Acta Neuropathol (1992) 84: 178 – 183

Acta Neuropathologica

Histochemical study of pituitary adenomas with Ki-67 and anti-DNA polymerase α monoclonal antibodies, bromodeoxyuridine labeling, and nucleolar organizer region counts*

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Received September 16, 1991/Revised, accepted March 4, 1992

Summary. The growth potential of 65 pituitary adenomas was determined by histochemical analysis with Ki-67 and anti-DNA polymerase α monoclonal antibodies, bromodeoxyuridine (BrdUdR) labeling, and counts of argyrophilic nucleolar organizer regions (Ag-NORs). The mean proliferating cell indices (PCIs) determined by Ki-67 and anti-DNA polymerase α and the BrdUdR labeling index (LI) were generally very low [1.0 ± 0.2%, 1.1 ± 0.2%, and 0.5 ± 0.1% (± SE), respectively]. Apart from adrenocorticotropic hormone-positive adenomas, which had significantly higher indices, there were no statistically significant differences in the indices among the other subtypes of pituitary adenomas. Recurrent tumors had higher Ki-67 and DNA polymerase α PCIs and BrdUdR LIs (3.6%, 4.2%, 1.4%) than primary tumors (0.8%, 0.8%, 0.3%; $P < 0.005$). The number of Ag-NORs did not correlate significantly with any of the three indices. The mean number of Ag-NORs was higher in nonfunctioning adenomas than in functioning adenomas (2.04 vs 1.66, $P < 0.005$); among prolactin-positive adenomas, those treated preoperatively with bromocriptine had more Ag-NORs than untreated tumors (1.75 vs 1.57, $P < 0.005$). These results suggest that the Ki-67 and DNA polymerase α PCIs and the BrdUdR LI predict the growth potential of individual pituitary adenomas, whereas the number of Ag-NORs appears to correlate with hormone production rather than with the proliferative potential.

Key words: Pituitary adenoma – Monoclonal antibody Ki-67 – Anti-DNA polymerase α monoclonal antibody – Bromodeoxyuridine – Nucleolar organizer regions

Most pituitary adenomas grow slowly and are considered benign [15, 17, 21, 28]. Some, however, show aggressive [1, 9] or invasive growth [28, 29] and even metastasis [2, 27]. The histopathological findings often do not predict the biological behavior of these atypical tumors [1, 28, 29].

Immunohistochemical studies with monoclonal antibodies have proven useful for determining the proliferative potential of various brain tumors. Monoclonal antibody Ki-67 [5, 6] and anti-DNA polymerase α monoclonal antibody [19, 22] recognize nuclear antigens expressed in G1-, S-, G2 and M-phase cells, but not in G0 cells. Monoclonal antibodies against bromodeoxyuridine (BrdUdR), a thymidine analog incorporated during DNA synthesis, can be used to determine the S-phase fraction. The proliferative potential estimated with these three antibodies reflects the biological behavior of various brain tumors [10, 11, 16, 30]. The number of argyrophilic nucleolar organizer regions (Ag-NORs) has also been shown to correlate with cellular proliferation in several tumors [8, 14, 23]. NORs are loops of ribosomal DNA that are transcribed by RNA polymerase I and are of central importance in the regulation of the protein synthesis of cells; they are readily demonstrated by argyrophilic staining techniques [25].

In this study, we analyzed the cellular proliferation of pituitary adenomas with these techniques to determine if there was a correlation with the biological behavior of individual tumors.

Materials and methods

Tissue preparation

Sixty-four pituitary adenomas and one recurrent pituitary carcinoma, all resected by the transsphenoidal approach, were examined. A portion of each tumor specimen was embedded in OCT compound (Miles, Elkhart, Ind.) and frozen with dry ice in ethanol. Other portions were fixed in 70% ethanol and embedded in paraffin for NOR study and in 10% buffered formalin for pathological evaluation and immunohistochemical identification

* Supported by grants CA-13525 and CA-50210 from the National Cancer Institute, by a grant from the Phi Beta Psi Sorority, and by Grant-in-Aid A 63771083 from the Ministry of Education, Science, and Culture of Japan

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of prolactin (PRL), growth hormone (GH), adrenocorticotropic hormone (ACTH), and thyroid stimulating hormone (TSH). Some specimens were labeled with BrdUdR in vitro as described by Sasaki et al. [26] and fixed in 70 % ethanol. Briefly, the specimens were cut into small fragments, incubated in Eagle's MEM supplemented with 20 % fetal calf serum and 100 μM/l BrdUdR for 1 h at 37 °C in carbogen (95 % O_2 and 5 % CO_2) at three atmospheres of pressure.

The tumors were classified according to their major clinical manifestations, serological studies, and immunohistochemical properties. Tumors without evidence of immunohistochemical markers and that did not cause endocrinological symptoms or abnormal increse of serum hormone levels were considered to be nonfunctioning tumors.

Immunohistochemistry with Ki-67 and anti-DNA polymerase α monoclonal antibody

The staining procedure has been described elsewhere [31]. Briefly, cryostat sections 4–6 μm thick were air dried and fixed in 4 % paraformaldehyde at 4 °C for 30 min. The sections were incubated for 1 h at room temperature with monoclonal antibody Ki-67 (Dako, Santa Barbara, Calif.) diluted 1:25 or with mouse anti-DNA polymerase α monoclonal antibody (CL-22-2-42B, MBL, Nagoya, Japan) diluted 1:20. After incubation in 0.3 % hydrogen peroxide in phosphate-buffered saline for 30 min to block endogenous peroxidase activity, the sections were incubated first for 30 min with biotinylated secondary antibody against mouse immunoglobulins (Dako) and then for 30 min with streptavidin-biotin-peroxidase complex (Dako). The sections were then stained with 3,3′-diaminobenzidine and lightly counterstained with 1 % methyl green.

Immunohistochemistry with anti-BrdUdR monoclonal antibody

The sections were deparaffinized, denatured with 4 N hydrochloric acid for 10 min at room temperature, and incubated for 30 min with mouse anti-BrdUdR monoclonal antibody (BR-3, Caltag, South San Francisco, Calif.) diluted 1:20,000 in phosphate-buffered saline containing 5 % normal goat serum and for 30 min with gold-conjugated goat anti-mouse immunoglobulin G (Amersham, Arlington Heights, Ill.) diluted 1:50. The sections were fixed with 2.5 % glutaraldehyde for 10 min, developed with a silver enhancement kit (IntenSE M, Amersham), and counterstained with 0.1 % nuclear fast red.

Evaluation of immunohistochemistry

The proliferating cell index (PCI) and the BrdUdR labeling index (LI) were calculated from each slide as the percentage of immunopositive nuclei; vascular components and hematogenous cells were excluded. More than 1,000 cells from each specimen were counted in several viable areas of tissue in which positive nuclei were evenly distributed.

Staining and counting of Ag-NORs

The procedure for staining Ag-NORs has been described in detail [4]. Briefly, ethanol-fixed, paraffin-embedded sections 4–6 μm thick were covered with a solution consisting of gelatin (2 g/100 ml) in aqueous formic acid (1 g/100 ml) mixed with aqueous silver nitrate (50 g/100 ml, 1:2 v/v) for 20 min in a dark room. Ag-NORs were counted in 200 cells in each case, and the mean number per cell was calculated.

Statistical analysis

The t test was used to determine the statistical significance of difference in the PCIs, BrdUdR LI, and the number of Ag-NORs in different tumor subtypes. Linear regression analysis was used to correlate the three indices with each other and with the number of Ag-NORs; the statistical significance of the correlation was determined by t test.

Results

The Ki-67 and DNA polymerase α PCIs and the BrdUdR LIs ranged from <0.1 % to 10.9 % (median 0.7 %), 14.1 % (median 0.6 %), and 4.4 % (median 0.3 %), respectively. The number of Ag-NORs ranged from 1.31 to 2.94 (median 1.73). The PCIs, BrdUdR LI, and the number of Ag-NORs did not correlate with the age or sex of the patient, the duration of symptoms, or tumor size determined from magnetic resonance images or computed tomography scans. Photomicrographs of nonfunctioning, PRL-positive, and ACTH-positive adenomas stained for Ag-NORs are shown in Fig. 1.

Linear regression analysis showed a statistically significant correlation between Ki-67 and DNA polymerase α PCIs (Fig. 2) as well as between the BrdUdR LIs and the Ki-67 and DNA polymerase α PCIs [$r = 0.52$, $P < 0.005$, $n = 37$; $r = 0.65$, $P < 0.005$, $n = 35$, respectively (data not shown)]. The number of Ag-NORs did not correlate with the Ki-67 or DNA polymerase α PCIs or with the BrdUdR LIs by linear regression analysis.

The Ki-67 and DNA polymerase α PCIs showed few significant differences among the various subtypes of tumors (Table 1). ACTH-positive adenomas had a significantly higher mean Ki-67 PCI than PRL-positive adenomas and a significantly higher mean DNA polymerase α PCI than nonfunctioning, GH-positive, or PRL-positive adenomas. There were no significant differences in the mean BrdUdR LIs of the various tumor subtypes.

The mean number of Ag-NORs was significantly higher in the 24 nonfunctioning pituitary adenomas than in the 38 functioning tumors (2.04 ± 0.05 vs 1.66 ± 0.03, $P < 0.005$), but there were no significant differences in the number of Ag-NORs in different subtypes of functioning tumors (Table 1). The Ag-NORs were smaller in nonfunctioning adenomas, larger in PRL- and TSH-positive tumors, and intermediate or mixed in GH- and ACTH-positive tumors.

The mean PCIs, BrdUdR LI, and number of Ag-NORs were significantly higher in recurrent tumors than in primary tumors (Table 2). The PCIs and BrdUdR LI were also higher in seven tumors (three recurrent) with atypical histological features, such as nuclear atypia or pleomorphism and mitotic figures, than in tumors without such features. There was no difference in the mean number of Ag-NORs of typical and atypical adenomas. Macroscopically invasive tumors had a higher mean DNA polymerase α PCI than tumors judged noninvasive at surgery. Invasiveness did not correlate

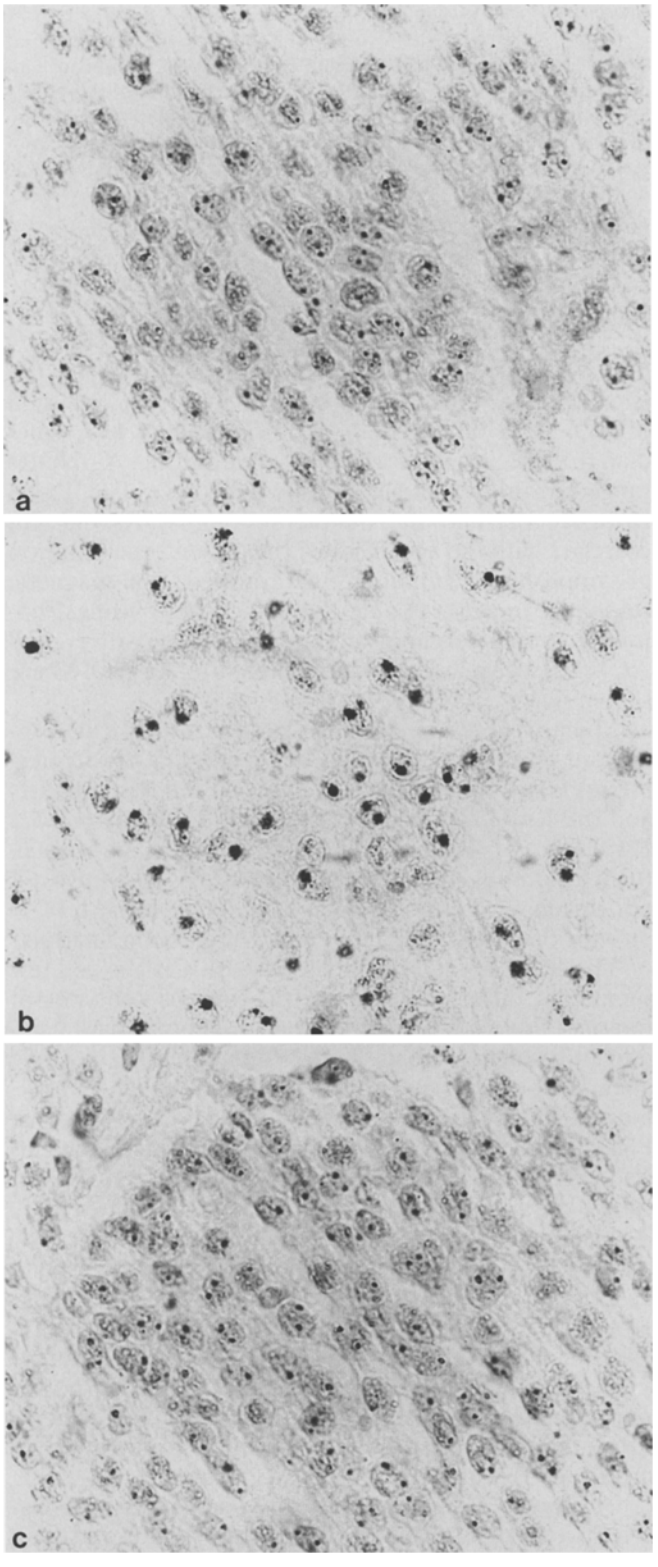

Fig. 1. Nucleolar organizer regions stained by argyrophil technique in (**a**) a nonfunctioning pituitary adenoma (1.97 ± 0.16, mean ± SD), (**b**) a prolactin-positive adenoma (1.34 ± 0.11), and (**c**) an adrenocorticotropic hormone cell adenoma (1.66 ± 0.17). The Ag-NORs were smaller in the nonfunctioning tumor, larger in the prolactin-positive tumor, and intermediate or mixed in adrenocorticotropic hormone-positive tumor. **a–c** original magnification × 600

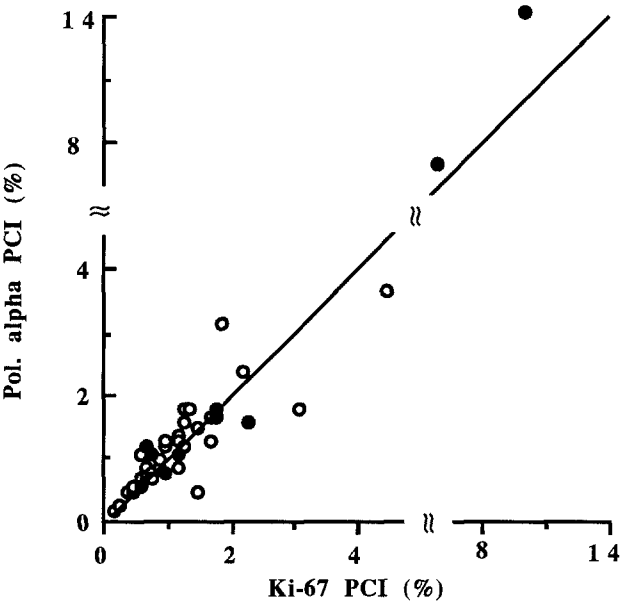

Fig. 2. The correlation between Ki-67 and DNA polymerase α (Pol. alpha) proliferating cell indices (PCI) in 62 pituitary tumors (61 adenomas and one carcinoma). Linear regression analysis showed a highly significant correlation: DNA polymerase α PCI = −0.10 + 1.18 Ki-67 PCI ($r = 0.97$, $P < 0.005$). (○) Primary tumor; (●) recurrent or histologically atypical tumor

with the other indices or with the number of Ag-NORs.

Among 20 PRL-positive adenomas, there were no significant differences in the Ki-67 and DNA polymerase α PCIs of tumors treated preoperatively with bromocriptine and those that were not (Table 3). The mean number of Ag-NORs, however, was higher in treated than in untreated tumors.

Discussion

This study showed that the proliferative potential of pituitary adenomas determined immunohistochemically with Ki-67, anti-DNA polymerase α, and anti-BrdUdR monoclonal antibodies was generally very low. Excluding one ACTH-positive adenoma with unusually high indices (Ki-67 and DNA polymerase α PCIs of 10.9% and 14.1%, respectively, and a BrdUdR LI of 1.5%) that recurred three times, the ACTH-positive adenomas had mean Ki-67 and DNA polymerase α PCIs of 1.08 ± 0.02% and 1.54 ± 0.38%, respectively, and a BrdUdR LI of 0.15 ± 0.15%. These values were significantly higher than those of nonfunctioning ($p < 0.005$) and GH-positive adenomas ($P < 0.05$). One of two adenomas in patients with Nelson's syndrome had relatively high Ki-67 and DNA polymerase α PCIs (1.7% and 3.0%, respectively). These observations agree with previous reports of high proliferative potentials in ACTH-positive adenomas and adenomas associated with Nelson's syndrome [15, 17, 21].

The number of Ag-NORs did not always correlate with the clinical aggressiveness or with the proliferative

Table 1. Proliferative potential of pituitary adenomas[a]

Tumor type	No. of cases	Ki-67 PCI (%)	DNA polymerase α PCI (%)	BrdUdR LI (%)	Mean no. of Ag-NORs
Nonfunctioning	24	0.82 ± 0.18 (24)	0.72 ± 0.10 (23)	0.56 ± 0.19 (20)	2.04 ± 0.05 (24)[b,c]
PRL-positive	20	0.91 ± 0.24 (20)	0.85 ± 0.20 (19)	0.18 ± 0.05 (8)	1.62 ± 0.05 (19)
GH-positive	10	0.69 ± 0.17 (10)	0.76 ± 0.19 (10)	0.43 ± 0.24 (3)	1.65 ± 0.06 (10)
ACTH-positive	7	2.49 ± 1.41[d] (7)	3.63 ± 2.12[e,f,g] (6)	0.60 ± 0.46 (3)	1.76 ± 0.09 (6)
TSH-positive	3	0.87 ± 0.34 (3)	0.70 ± 0.21 (3)	0.35 ± 0.05 (2)	1.66 ± 0.13 (3)
Total	64	1.01 ± 0.19 (64)	1.05 ± 0.23 (61)	0.47 ± 0.13 (36)	1.80 ± 0.04 (62)
Pituitary carcinoma	1	6.3	7.2	1.0	2.94

[a] Values are mean ± SE; numbers in parentheses are numbers of cases; PCI, proliferating cell index; BrdUdR LI, bromodeoxyuridine labeling index; Ag-NORs. argyrophilic nucleolar organizer regions, PRL, prolactin; GH, growth hormone; ACTH, adrenocorticotropic hormone; TSH: thyroid stimulating hormone
[b] $P < 0.005$ vs PRL- and GH-positive adenomas
[c] $P < 0.01$ vs ACTH- and TSH-positive adenomas
[d] $P < 0.05$ vs PRL-positive adenomas
[e] $P < 0.005$ vs nonfunctioning adenomas
[f] $P < 0.05$ vs GH-positive adenomas
[g] $P < 0.025$ vs PRL-positive adenomas

Table 2. Proliferative potential of various pituitary adenomas[a]

Tumor type	No. of cases	Ki-67 PCI (%)	DNA polymerase α PCI (%)	BrdUdR LI (%)	Mean no. of Ag-NORs
Primary	58	0.80 ± 0.10 (58)	0.83 ± 0.09 (56)	0.29 ± 0.04 (31)	1.79 ± 0.04 (57)
Recurrent	7[b]	3.56 ± 1.48[c] (7)	4.17 ± 2.24[c] (6)	1.40 ± 0.64[c] (6)	2.07 ± 0.18[d] (7)
Typical	58	0.77 ± 0.10 (58)	0.81 ± 0.09 (56)	0.34 ± 0.06 (34)	1.80 ± 0.04 (57)
Atypical[e]	7[f]	3.76 ± 1.41[c] (7)	4.32 ± 2.20[c] (6)	1.97 ± 1.22[c] (3)	2.00 ± 0.18 (7)
Noninvasive[g]	31	0.80 ± 0.19 (31)	0.71 ± 0.13 (30)	0.58 ± 0.26 (16)	1.78 ± 0.05 (31)
Invasive	19	1.19 ± 0.31 (19)	1.46 ± 0.41[d] (17)	0.31 ± 0.12 (10)	1.82 ± 0.08 (18)

[a] Values are mean ± SE; numbers in parentheses are numbers of cases; PCI, Proliferating cell index; BrdUdR LI, bromodeoxyuridine labeling index; Ag-NORs, argyrophilic nucleolar organizer regions
[b] Three nonfunctioning, two adrenocorticotropic hormone-positive, and one prolactin-positive adenomas and one pituitary carcinoma
[c] $P < 0.005$, recurrent vs primary, atypical vs typical
[d] $P < 0.025$, recurrent vs primary, invasive vs noninvasive
[e] Histologically atypical featurers, such as nuclear atypia or pleomorphism, and mitotic figures
[f] Two nonfunctioning, one growth hormone-positive, one thyroid-stimulating hormone-positive, and two adrenocorticotropic hormone-positive adenomas and one pituitary carcinoma
[g] Observed at operation

Table 3. Proliferative potential of prolactin-positive adenomas[a]

Preoperative treatment	No. of cases	Ki-67 PCI (%)	DNA polymerase α PCI (%)	BrdUdR LI (%)	Mean no. of Ag-NORs
Bromocriptine	7	1.01 ± 0.38 (7)	0.73 ± 0.26 (7)	ND	1.75 ± 0.09[b] (6)
Untreated	13	0.85 ± 0.31 (13)	0.93 ± 0.28 (12)	0.18 ± 0.05 (8)	1.57 ± 0.04 (13)

PCI, proliferating cell index; BrdUdR LI, bromodeoxyuridine labeling index; Ag-NORs, argyrophilic nucleolar organizer regions; ND, not done
[a]Values are mean ± SE; numbers in parentheses are numbers of cases
[b]$P < 0.05$ vs untreated tumors

potential reflected by the PCIs and the BrdUdR LIs [7, 18], although a recurrent pituitary carcinoma had the highest number of Ag-NORs. The aggressive ACTH-positive adenoma described above had only 1.55 Ag-NORs. The mean number of Ag-NORs was significantly higher in nonfunctioning adenomas than in functioning adenomas but was similar in the various subtypes of functioning tumors, as previously reported [20, 32].

Although only macroscopic invasiveness was evaluted in this study, the mean DNA polymerase α PCI was

higher in tumors judged invasive at surgery than in those that were not [15, 17]. Thus, invasive pituitary adenomas may be considered to have a biological behavior intermediate between those of noninvasive adenomas and pituitary carcinomas [28].

Seven patients with PRL-positive adenomas received bromocriptine (2.5 to 10 mg/day) for more than 1 month before surgery. The proliferative potential of PRL-positive adenomas was not changed by the preoperative administration of bromocriptine [17], even though high concentrations of bromocriptine have been reported to inhibit DNA synthesis [24] and delay the cell cycle [12, 13]. The mean number of Ag-NORs, however, was higher in bromocriptine-treated adenomas than in untreated tumors. Thus, the number of Ag-NORs may reflect the effect of bromocriptine on hormone production rather than the proliferative potential. These results are not in agreement with those of a previous report [32]. Further studies may be necessary to evaluate the effect of bromocriptine on the number of Ag-NORs.

We cannot determine from the results of the present study the values at which the PCIs and BrdUdR LIs of pituitary adenomas indicate aggressive tumor growth. A PCI >1.5 % or a BrdUdR LI >1 % appears to correlate with rapid growth and the likelihood of recurrence (Fig. 3 and Table 2). In such cases, postoperative radiation therapy should be considered [3, 33], especially when there is residual tumor or invasive growth is suspected. The Ki-67 and DNA polymerase α PCIs and the BrdUdR LI may provide information that is useful for predicting the biological behavior of individual pituitary adenomas and for planning follow-up studies and treatment after surgical resection. The BrdUdR LI, however, may be too low to accurately estimate the proliferative potential of individual pituitary adenomas. Therefore, the PCIs may have some advantages over BrdUdR LI in estimating the proliferative potential of individual pituitary adenomas. The number and size of Ag-NORs may be more useful for evaluating hormone production than for estimating the proliferative potential of pituitary adenomas.

Acknowledgements. The authors thank Cheryl Christensen for manuscript preparation and Stephen Ordway for editorial assistance.

References

1. Anniko M, Holm L-E, Wersäll J (1983) Aggressive pituitary tumor growth. Arch Otorhinolaryngol 238:53–62
2. Asai A, Matsutani M, Funada N, Takakura K (1988) Malignant growth hormone-positive pituitary adenoma with hematogeneous dural metastasis: case report. Neurosurgery 22:1091–1094
3. Chun M, Masko GB, Hetelekidis S (1988) Radiotherapy in the treatment of pituitary adenomas. Int J Radiat Oncol Biol Phys 15:305–309
4. Crocker J, Nar P (1987) Nucleolar organizer regions in lymphomas. J Pathol 151:111–118
5. Gerdes J, Schwab U, Lemke H, Stein H (1983) Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 31:13–20
6. Gerdes J, Lemke H, Baisch H, Wacker H-H, Schwab U, Stein H (1984) Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 133:1710–1715
7. Giri DD, Nottingham JF, Lawry J, Dundas SAC, Underwood JCE (1989) Silver-binding nucleolar organizer regions (Ag-NORs) in benign and malignant breast lesions: correlations with ploidy and growth phase by DNA flow cytometry. J Pathol 157:307–313
8. Hall PA, Crocker J, Watts A, Stansfeld AG (1988) A comparison of nucleolar organizer region staining and Ki-67 immunostaining in non-Hodgkin's lymphoma. Histopathology 12:373–381
9. Horvath E, Kovacs K, Killinger DW, Smyth HS, Platts ME, Singer W (1980) Silent corticotropic adenomas of the human pituitary gland. A histologic, immunocytologic and ultrastructural study. Am J Pathol 98:617–638
10. Hoshino T, Nagashima T, Murovic JA, Wilson CB, Edwards MSB, Gutin PH, Davis RL (1986) In situ cell kinetics studies on human neuroectodermal tumors with bromodeoxyuridine labeling. J Neurosurg 64:453–459
11. Hoshino T, Prados M, Wilson CB, Cho KG, Lee K-S, Davis RL (1989) Prognostic implications of the bromodeoxyuridine labeling index of human gliomas. J Neurosurg 71:335–341
12. Johansen PW, Clausen OPF, Haug E, Fossum S, Gautvik KM (1985) Effects of bromocriptine on cell cycle distribution and cell morphology in cultured rat pituitary adenoma cells. Acta Endocrinol (Copenh) 110:319–328
13. Johansen PW, Haug E, Gautvik KM (1985) Effects of bromocriptine on hormone production and cell growth in cultured rat pituitary cells. Acta Endocrinol (Copenh) 110:200–206
14. Kajiwara K, Nishizaki T, Orita T, Nakayama H, Aoki H, Ito H (1990) Silver colloid staining technique for analysis of glioma malignancy. J Neurosurg 73:113–117
15. Knosp E, Kitz K, Perneczky A (1989) Proliferation activity in pituitary adenomas: measurement by monoclonal antibody Ki-67. Neurosurgery 25:927–930
16. Kunishio K, Mishima N, Matsuhisa T, Tsuno K, Matsumi N, Satoh T, Matsumoto K, Furuta T, Nishimoto A, Shiraishi T (1990) Immunohistochemical demonstration of DNA polymerase α in human brain-tumor cells. J Neurosurg 72:268–272
17. Landolt AM, Shibata T, Kleihues P (1987) Growth rate of human pituitary adenomas. J Neurosurg 67:803–806
18. Maier H, Morimura T, Öfner D, Hallbrucker C, Kitz K, Budka H (1990) Argyrophilic nucleolar organizer region proteins (Ag-NORs) in human brain tumors: relations with grade of malignancy and proliferation indices. Acta Neuropathol 80:156–162
19. Masaki S, Shiku H, Kaneda T, Koiwai O, Yoshida S (1982) Production and characterization of monoclonal antibody against 10S polymerase α from calf thymus. Nucleic Acids Res 10:4703–4713
20. McNicol AM, Colgan J, McMeekin W, Teasdale GM (1989) Nuclear organiser regions in pituitary adenomas. Acta Neuropathol 77:547–549
21. Nagashima T, Murovic JA, Hoshino T, Wilson CB, DeArmond SJ (1986) The proliferative potential of human pituitary tumors in situ. J Neurosurg 64:588–593
22. Nakamura H, Morita T, Masaki S, Yoshida S (1984) Intracellular localization and metabolism of DNA polymerase α in human cells visualized with monoclonal antibody. Exp Cell Res 151:123–133
23. Orita T, Kajiwara K, Nishizaki T, Ikeda N, Kamiryo T, Aoki H (1990) Nucleolar organizer regions in meningioma. Neurosurgery 26:43–46

24. Prysor-Jones RA, Jenkins JS (1981) Effect of bromocriptine on DNA synthesis, growth and hormone secretion of spontaneous pituitary tumours in rats. J Endocrinol 88:463–469
25. Rüschoff J, Plate K, Bittinger A, Thomas C (1989) Nucleolar organizer regions (NORs). Basic concepts and practical application in tumor pathology. Pathol Res Pract 185:878–885
26. Sasaki K, Ogino T, Takahashi M (1986) Immunological determination of labeling index on human tumor tissue sections using monoclonal anti-BrdUrd antibody. Stain Technol 61:155–161
27. Scheithauer BW, Randall RV, Laws ER Jr, Kovacs KT, Horvath E, Whitaker MD (1985) Prolactin cell carcinoma of the pituitary. Clinicopathologic, immunohistochemical, and ultrastructural study of a case with cranial and extracranial metastases. Cancer 55:598–604
28. Scheithauer BW, Kovacs KT, Laws ER Jr, Randall RV (1986) Pathology of invasive pituitary tumors with special reference to functional classification. J Neurosurg 65:733–744
29. Selman WR, Laws ER Jr, Scheithauer BW, Carpenter SM (1986) The occurrence of dural invasion in pituitary adenomas. J Neurosurg 64:402–407
30. Shibuya M, Hoshino T, Ito S, Wacker MR, Prados MD, Davis RL, Wilson CB (1992) Meningiomas: clinical implications of a high proliferative potential determined by bromodeoxyuridine labeling. Neurosurgery 30: 494–498
31. Shibuya M, Miwa T, Hoshino T (1992) Embedding and fixation techniques for immunohistochemical staining with anti-DNA polymerase α and Ki-67 monoclonal antibodies to analyze the proliferative potential of tumors. Biotechnic Histochem (in press)
32. Stefaneanu L, Horvath E, Kovacs K (1989) Argyrophil organizer region proteins (AgNORs) in adenohypophysial cells and adenomas of the human pituitary. Mod Pathol 2:192–199
33. Vlahovitch B, Reynaud C, Rhiati J, Hammoud F (1988) Treatment and recurrence in 135 pituitary adenomas. Acta Neurochir (Wien) [Suppl] 42:120–123