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Research Paper

Meningioma growth dynamics assessed by radiocarbon retrospective birth dating

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ABSTRACT

It is not known how long it takes from the initial neoplastic transformation of a cell to the detection of a tumor, which would be valuable for understanding tumor growth dynamics. Meningiomas show a broad histological, genetic and clinical spectrum, are usually benign and considered slowly growing. There is an intense debate regarding their age and growth pattern and when meningiomas should be resected. We have assessed the age and growth dynamics of 14 patients with meningiomas (WHO grade I: n = 6 with meningothelial and n = 6 with fibrous subtype, as well as n = 2 atypical WHO grade II meningiomas) by combining retrospective birth-dating of cells by analyzing incorporation of nuclear-bomb-test-derived ^{14}C, analysis of cell proliferation, cell density, MRI imaging and mathematical modeling. We provide an integrated model of the growth dynamics of benign meningiomas. The mean age of WHO grade I meningiomas was 22.1 ± 6.5 years, whereas atypical WHO grade II meningiomas originated 1.5 ± 0.1 years prior to surgery ($p < 0.01$). We conclude that WHO grade I meningiomas are very slowly growing brain tumors, which are resected in average two decades after time of origination.

1. Introduction

It is not known how long it takes from the initial neoplastic transformation of a cell to the detection of a tumor, which would be valuable for understanding tumor growth dynamics and to establish at what stage therapy may be needed. Along with gliomas, meningiomas are the most frequent CNS tumors with an annual incidence of about 4–5/100.000 individuals (Wiemels et al., 2010; Whittle et al., 2004). Meningiomas arise from arachnoidal cap cells and show a broad histological, genetical and clinicopathological spectrum (Riemenschneider et al., 2006). The dignity of the various subtypes is graded according to the World Health Organization (WHO) classification, the vast majority of meningiomas (~80%) being benign, slowly growing, WHO grade I tumors (Louis et al., 2016; Riemenschneider et al., 2006). There has been an intensive debate regarding the age of meningiomas (Braunstein and Vick, 1997; Herscovici et al., 2004; Nakasu et al., 2011). Estimates were often based on consecutive MRI imaging of patients with incidental and recurrent meninigiomas (Nakasu et al., 2011; Nakamura et al., 2003; Herscovici et al., 2004; Nakamura et al., 2005). However, it has been difficult to establish their growth pattern and to decide when meningiomas should be resected (Braunstein and Vick, 1997; Herscovici et al., 2004; Nakasu et al., 2011).

We used retrospective birth dating (Spalding et al., 2005) to assess the age of meningioma cells. This strategy takes advantage of the dramatically elevated levels of the isotope ^{14}C caused by above ground nuclear bomb testing during the Cold War (De Vries, 1958; Nydal and Lövseth, 1965; Levin and Kromer, 2004). ^{14}C together with oxygen

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forms $^{14}CO_2$ and enters the food chain through plant photosynthesis such that the ^{14}C concentration in the human body closely corresponds to that in the atmosphere at any given time (Libby et al., 1964; Harkness, 1972). During cell division ^{14}C is integrated into genomic DNA, resulting in a stable date mark (Spalding et al., 2005). We have previously used this technique to establish the age and cell renewal rates of various human cell types including neurons, oligodendrocytes, heart muscle and fat cells (Bergmann et al., 2009; Huttner et al., 2014; Yeung et al., 2014; Bergmann et al., 2015b; Bergmann et al., 2015a; Spalding et al., 2013; Spalding et al., 2008).

We have assessed the age and growth dynamics of meningiomas by combining ^{14}C-based retrospective birth dating, analysis of cell proliferation, cell density, MRI imaging and mathematical modeling. We provide an integrated model of the growth dynamics of benign meningioma and show that growth curve estimation models may benefit from incorporating ^{14}C data to predict future progress and facilitate the decision of whether to remove a detected meningioma.

2. Material and Methods

2.1. Patient Selection and Tumor Sample Preparation

All investigated samples were taken from tumor tissue bio-banks of the Departments of Neurosurgery of the University Hospitals Hannover and Erlangen, Germany. Upon meningioma surgery routine sample handling included (i) instantaneous section analysis, (ii) paraffin embedding of the gross of the sample for subsequent neuropathological characterization as well as (iii) snap freezing a representative tumor part (roughly 0.5 × 0.5 × 0.5 cm) and unfixed storage of this tumor aliquot at −80 °C immediately after surgery. All subjects gave written informed consent and the study was approved the local institutional review boards (Ethics Committee Approval No: 104_13B).

2.2. Clinical Characteristics and Imaging

Demographic and baseline clinical information were collected by screening the institutional electronic databases and by reviewing the patients´ medical charts. All patients underwent MR imaging and examinations were reviewed on a PACS workstation using FDA-approved software (syngo.via, MR Onco Brain, Siemens, Erlangen, Germany). Evaluation of each examination was performed on contrast-enhanced T1-weighted images with a slice thickness of 5 mm or less. For volume measurement, the semi-automated lesion segmentation of the software based on signal intensity values was used. The appropriate lesion detection was approved by two neuroradiologists in consensus and the volume was calculated by the software for each examination and time point.

2.3. Histology and Immunohistochemistry

Initial histological analysis for verification of tumor type and WHO classification (Louis et al., 2016) was performed by local experienced neuropathologists on formalin embedded tissue pieces (frozen tumor tissue samples, 20 μm microtome sectioning and fixed in 4% formaldehyde buffered in PBS for 30 min). Standard HE staining confirmed meningothelial and fibrous/fibromatous tumor types. Sections were incubated with the primary antibody overnight at 4 °C: mouse Ki-67 (Mib1 monoclonal, 1:200, Thermo Fischer Scientific), phospho-Histone H3 (rabbit, Abcam, 1:1000) and visualized with the matching secondary antibody conjugated to Alexa 488, 546 or 647 (Invitrogen, 1:500). Apoptosis was detected with TUNEL staining (Click-It Alexa-Flour, Invitrogen). The indices for positive cells labeled with, Ki-67, phospho-Histone H3 (p-HH3), and TUNEL respectively, were calculated referring to overall DAPI-positive nuclei and averaged on 5 arbitrarily chosen high power fields (400× magnification) (Riemenschneider et al., 2006; Vranic et al., 2010; Louis et al., 2016). Immunohistochemistry was analyzed using a confocal microscope (LSM 710, Carl Zeiss Jena, Germany using ZEN software).

2.4. Isolation of Nuclei

Tumor tissue samples were thawed at room temperature and homogenized in 10 ml lysis buffer (0.32 M sucrose, 5 mM $CaCl_2$, 3 mM magnesium acetate, 0.1 mM EDTA, 10 mM Tris-HCl [pH 8.0] and 1 mM DTT) using a T25-digital-Ultra-Turrax® disperser (www.ika.com). Samples were then diluted with additional 190 ml lysis buffer, dounce homogenized, filtered twice in 100 μm and 60 μm Nylon Net Filters (Millipore), equally distributed into 4 tubes with 50 ml and subsequently centrifuged (700 ×*g* for 10 min at 4 °C). The pellets of each of the four tubes were resuspended in 2.5 ml lysis buffer, pooled and added to 20 ml of sucrose solution (1.7 M sucrose, 3 mM magnesium acetate, 1 mM DTT, and 10 mM Tris-HCl [pH 8.0]). As described previously (Bergmann et al., 2012; Huttner et al., 2014), the samples were subjected to a gradient centrifugation after layered onto a cushion of 10 ml sucrose solution (36,500 ×*g* for 2.20 h at 4 °C). The isolated nuclei were resuspended in 10 ml nuclei storage buffer (NSB; 10 mM Tris [pH 7.2], 2 mM $MgCl_2$, 70 mM KCl, and 15% sucrose) and again centrifuged for 10 min at 700 x g at 4 °C. The resulting pellet was transferred to glassware for consecutive DNA extraction.

2.5. Extraction of DNA

As published earlier (Bergmann et al., 2012; Huttner et al., 2014), DNA extraction experiments were carried out in a clean room (ISO8) to prevent any carbon contamination. All used instruments and glassware were prebaked at 450 °C for 4 h. DNA isolation was performed according to (Bergmann et al., 2012, Huttner et al., 2014); briefly summarized here: to each sample of collected nuclei 500 μl of DNA lysis buffer (100 mM Tris [pH 8.0], 200 mM NaCl, 1% SDS, and 5 mM EDTA) and 6 μl Proteinase K (20 mg/ml) were added and incubated overnight at 65 °C. Three μl RNase cocktail (Ambion) was added and incubated at 65 °C for 45 min. We added half of the existing volume of 5 M NaCl solution, vortexed for 15 s and the solution was centrifuged for 3 min at 13,000 rpm for 3 min. The supernatant containing the DNA was transferred to a 12 ml glass vial. Three times the volume of absolute ethanol was added, and the glass vial was inverted several times to precipitate the DNA. The DNA precipitate was washed three times in DNA washing solution (70% Ethanol [v/v] and 0.5 M NaCl) and dried out overnight at 65 °C. Finally, 500 μl DNase/RNAase free water (GIBCO/Invitrogen) was added to the glassware allowing the crystallized DNA to be dissolved entirely during another 24 h incubation at 65 °C. The DNA was quantified and DNA purity verified by UV spectroscopy (NanoDrop).

2.6. Accelerator Mass Spectrometry (AMS)

As described previously, all AMS analyses were performed blind to age and clinical information (Huttner et al., 2014). Purified DNA samples suspended in water were combusted to CO_2, and reduced to graphite according to the procedures described previously (Spalding et al., 2013). ^{14}C AMS measurements of graphitized samples were carried out at the Tandem Accelerator Laboratory in Uppsala, Sweden using a 5 MV Pelletron tandem accelerator. ^{14}C measurement results and carbon background subtraction are reported as described earlier (Spalding et al., 2013; Salehpour et al., 2013; Salehpour et al., 2015). Age calibration of ^{14}C concentrations was performed using the software CALIbomb (http://calib.qub.ac.uk/CALIBomb) with the following parameters: smoothing in years, 1 year; resolution, 0.2; ^{14}C calibration, two sigma.

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