IMAGING OF SYNGENEIC RAT BRAIN TUMOR MODEL

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Introduction

The ability to image rat brain tumors serves several purposes for our research. The first is to verify the presence of the tumor before any experimentation is initiated. The second is verification of the location of the tumor for stereotactic injection. The last is monitoring the tumor growth rate by calculating the tumor volume. The importance of the last experimental feature is that it allows growth rates to be determined rather than a comparison of reduction in tumor size at a fixed time point or time points. This reduces the number of rats needed for an efficacy study and decreases the experimental variables.

Experimental

Fisher rats were anesthetized with 2% isofluorane administered using an anesthesia machine. Rats were mounted in a stereotaxic device and a 3-4 mm long incision is made over the skull. The periostium was removed and the skull dried to reveal the bone sutures. Holes were drilled based on atlas coordinates using a dissecting microscope to avoid damage of the dura mater. The dura mater was cut using a 25-g hypodermic needle to allow insertion of a micropipette into the brain. 2 uls of RG2 cell suspension (10^5 cells) was injected into the striatum at a rate of 1ul/min with the incisor bar set at –3.3 mm below the intra-aural line. The cell suspension was injected through a 5 ul Hamilton syringe fitted with a pulled micropipette with a diameter of 80-100 um. One minute after the cessation of the injection, the needle was retracted 1mm and then left in place for 2 minutes before being slowly withdrawn from the brain. Rats were imaged to determine brain tumor location and size 6 to 10 days after tumor administration. Rats were anesthetized with isofluorane and fitted to the MRI gurney. Anesthesia was continued during the imaging of the rat. An anal probe was inserted to monitor body temperature and an oxygen detector was clipped to the rear paw to monitor respiration during imaging. Coils were fitted to the rat and placed in a 4.7 Tesla MRI spectrometer. Imaging parameters were set by trained personnel assisting in the imaging. Coronal and axial sections were obtained that were 1 mm thick.

Results and Discussion

The imaging results were to verify that the rats had tumors. These rats were then used for the following studies.

1. Location of the tumor verified that the coordinates used to implant the tumor cells could then be used for an intraumoral injection of a polymer formulated green fluorescent protein DNA expression plasmid. Five ul injections of 1 mg/ml DNA plasmid with increasing amounts of a block copolymer were carried out and expression was assayed by fluorescence microscopy. Unfortunately, no correlation was observed between increased fluorescence and an increase in the polymer formulation.

2. The second study was to remove the rat brain following imaging and create a 300 um organ slice culture of the portion of the rat brain containing the tumor. This was successful and the tumor could be maintained in culture for 8 days resulting in the tumor completely taking over the organ slice culture in that time period.

3. The last experiment which is ongoing involved the determination of the tumor growth rate in the organ culture vs. in the rat. The growth rates are being determined by volumetric reconstruction of the histological sections as a function of time.

Conclusions

The ability to image the rats prior to performing an experiment ensures that the tumors are of a particular size before the experiment is initiated. We have been able to establish the organ slice brain tumor culture and the next set of experiments will be to compare the growth rates in vitro with that obtained from the tumors growing in vivo. The organ culture is used to optimize transfection conditions in vitro and then these will be tested in vivo. We have a cytotoxic gene construct that will be tested and the affect on tumor volume will be followed by MRI imaging.

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