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A syngeneic glioma model to assess the impact of neural progenitor target cell age on tumor malignancy

Andrei M Mikheev^{1,2}, Elizabeth A Stoll^{1,2}, Svetlana A Mikheeva^{1,2}, John-Patrick Maxwell^{1,2,*}, Pawel P Jankowski^{1,2}, Sutapa Ray^{1,2}, Takuma Uo¹, Richard S Morrison¹, Philip J Horner^{1,2}, and Robert C Rostomily^{1,2,#}

¹Department of Neurological Surgery, University of Washington School of Medicine, Seattle, WA

²Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA

Summary

Human glioma incidence, malignancy and treatment resistance are directly proportional to patient age. Cell intrinsic factors are reported to contribute to human age-dependent glioma malignancy but suitable animal models to examine the role of aging are lacking. Here we developed an orthotopic syngeneic glioma model to test the hypothesis that the age of neural progenitor cells (NPCs), presumed cells of glioma origin, influences glioma malignancy. Gliomas generated from transformed donor 3-, 12-, and 18-month-old NPCs in same-aged adult hosts all formed highly invasive glial tumors that phenocopied the human disease. Survival analysis indicated increased malignancy of gliomas generated from older 12- and 18-month-old transformed NPCs compared with their 3-month counterparts (median survival of 38.5 and 42.5 vs. 77 days, respectively). This study showed for the first time that age of target cells at the time of transformation can affect malignancy and demonstrated the feasibility of a syngeneic model using transformed NPCs for future examination of the relative impacts of age-related cell intrinsic and cell-extrinsic factors in glioma malignancy.

Keywords

Gliomagenesis; aging; progenitor; malignancy; tumor suppressor; animal model

Increased age has a negative impact on human malignant glioma survival and directly correlates with increased incidence, malignancy and treatment resistance (Curran et al. 1993; Barker et al. 2001; CBTRUS 2005). However, little is known about age-related mechanisms that impact glioma malignancy. Previous animal studies reported conflicting impacts of host age related immune function and neural progenitor cell (NPC) recruitment on survival (Wheeler et al. 2003; Glass et al. 2005) while human data suggests that age-related cell-intrinsic factors may affect glioma malignancy (Rosenblum et al. 1982). To examine the role of host age, we implanted GL261 glioma cells into syngeneic 3- and 18-month-old host C57Bl/6 mice and found no difference in survival (see supplemental data). Since NPCs are presumed glioma cells of origin (Stiles & Rowitch 2008), we tested how NPC age influences glioma malignancy. NPCs isolated from 3-, 12-, and 18-month-old C57Bl/6 mice formed neurospheres and demonstrated multi-potent differentiation (Fig 1A, B). To validate

*corresponding author Robert C Rostomily, University of Washington School of Medicine, Department of Neurological Surgery, Institute for Stem Cell and Regenerative Medicine, Box 356470, 1959 NE Pacific St, Seattle, WA 98195, Fax; 206-543-8315, Phone; 206-543-3570, rosto@u.washington.edu.

current address: University of Toledo College of Medicine, Toledo, OH

molecular changes reported in aging NPCs (Molofsky et al. 2006), we confirmed that p16 tumor suppressor expression increased with NPC age (Fig 1C,D). Like p16, loss of p53 tumor suppressor (TS) activity contributes significantly to human glioma pathogenesis, but in a non-overlapping set of gliomas (Ohgaki & Kleihues 2005). We found a striking age-related inverse relationship between p16 and p53 expression levels, with marked reductions in p53 expression and activity with increasing NPC age (Fig 1E). These results indicated that NPCs of all ages demonstrated similarities but were distinguished by unique profiles of TS expression/activity highly relevant to human glioma pathogenesis.

To determine the impact of NPC age on glioma malignancy, NPCs of different ages were transformed by over-expression of activated Ha-Ras (Ha-RasV12) and HPV18 E6E7 (to inhibit both p53 and the p16/Rb axis). All cultures demonstrated equivalent levels of exogenous Ras expression (Fig S2), and expected alterations in p53 and Rb expression *in vitro* (data not shown). Intracranial implantation of equal numbers of transformed 3-, 12-, and 18-month-old NPCs into 4- to 6-month-old adult C57Bl/6 mice produced highly invasive GFAP and Olig2-positive glial tumors in all cases (Fig 2A, B, C). Gliomas derived from 12- and 18-mo transformed NPCs were distinguished by increased cellularity, less differentiated morphology, increased proliferation (Ki-67 LIs) and a distinct pattern of invasion with large cell aggregates as well as diffuse cellular invasion (Fig 2B, C, D). Most importantly, animals with gliomas derived from 3-mo NPCs survived nearly twice as long as those with gliomas from 12- and 18-mo NPCs (77 vs. 38.5 and 42.5 days, respectively; log rank $p=0.01132$ and 0.002 , respectively; Fig 2D).

This study of gliomas provides the first experimental evidence that increased age of transformation impacts the malignant potential of cancer target cells. The current findings are concordant with clinical observations that age-related cell intrinsic factors may play a leading role in the impact of age on glioma malignancy. The highly invasive glial growth patterns further support the clinical relevance of this syngeneic model and its usefulness for future studies to i) determine how specific oncogenic mechanisms affect age-related malignancy, ii) examine the role of p53 activity in NPC malignant potential, iii) define the relative importance of target cell versus host age on glioma malignancy, and iv) determine how age-related cell-extrinsic host mechanisms such as immune function and recruitment of endogenous progenitor cells impact glioma malignancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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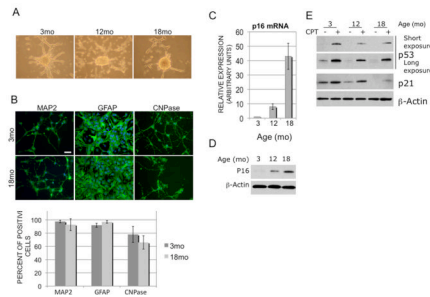


Figure 1. Isolation and characterization of NPCs from different-aged donor brain

(A) Representative neurospheres generated from NPCs isolated from 3-, 12- and 18- mo mouse forebrain cultures. (B) Multipotency in 3- and 18-mo NPC cultures under differentiation conditions assayed by neuronal (MAP2), astrocytic (GFAP) and oligodendroglia (CNPase) marker expression (quantified in histogram below), scale bar = 20 μ m. Expression levels of p16 message (C) and protein (D) increase proportionally with NPC age. E) Basal and CPT-induced expression of p53 and its target gene, p21WAF1, decrease with increased NPC age. Long exposure panel demonstrates steady state p53 expression.

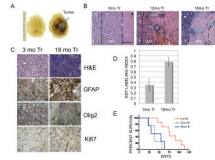


Figure 2. Age-dependent malignancy of transformed NPCs in vivo

(A) Macroscopic appearance of representative 18-mo tumor (arrow) compared to normal brain (left- ruler units=1mm) (B) Distinct age-related invasive growth patterns indicated by aggregate (arrows) and diffuse cell invasion in 12- and 18 mo gliomas and lack of aggregate invasion in 3 mo tumors. T-solid tumor, INV-invasive margin (dashed lines). Scale bar 170 μ m. (C) Top panel- H&E histology demonstrates increased cellularity and less differentiated morphology of 18-versus 3-mo gliomas. Middle panels- GFAP and Olig2 expression confirm glial differentiation in both 3- and 18-mo tumors. Lower panel- Representative images of Ki-67 positive cells (brown nuclei of various intensity) indicates increased proliferation in 18 mo versus 3 mo glioma. Scale bars 90 μ m/50 μ m (top 3/ lower panel respectively). (D) Histogram demonstrates significant difference in mean Ki-67 LIs between 3 and 18 mo gliomas (35% vs 78%, respectively, $p < 0.0026$). (E) Kaplan-Meier survival analysis. Animals injected with 3-mo transformed cells survived significantly longer than animals injected with 12- or 18-mo transformed NPCs (77 vs 38.5 and 42.5 days, respectively; log rank $p = 0.01132$ and 0.002 , respectively).