Development and Evolution of Glioma after ENU exposure: A Model of Malignant Transformation of Low Grade Gliomas

B. N. Sathy[1], Y-H. Hsu[1], T. Jang[2], L. Recht[2], C. Chang[1]

[1]Functional and Micro-MRI Center, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [2]Departments of Neurology and Clinical Neurosciences, Stanford University, Stanford, California, United States

Synopsis:

MRI was used to detect the evolution of chemically induced experimental brain tumors in rats after *in utero* exposure to ethyl-nitrosourea (ENU). Both T2WI and DWI were used to acquire brain images every 15 days from postnatal day 30 to day 210. T2WI images of early and advanced gliomas were correlated with histology. Tumor growth rate and CNR were serially assessed during tumor development.

Introduction:

Low grade gliomas are a relatively common clinical problem that usually presents in young middle age [1]. Tumor size and character tends to remain relatively constant on MR imaging for several years until terminating in a phase of malignant transformation [2] in which tumor growth rapidly accelerates. Preventing such transformations should therefore lead to better clinical outcome for these patients. Because it is clinically difficult to study gliomas at early (i.e., preclinical) stages, the natural history of glioma development, especially at early time points, must be addressed experimentally. Because gliomas invariably develop two to six months after a single *in utero* exposure, the ENU model of neurocarcinogenesis [3] provides an optimal framework with which to study glioma's evolution. We performed serial MRI's at 15 day intervals on ENU-exposed rats to assess the earliest times that we could detect tumor formation and to study their growth patterns.

Materials and Methods

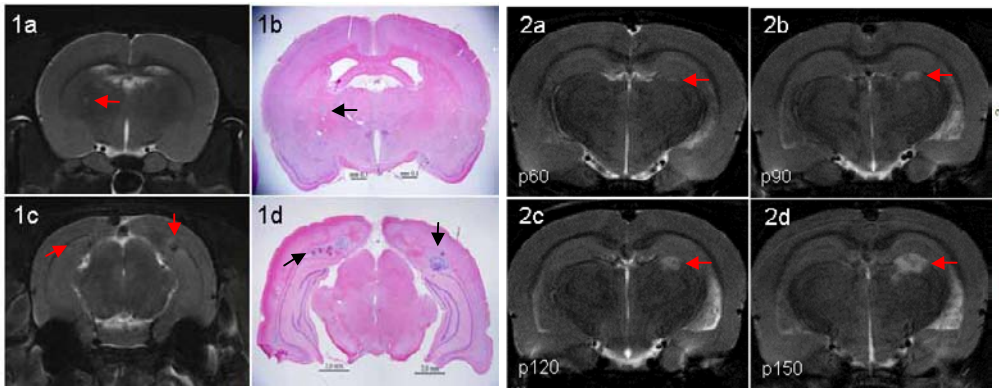
We serially examined 20 gliomas in 15 male Sprague-Dawley rats that were exposed to ENU prenatally. Rat brain images were acquired every 15 day starting at postnatal day 30 (P30). Rats were anesthetized with isoflurane/oxygen mixture (isoflurane 5%) and maintained in anesthetized state (isoflurane 1.5 – 2 %) throughout the experiment. All MRI experiments were performed on a 7T spectrometer equipped with an active shielding gradient (30 G/cm in 150 µs). Axial T2WI images and DWI images were acquired in the same location, with a FOV of 3 cm, slice thickness of 1mm and a slice number of 28 with no interslice gap, to cover the entire brain for tracing every abnormal cell proliferation. T2WI images were acquired using RARE sequence with a TR of 5100 ms, TE of 70 ms, ETL of 8, NEX of 6 and matrix size of 256*256. DWI images were acquired using spin-echo sequence with a TR of 1500 ms, TE of 32 ms, high b-factor (b = 1100 s/mm^2), NEX of 4 and matrix size of 256*192 (zero- filling to 256*256).The 3D tumor volumes were obtained from delineating the multislice axial T2WI images. Tumor region on each slice were manually outlined. The final volume for tumors which occupied more than one slice is the sum of tumor regions from each individual slices. All 3D processing were carried out using manual trace tool and edge editing function provided by the ANALYZE (Biomedical imaging Resource, Rochester, Minnesota, USA). All other data were processed using commercially available image analysis package, MR Vision (MR Vision Co., Menlo Park, CA). CNR (SNR_A – SNR_B) was calculated by the comparison of the tumors and their correspondent tumor-free area on the opposite side of the same brain. MR images were mapped to the bregma to estimate tumor locations. A parallel and independent experiment was used for histology validation of MRI findings.

Results:

Gliomas became visible at a median age of 60 days (range 45-120 days) when their median volume was 0.260 mm^3 (range 0.14-1.05). T2WI was more sensitive and was able to detect lesions at 0.2 mm^3. A close correlation between areas of abnormality and cellular proliferations were noted in independent subsequent experiments, confirming that the lesions in question represented early gliomas, primarily astrocytomas (Figure 1). Gliomas grew slowly initially but then underwent a marked acceleration once their volumes exceeded 10 mm^3 (Figure 2). Only 53% of rats survived at 210 days. Although tumors increased steadily in size, CNR generally peaked just before tumor acceleration, after which values decreased, presumably because of the increased heterogeneity in tumor masses.

Discussion:

Based on these findings, we conclude that this model faithfully reproduces the clinical situation of the low grade glioma, including appearance in young middle age, a slow growth phase lasting several weeks followed by a terminal acceleration that results in death. T2WI is sensitive and specific enough to detect early neoplastic lesions without enhancement. This model should therefore prove very useful in determining both the mechanisms underlying this growth acceleration as well as developing potential therapeutic strategies.



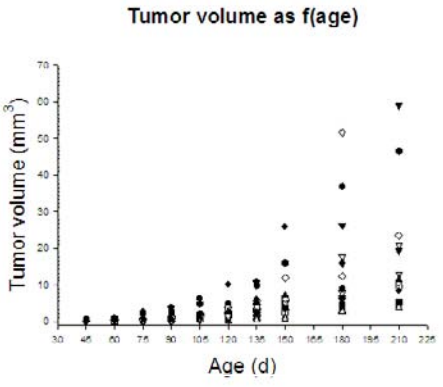


Figure 1. T2WI images (1a, 1c) and correspondent H&E stained brain sections (1b, 1d) of ENU-exposed rats of early (1a and 1b, arrows) and advanced astrocytomas (1c and 1d, arrows). The early tumor in T2WI was 0.4 mm^3 (1a) .Figure 2. T2WI images of ENU-exposed rat brain from p60 to p150 (1a-2d) showing that tumor evolutes from a tiny homogeneous nodule (2a and 2b, arrows) to a large and heterogeneous mass (C, D, arrows).Figure 3. ENU-induced tumor volumes up to postnatal day 210, n =10.

References:

1. Whittle, I.R. J Neurol Neurosurg Psychiatry 75:31-36, 2004. [2].Rees, J.H. Curr Opin Neurol 15:657–661, 2002. [3]. Ross, D.A et al., J Neuro Oncol 40:29-38, 1998.