vivo* BLI is used for histological assessment.

Behavioral : testing Spontaneous locomotor activity

Step 3.

- Spontaneous global locomotor activity are quantified by the Infrared (IR) Actimeter. The apparatus comprises a two-dimensional (X-axis and Y-axis) square frame of 450 × 450 mm^2,

surrounded by transparent walls of 30 cm high, a frame support, and a control unit.
- Each animal are placed in the center of the arena, and the spontaneous locomotor behavior is tracked for 5 min.
- The six parameters used for comparison between groups and sessions are slow movements (S-MOV), fast movements (F-MOV), slow stereotyped (S-STE), fast stereotyped (F-STE) slow rearing (S-REA), and fast rearing (F-REA).

Behavioral : Gait assessment by CatWalk

Step 4.

- The gait is analyzed by using a “Catwalk” (Noldus Information Technology, Netherlands) apparatus that comprises a 1.3-m long glass platform illuminated by fluorescent lights that are reflected downward while pressure is applied from above.
- During data analysis, each the print on paw contact is automatically classified as right forepaw (RF), right hindpaw (RH), left forepaw (LF), and left hindpaw (LH).
- After the identification of individual footprints, we performed an automated analysis of wide-range parameters. Data are classified as follows:
- **Spatial parameters:** print positions (PP), the distance between the placement of a hindpaw and the ipsilateral frontpaw placed just before it; base of support (BOS), the distance between the center points of the two fore or hind paws (both represent inter-limb coordination measures); stride length (SL), the distance between successive placements of the same paw (dynamic gait parameters); and maximum contact area (mCA), the maximum area of a paw that comes into contact with the glass plate (static gait parameters).
- **Temporal parameters: stand**, the duration in seconds of contact of a paw with the glass plate; **step cycle (SC)**, the duration of stance and swing phases combined; **cadence**, steps per second; and **duration**, total time of entire run.