Laboratory Investigation

Comparison of bromodeoxyuridine uptake and MIB 1 immunoreactivity in medulloblastomas determined with single and double immunohistochemical staining methods

Kiyoshi Onda, Richard L. Davis and Michael S.B. Edwards

1 Brain Tumor Research Center of the Department of Neurological Surgery and 2 Department of Pathology, School of Medicine, University of California, San Francisco, CA 94143, USA

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Summary

We examined the growth potential of 17 medulloblastomas by single and double immunohistochemical staining with bromodeoxyuridine (BUDR) and MIB 1, a monoclonal antibody for Ki-67 protein, in serial sections of ethanol-fixed, paraffin-embedded tissues; we also assessed the heterogeneity of the immunoreactivity in the tumors. In the most active areas, the BUDR labeling index (LI) was 6.8 to 26.9% (HCl hydrolysis) and 7.5 to 28.8% (microwave heating), and the MIB 1 proliferating cell index (PCI) was 14.9 to 56.5%. Linear regression analysis showed that the BUDR LI correlated with the MIB 1 PCI (p < 0.001). The ratio of MIB 1-positive to BUDR-positive cells was 2.2 ± 0.4 by both single and double staining. BUDR-positive nuclei were heterogeneously distributed in all cases, especially in areas with scattered foci of necrosis. Three tumors had areas with many MIB 1-positive but few BUDR-positive nuclei; these areas were associated with recent tumor necrosis. However, in most of the tumors, the densities of BUDR-positive and MIB 1-positive cells changed concomitantly from area to area. These changes were clearly shown by double immunostaining. Thus, transcapsillary passage of BUDR does not appear to be impeded in most medulloblastomas. This study suggests that MIB 1 immunostaining provides essentially the same data as BUDR labeling for assessing the proliferative potential of medulloblastomas.

Introduction

Medulloblastomas are undifferentiated neuroepithelial tumors of the cerebellum that occur mainly in children [1, 2]. Histologically, most medulloblastomas are more uniform than glioblastoma multiforme. However, the S-phase fraction of medulloblastomas, as determined by [3H]-thymidine autoradiography and recently by immunohistochemical staining of bromodeoxyuridine (BUDR) has varied from 3.9 to 38.2% [3, 4]. Cell kinetics studies with Ki-67, a monoclonal antibody that recognizes a protein in proliferating cells, have shown that cycling cells are more heterogeneously distributed in medulloblastomas than in glioblastomas [5]. So far few studies have addressed comparison between BUDR uptake and immunohistochemical staining of Ki-67 protein in medulloblastomas. This comparison will clarify the ratio of S-phase cells in the growth fraction and thus be helpful for better understanding of cell kinetics of the tumors.

Immunohistochemical staining of S-phase cells labeled with BUDR [6] has been used successfully to study the cell kinetics of brain tumors, including medulloblastomas [4, 7]. It has been suggested that the heterogeneous distribution of BUDR-positive cells may reflect nonuniform distribution of BUDR to the tumor [8]. Ki-67 immunostaining [9, 10] is an-
other technique for quantifying the growth fraction of brain tumors [11]. Unfortunately, the Ki-67 proliferating cell index (PCI) has varied greatly in different studies of histologically similar tumors [12], and the antigenicity of Ki-67 protein may be lost during air drying, storage, and fixation of the frozen sections [5, 13].

Recently, heating paraffin-embedded tumor tissue in a microwave oven [14] has been shown to re-activate Ki-67 antigen, which can then be identified by immunostaining with monoclonal antibody MIB 1 [15–17]. This technique has obvious advantages over immunostaining of frozen sections with Ki-67 monoclonal antibody, including more intense staining and better histological preservation [15]. Using this technique, we have shown a close correlation between regional BUdR uptake and MIB 1 immunoreactivity in glioblastomas [18]. In this study, we evaluated regional heterogeneity in the growth potential of medulloblastomas by single and double staining with anti-BUdR and MIB 1 monoclonal antibodies in serial sections of paraffin-embedded tissues. The goals of the study were to compare the patterns of BUdR uptake and MIB 1 immunoreactivity and to assess the uniformity of BUdR delivery to the tumor.

Materials and methods

Tumor specimens were obtained from 17 patients undergoing resection of medulloblastomas at the University of California, San Francisco (UCSF). Permission to administer BUdR was received from the Human Experimentation Committee, UCSF, and from the National Cancer Institute. Informed consent was obtained from each patient or a responsible relative. BUdR, 150 to 200 mg/sq m (maximum dose, 400 mg), was administered for 30 to 60 minutes by intravenous infusion beginning shortly after the induction of anesthesia.

Tissue preparation and immunohistochemistry

A portion of each tumor specimen was fixed in chilled 70% ethanol for at least 12 hours, embedded in paraffin, and sliced 4 to 6 µm thick. Serial sections were stained with hematoxylin and eosin and with anti-BUdR and MIB 1 monoclonal antibodies, as described below. Thus, staining with all three techniques could be examined and compared in the same areas of each tumor.

Paraffin-embedded tissue sections were processed by one of two methods before reaction with the primary antibody to identify BUdR-labeled nuclei. In one method, the tissue sections were deparaffinized, immersed for 30 minutes in methanol containing 0.3% hydrogen peroxide, and denatured for 12 minutes with 4N hydrochloric acid as described previously [18–20]. In the other, the sections were deparaffinized, placed in 300 ml of citrate buffer (pH 6.0), and heated four times for 5 minutes each at full power in a 650 W microwave oven [14–16, 18]. Thereafter, all sections were reacted for 1 hour with anti-BUdR monoclonal antibody (IU-4; Caltag Laboratories, South San Francisco, CA) diluted 1:200 in phosphate-buffered saline (PBS) containing 1% normal rabbit serum. The sections were then reacted for 30 minutes with biotinylated rabbit secondary antibody against mouse immunoglobulins (DAKO, Santa Barbara, CA) and for 30 minutes with avidin-biotin complex (ABC) (DAKO), developed with diaminobenzidine (DAB), and counterstained with hematoxylin.

Single-staining of Ki-67 protein with MIB 1 in paraffin sections was done as previously described [18]. After deparaffinization, the sections were heated in the microwave oven as described above. The sections were then reacted for 1 hour with MIB 1 (AMAC, Inc., Westbrook, ME) diluted 1:200 in phosphate-buffered saline (PBS) containing 1% normal rabbit serum, stained by the ABC technique as described above, developed with DAB, and counterstained with hematoxylin.

Double-staining with anti-BUdR and MIB 1 monoclonal antibodies was performed using immunohistochemical techniques described by Shibui et al. [21]. Briefly, sections were deparaffinized, heated as described above, and reacted for 1 hour with IU-4 diluted 1:200 in PBS containing 1% normal rabbit serum. The sections were then stained by the ABC technique and developed with DAB. After immersion in 5% acetic acid overnight, the sections were reacted for 1 hour with MIB 1 diluted 1:50 in