

A rapid intraoperative estimation of the proliferative activity in brain tumors

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Summary. A technique for staining the nucleolar organizer regions (NORs) in tumor cells applied to smears from brain tumor biopsy specimens is described. This technique provides a rapid intraoperative evaluation of the proliferative activity in cerebral neoplasms and is a valuable complement to hematoxylin-eosin stained smears, supporting the criteria of benignity or malignancy in these tumors.

Key words: Smear technique, Brain tumor, Nucleolar organizer region, Stereotactic biopsy, Cell proliferation, Pathological diagnosis

Introduction

The routine histological techniques based on nuclear pleomorphism, on the increase of cellularity and on the presence or absence of mitoses, have proved to be inaccurate in the biological prognosis of brain tumors. For this reason, the techniques for the study of proliferative activity in neoplastic cells have gained increasing interest (Nagashima et al., 1985; Fukui et al., 1986; Hoshino et al., 1986, 1988, 1989; Giangaspero et al., 1987; Ironside et al., 1987; Roggendorf et al., 1987; Nishizaki et al., 1988, 1989; Zuber et al., 1988; Decker et al., 1989; Detta and Hitchcock, 1990; Hara et al., 1990; Kunishio et al., 1990; Orita et al., 1990). The most usual techniques are the labelling index for bromodeoxyuridine (BrdU) (Nagashima et al., 1985; Fukui et al., 1986; Hoshino et al., 1986, 1988, 1989; Nishizaki et al., 1988, 1990; Detta and Hitchcock, 1990; Orita et al., 1990), Ki-67 (Giangaspero et al., 1987; Roggendorf et al., 1987; Zuber et al., 1988; Deckert et al., 1989; Nishizaki et al., 1989; Hara et al., 1990), DNA polymerase alpha (Kunishio et al., 1990), or flow cytometric DNA analysis

(Ironside et al., 1987; Nishizaki et al., 1989).

Recently, the study of nucleolar organizer regions (NORs) has proved to be less expensive, faster, easier and as accurate as other techniques for the study of the proliferative activity of brain tumors. NORs are loops of DNA that transcribe to ribosomal RNA (rRNA); they are not related to the cell cycle and can be located by staining with silver nitrate. The number of NORs within the tumor cells correlates with the BrdU or Ki-67 labelling indexes and reflects the cellular proliferation or malignancy (Hara et al., 1990; Orita et al., 1990).

We now report our experience with the use of NOR staining in smear techniques, which allows a rapid evaluation of the proliferative activity or malignancy during the intraoperative histological study of brain tumors.

Materials and methods

At present, we have studied by means of smear techniques more than 150 stereotactic brain tumor biopsies and brain biopsy specimens obtained from open craniotomies. These specimens were obtained fresh, smeared and currently stained with hematoxylin-eosin (H&E) technique. In the latter 34 cases, in addition to H&E, a silver-stain technique was carried out in order to identify NORs on tumoral cells. The following method was used: biopsy specimens were prepared by placing a small sample of tissue on an alcohol-cleaned microscope slide and applying gentle but firm pressure with the edge of a second slide; the two slides being drawn apart in opposite directions to smear the tissue. Both smears were subsequently fixed for 2-3 minutes in 96° alcohol, and then washed in distilled water. One of the slides was stained with H&E, while the second one was placed into a silver colloidal solution for 10-15 minutes, isolated from light. The silver colloidal solution was prepared just before its use by mixing two fractions of 50% silver nitrate and one fraction of 2% gelatine in 1% formic acid solution. The slides were subsequently washed in

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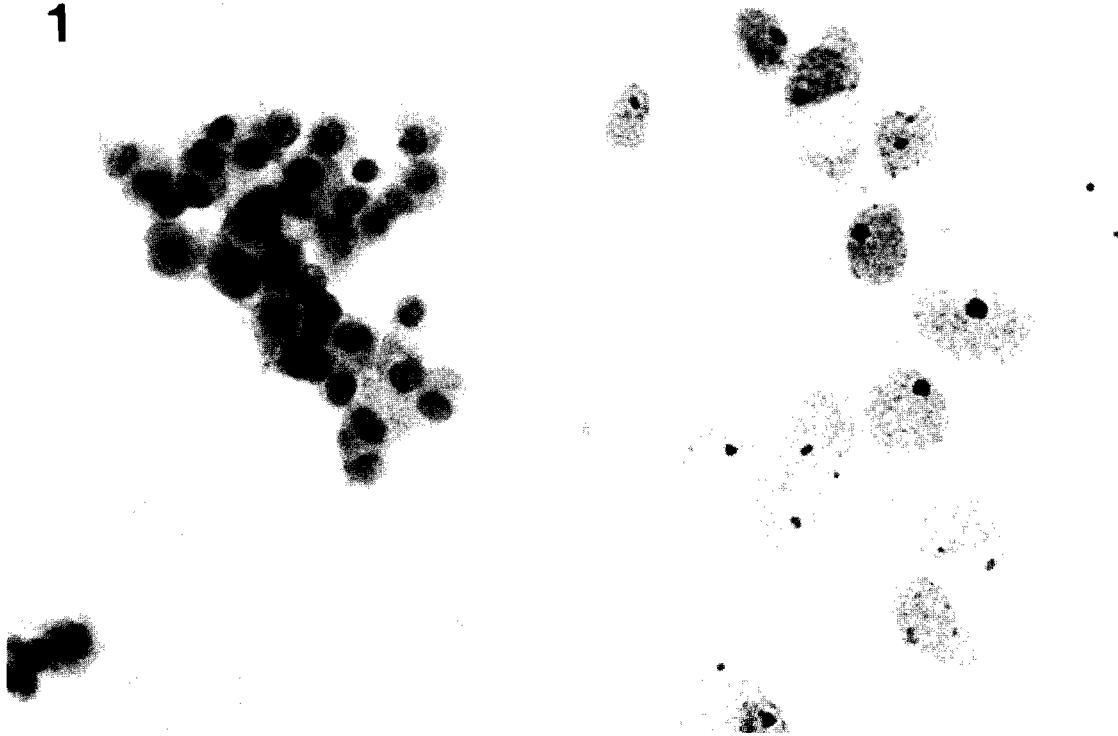


Fig. 1. Smears from a parasellar mass. On the left, the staining with H&E shows clusters of epithelial cells, suggesting the diagnosis of pituitary adenoma, but the possibility of a metastatic adenocarcinoma must be considered. On the right silver colloidal technique shows only one nucleolar organizer region (NOR) in tumor cells suggesting benignity and supporting the diagnosis of pituitary adenoma. Original magnification $\times 400$ (left) and $\times 1,000$ with oil-immersion lens (right).

2

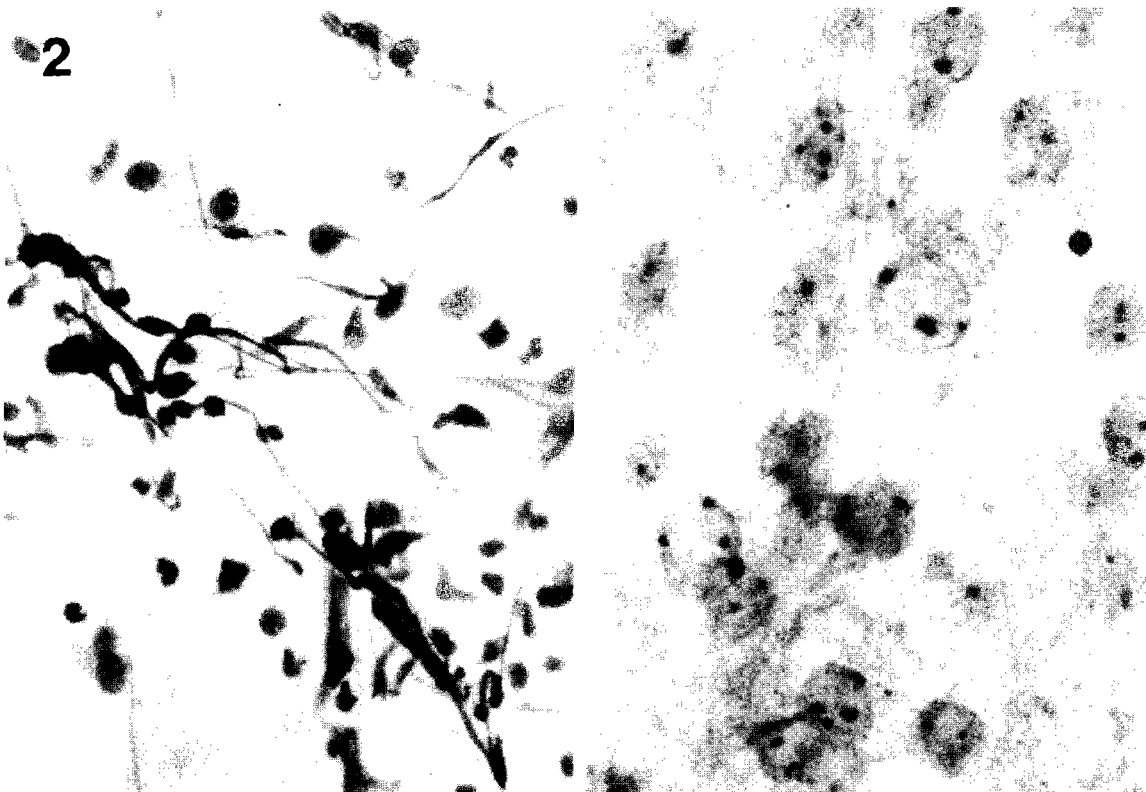


Fig. 2. Smears from stereotactic biopsy of a brain hemispheric mass. It is hypodense, without enhancement after contrast administration, on CT-scan study. On the left, H&E technique shows tumor cells with long processes suggesting an astrocytoma, but the small areas of tissue make the study of criteria for malignancy difficult. On the right silver colloidal technique reveals two or more nucleolar organizer regions (NORs) in most tumor cells, supporting a diagnosis of malignancy. A grade III astrocytoma is diagnosed on paraffin-embedded samples. Original magnification $\times 400$ (left) and $\times 1,000$, with oil-immersion lens (right).

distilled water and mounted with geltol permanent aqueous mounting medium (Lipshaw, Detroit, MI). They were then examined under optic microscope. NORs were identified on the second slide as clear black dots within the cells nuclei.

Results

Following this method, about 15 minutes after obtaining the tissue, two slides from the same region of the tumor were examined in each case, one stained with H&E technique and the other one processed for identification of NORs. When most cell nuclei showed only one NOR, a benign prognosis has been suggested. On the other hand, when most cell nuclei showed two or more NORs, we have suggested a malignant prognosis of the tumor.

In 6 cases (17.6%), the technique employed for NOR identification was considered as useless, due to silver precipitate. In the remaining cases (82.4%), NORs were clearly disclosed on tumoral cells. When the 29 cases simultaneously studied by H&E and NOR techniques were considered, tumoral diagnosis was clearly established on H&E-stained smear, in 22 (17 malignant astrocytomas, 3 oligodendrogliomas and 2 benign astrocytomas). In these cases, the NOR technique corroborated the morphological criteria for benignity or malignancy of the H&E stained slide. In 7 cases, intraoperative diagnosis was considered as doubtful on the basis of H&E-stained smears. Nevertheless, in these cases, NOR study suggested a diagnosis of benignity in 2 cases and a diagnosis of malignancy in 5 cases (Figs. 1, 2). In the seven cases this presumption was corroborated when permanent paraffin-embedded specimens were examined (1 pituitary adenoma, 1 benign astrocytoma and 5 malignant astrocytomas).

Discussion

The preparation of smears from brain biopsy material is a well-established technique for rapid intraoperative tumor diagnosis (Morris, 1947; Adams et al., 1981; Hitchcock et al., 1986; Chandrasoma and Apuzzo, 1989; Nguyen et al., 1989). The increasing importance of stereotactic biopsy has encouraged neurosurgeons and neuropathologists to attempt this technique with the very small specimens obtained in these procedures, because conventional techniques such as frozen or paraffin sections are sometimes difficult with such small fragments (Hitchcock et al., 1986; Nguyen et al., 1989). Nevertheless, sometimes the diagnosis of a brain tumor as «malignant» or «benign» based on the smears stained by H&E technique is very difficult because there are small areas of tissue to be examined. In such cases, the identification of the number of NORs within the nuclei of the tumor cells gives valuable information about the benignity or malignancy of the lesion. When the diagnosis has been well-established by H&E-stained

smear the study of NORs has a complementary role.

At present, we have studied 34 smears of tumors with the described NOR staining technique and our findings confirm previous reports about NOR staining in human brain tumors and normal brain tissue. Normal cells show one NOR within their nuclei and very occasionally two, while malignant cells usually show two or more NORs within their nuclei (Hara et al., 1990).

Our present experience suggests that the use of simultaneous H&E and NOR staining techniques on the smears from the same tissue fragment of the tumoral biopsies is a useful and rapid procedure that allows a more accurate intraoperative histological diagnosis of brain tumors.

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