Title	Expression of O6-methylguanine DNA methyltransferase (MGMT) and immunohistochemical analysis of 12 pineal parenchymal tumors
Author(s)	Kanno, Hiromi; Nishihara, Hiroshi; Oikawa, Mitsuteru; Ozaki, Yoshimaru; Murata, Junichi; Sawamura, Yutaka; Kato, Masahito; Kubota, Kanako; Tanino, Mishie; Kimura, Taichi; Nagashima, Kazuo; Itoh, Tamio; Tanaka, Shinya
Citation	Neuropathology, 32(6), 647-653 https://doi.org/10.1111/j.1440-1789.2012.01315.x
Issue Date	2012-12
Doc URL	http://hdl.handle.net/2115/54012
Rights	The definitive version is available at wileyonlinelibrary.com
Type	article (author version)
File Information	Neu32-6_647-653.pdf

[Instructions for use](#)

Original Article

Expression of O⁶-methylguanine DNA methyltransferase (MGMT) and immunohistochemical analysis of 12 pineal parenchymal tumors.

Hiromi Kanno¹, Hiroshi Nishihara², Mitsuteru Oikawa³, Yoshimaru Ozaki³, Junichi Murata⁴, Yutaka Sawamura⁵, Masahito Kato⁶, Kanako Kubota⁷, Mishie Tanino¹, Taichi Kimura¹, Kazuo Nagashima⁸, Tamio Itoh³, and Shinya Tanaka^{1, 2}.

¹Laboratory of Cancer Research, Department of Pathology, Hokkaido University School of Medicine, ²Laboratory of Translational Pathology, Hokkaido University School of Medicine, ³Nakamura Memorial Hospital, ⁴Sapporo Azabu Neurosurgical Hospital, ⁵Sawamura Neurology and Neurosurgery Clinic, ⁶Hokkaido Neurosurgical Memorial Hospital, ⁷Department of Surgical Pathology, Hokkaido University Hospital and ⁸Department of Pathology, Sapporo Higashi Tokushukai Hospital, Sapporo, JAPAN.

Running title: MGMT expression in pineal parenchymal tumors.

Correspondence: Hiroshi Nishihara, MD., PhD.

Laboratory of Translational Pathology, Hokkaido University School of Medicine, North
15, West 7, Kita-ku, Sapporo, 060-8638, Japan.

Tel. +81-11-706-5053

Fax +81-11-706-5902

E-mail: hnishihara@s5.dion.ne.jp

Funding: No fundings.

Conflict of interest: No

Unpublished papers cited: Nothing

Abstract

Pineal parenchymal tumors (PPTs) are rare neoplasms which occupy less than 1% of primary central nervous system tumors. Because of their rare incidence, the previous reports on PPTs are limited in number and the useful molecular markers for deciding the histological grading and even selecting chemotherapy are undetermined. In this study, we conducted immunohistochemical analysis of 12 PPT specimens, especially for expression of O⁶-methylguanine DNA methyltransferase (MGMT) to assess whether temozolomide (TMZ) could serve as a possible alternative therapy for PPTs. We analyzed 12 PPTs consisted of 3 pineocytomas, 6 pineal parenchymal tumors of intermediate differentiation (PPTIDs), and 3 pineoblastomas. Immunohistochemical analysis was performed using antibody against MGMT, synaptophysin, neurofilament protein (NF), p53, and NeuN. Immunohistochemically, 11 out of 12 cases were positive for MGMT. The mean MIB-1 labeling index was less than 1 % in pineocytoma, 3.5 % in PPTID, and 10.5 % in pineoblastoma. All 12 cases were positive for synaptophysin and 11 cases except 1 PPTID case showed positive for NF. Nuclear staining of NeuN was negative in all cases although cytoplasmic stain of NeuN was observed in 5 cases. No case was positive for p53. Eleven out of 12 cases of PPTs demonstrated MGMT expression, suggesting the chemoresistance to TMZ treatment. This is the first report showing MGMT expression in PPTs. In addition, MIB-1 labeling index correlated with WHO grade, although the immunoreactivity of synaptophysin, NF, NeuN, and p53 did not correlate with the histological grade. (240 words)

Keywords; pinealoma, immunohistochemistry, MGMT, temozolomide, MIB-1

Introduction

Pineal parenchymal tumors (PPTs) are rare neoplasms arising from the pineal gland, and account for less than 1% of primary central nervous system (CNS) tumors ¹. According to the latest edition of the World Health Organization (WHO) Classification of CNS Tumors ², PPTs are subdivided into pineocytoma, pineal parenchymal tumor of intermediate differentiation (PPTID) and pineoblastoma, which correspond to Grade I, Grade II or III and Grade IV, respectively. Except for pineocytoma, PPTs are potentially aggressive tumors and sometimes demonstrate craniospinal seeding³. The biological characteristics of PPTs are still to be understood; however, the histopathological studies for PPTs which indicate prognostic factor or predictive factor are limited because of the rare incidence of PPT. Moreover, the therapeutic modality to high grade PPTs have yet to be standardized; these range from localized radiotherapy to cranio-spinal radiotherapy, radical resection and multidrug chemotherapy ⁴. Temozolomide (TMZ) is an oral alkylating agent which is widely used in the treatment of glioblastoma. Recently, TMZ has been applied to other neuro-oncological tumors including pituitary tumor, melanomas, neuroendocrine tumors and metastatic tumors ⁵⁻¹⁰. O⁶-methylguanine DNA methyltransferase (MGMT) is a DNA repair protein that reverses alkylation at the O⁶ position of guanine and counteracts the anti-neoplastic effect of TMZ. As previous studies showed an inverse correlation between MGMT expression and responsiveness to TMZ ¹¹⁻¹³, the examination of MGMT expression is important to predict the response to TMZ therapy. PPTs could be one of the potential therapeutic targets for TMZ; however, no data is available about MGMT expression in PPTs so far.

In this study, we evaluated the expression of MGMT in 12 cases of PPT to assess whether TMZ could be one of the possible therapeutic agents for PPTs. In addition, to clarify the state of differentiation or biological characteristics of each grades of PPTs, we also evaluated the immunohistochemical molecular expression of PPTs including MIB-1 labeling index as a proliferation marker, and the major prognostic markers of PPTs reported in previously ^{14, 15} such as Neurofilament (NF); the most famous tumor suppressor, p53; and NeuN, the neuronal marker.

Materials and Methods

Between 1992 and 2011, 12 patients were diagnosed as PPT in our faculty. The microscopic slides, paraffin-embedded tissue and clinical information of these patients were collected retrospectively. For histological examinations, formalin-fixed, paraffin-embedded tissue was sectioned to 3 μm , and stained using hematoxylin and eosin (H&E). We reassessed the histological diagnoses according to the latest WHO classification. The differentiation between PPTID Grade II and PPTID Grade III was defined according to morphologic and immunohistochemical features described in the previous report [Jouvet, 2000 #18]. Mitoses were counted in 10 randomly selected high power fields (HPFs, x400) and expressed as the number of mitoses per 10 HPFs as previously described [Jouvet, 2000 #18].

Immunohistochemistry was performed according to standard procedures. Briefly, tissue sections of 5 μm were deparaffinized. Antigen retrieval was carried out using pressure cooking (in citrate buffer for 3 min). Endogenous peroxidase activity was blocked by incubating sections in 3% hydrogen peroxide for 5 min. After blocking, tissue sections were incubated with the primary antibodies listed in Table 1. The EnVision kit from Dako (Denmark) was used to detect the staining. The evaluation of staining of each antibody was scored as 0 (no staining), 1 (<1/3 of cells positive), 2 (between 1/3 and 2/3 of cells positive), and 3 (>2/3 cells positive). The MIB-1 index was calculated in the field of maximal activity as percentage of positive cells.

Results

Histological examination

Characteristics of the patients and immunohistochemical results are summarized in Table 2. The patients consisted of 3 males and 9 females with a median age of 53.5 years (range, 24-71). Three cases were classified as pineocytoma (Cases 1-3), six as PPTID (Cases 4-9), and three as pineoblastoma (Cases 10-12).

Pineocytomas demonstrated low to moderate cellularity and large pineocytomatous rosettes with abundant cell processes. The cells were uniform and had round-to-oval nuclei (Fig. 1a). PPTIDs showed moderate to high cellularity and rosette-like formation but lacked conspicuous pineocytomatous rosettes. The nuclear atypia was mild to moderate (Fig. 2a). There were no mitotic figures or necroses in pineocytomas and PPTIDs. Pineoblastomas were composed of highly cellular, patternless sheets of small undifferentiated cells. The cells showed high nuclear cytoplasmic ratio and hyperchromatic nuclei (Fig. 3a). Mitoses were seen in 2 pineoblastoma cases, which corresponded to 3 and 1 /10 HPFs, respectively, and one case demonstrated necrosis. The histological appearances of the other 9 cases are indicated in the Supplementary figure.

Immunohistochemistry

MGMT expression was detected in almost all cases except 1 pineocytoma (Fig. 1b, 2b, 3b and Supplementary figures 2c, 2f, 2i, 2l, 2o, 2r, 2u, 2x, 2A); in particular, all PPTIDs and pineoblastomas showed maximum staining intensity. The mean MIB-1

labeling index was less than 1 % (range: 0) in pineocytoma, 3.5 % (range: 0.7 - 6.4 %) in PPTID, and 10.5 % (range: 8.4 - 12.7 %) in pineoblastoma (Figs. 2e, 3e and Supplementary figures 1b, 2b, 2e, 2h, 2k, 2n, 2q, 2t, 2w, 2z). Synaptophysin was positive in all PPTs (Fig. 1e, Supplementary figures 1c, 1e), and NF staining was present in almost all cases except the 1 PPTID case (Figs. 1c, 2c, 3c). There was no difference in NF-positivity between pineocytomas and the other types of PPTs. Nuclear staining of NeuN was not observed in any cases (Figs. 1d, 2d, 3d), although 4 PPTIDs and 1 pineoblastoma showed cytoplasmic staining in a dot-like manner (Figs. 2d). p53 immunolabeling was negative in all PPTs (Supplementary figures 1b, 1d, 1f).

Discussion

The prognosis of pineocytomas is considered to be favorable and the 5-year survival rate is 86 to 100 % ^{16, 17}, while PPTIDs and pineoblastomas sometimes show poor prognosis. In fact, the reported five-year survival of PPTIDs and pineoblastoma is 39 to 74 % ¹⁷ and 58 % ³, respectively. The therapeutic spectrum includes surgery, localized or craniospinal radiotherapy and multidrug chemotherapy such as etoposide, cisplatin, hydroxyurea, carboplatin, cyclophosphamide, vinblastine and ifosfamide ^{4, 18}; however, an evident standardized therapeutic protocol for PPTIDs and pineoblastoma has not been established.

TMZ, a widely used alkylating agent in the treatment of glioblastoma, could be a possible candidate of therapeutic agents for PPTs, because this drug is oral-available and effectively delivered into the tumors through the blood-brain-barrier. In fact, it has been applied for the treatment of aggressive pituitary adenoma ^{9, 10} as well as melanomas, neuroendocrine tumors and metastatic tumors ⁵⁻⁸. Previous reports demonstrated 13 to 54 % of pituitary tumors show low MGMT expression ¹⁹⁻²¹[Fevre-Montange, 2011 #64], and low MGMT expression has been shown to correlate with TMZ response ^{21, 22}. In this study, we have performed the immunohistochemical evaluation for MGMT expression because the assessment of MGMT expression as a predictive factor for responsiveness to TMZ has been proposed in various types of the tumor ²³⁻²⁵. As a result, 11 out of 12 PPTs in our series showed positivity for MGMT. Furthermore, all PPTIDs and pineoblastomas demonstrated maximum staining intensity of MGMT; therefore, our results may indicate the

insusceptibility of TMZ treatment for high grade PPTs, although the fact that the one case of pineocytoma was negative for MGMT might indicate the presence of TMZ-sensitive cases among the low grade pineal tumors such as PPTIDs and pineocytomas. We didn't examine the methylation status of MGMT gene promoter because all our samples were paraffin-embedded material. The methylation status should be examined by using fresh frozen samples in the future. In addition, we have to consider the possibility that the treatment-resistant mechanism of MGMT expression can't be applied to PPTs.

Here we also evaluated other immunohistochemical markers, synaptophysin, NF, p53, NeuN, and MIB-1 to specify the neuronal differentiation and biological characteristics among each grades of PPTs. Because PPTs comprise a spectrum of increasing malignancy, and thus it is sometimes difficult to obtain the proper diagnosis for each tumor grades. Moreover, even though the WHO defined PPTIDs as grade II or III, more definite grading criteria have yet to be established. The Recent immunohistochemical studies of pineal parenchymal tumors are summarized in Table 3. Our result of the mean MIB-1 labeling index was consistent with the previous studies which reported the mean MIB-1 labeling indices as 0.27 - 3.9 % in pineocytoma, 2.7 - 10.1 % in PPTID, and 6.49 – 36.4 % in pineoblastoma ^{14, 15, 26}. We failed to obtain the positive staining of p53 in immunohistochemistry as shown in previous report ²⁷. Meanwhile, our staining result of NF was not relevant to previous reports. Jouvett et al reported that PPTIDs and pineoblastomas were less positive for NF, and positive immunostaining for NF was associated with a better survival ¹. In this study, immunoreactivity of NF was observed in all types of PPTs not associated with the histological grade, although we have to consider the limited number of the cases in our

study.

We also analyzed the expression of NeuN, which is a neuron-specific nuclear protein widely used in neurodevelopmental research and histopathologic diagnosis^{28, 29}. Immunohistochemically, NeuN was not detected in the nucleus of any PPTs, while some PPTIDs and pineoblastomas demonstrated cytoplasmic staining of NeuN in a dot-like manner (Fig. 2d). Although some previous reports suggest the cytoplasmic expression of NeuN, the functional significance has not been determined³⁰.

In conclusion, our immunohistochemical result of MGMT expression in the majority of PPTs let us endeavor to find the effective chemotherapeutic agents for high grade PPTs other than TMZ. In addition, the MIB-1 labeling index will help to reach to proper WHO grade of PPTs.

Figure Legends

Fig. 1 A representative case of pineocytoma. (Case 2)

a. Pineocytoma shows low to moderate cellularity and multiple pineocytomatous rosettes which are composed of abundant tumor cell processes. The nuclei of tumor cells are moderate-sized, and nucleoli are conspicuous. There are no mitotic figures or necroses. b. Some tumor cells show weak positivity for O⁶-methylguanine DNA methyltransferase (MGMT). c. Neurofilament protein (NF) highlights a few cell processes, although the majority of tumor cells were negative for NF. d. Tumor cells were negative for NeuN. e. Synaptophysin is strong positive in cytoplasm of tumor cells or pineocytomatous rosettes. a-e: x400

Fig. 2 A representative case of pineal parenchymal tumor of intermediate differentiation (PPTID). (Case 4)

a. PPTID is more cellular than pineocytoma and less cellular than pineoblastoma. The rosette-like formations were found, while the conspicuous pineocytomatous rosettes were lacking. Nuclei are smaller and denser than those in pineocytoma, and nuclear: cytoplasmic ratio is higher. There are no mitotic figures or necroses. b. Almost all tumor cells demonstrate intense positivity for MGMT. c. Some tumor cells show positivity for NF. d. Nuclear staining of NeuN is negative; however, cytoplasmic staining is observed in a dot-like manner. e. MIB-1 labeling index is 3.7 %. a-e: x400

Fig. 3 A representative case of pineoblastoma. (Case 10)

a. Pineoblastoma is composed of highly cellular, densely packed small cells, which is reminiscent of medulloblastoma. The tumor cells are highly nuclear: cytoplasmic ratio, hyperchromatic nuclei. b. The majority of tumor cells show positivity for MGMT. c. A few tumor cells are positive for NF. d. Tumor cells are negative for NeuN. e. MIB-1 labeling index is 12.7 %. a-e: x400

References

- 1 Jouvett A, Saint-Pierre G, Fauchon F, Privat K, Bouffett E, Ruchoux MM, *et al.* Pineal parenchymal tumors: a correlation of histological features with prognosis in 66 cases. *Brain Pathol.* 2000; **10**: 49-60.
- 2 Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (eds). WHO Classification of Tumours of the Central Nervous System, 4th Edition, IARC Press, Lyons, 2007.
- 3 Schild SE, Scheithauer BW, Schomberg PJ, Hook CC, Kelly PJ, Frick L, *et al.* Pineal parenchymal tumors. Clinical, pathologic, and therapeutic aspects. *Cancer.* 1993; **72**: 870-80.
- 4 Lutterbach J, Fauchon F, Schild SE, Chang SM, Pagenstecher A, Volk B, *et al.* Malignant pineal parenchymal tumors in adult patients: patterns of care and prognostic factors. *Neurosurgery.* 2002; **51**: 44-55; discussion 55-6.
- 5 Quirt I, Verma S, Petrella T, Bak K, Charette M. Temozolomide for the treatment of metastatic melanoma: a systematic review. *Oncologist.* 2007; **12**: 1114-23.
- 6 Ekeblad S, Sundin A, Janson ET, Welin S, Granberg D, Kindmark H, *et al.* Temozolomide as monotherapy is effective in treatment of advanced malignant neuroendocrine tumors. *Clin Cancer Res.* 2007; **13**: 2986-91.
- 7 Kulke MH, Stuart K, Enzinger PC, Ryan DP, Clark JW, Muzikansky A, *et al.* Phase II study of temozolomide and thalidomide in patients with metastatic neuroendocrine tumors. *J Clin Oncol.* 2006; **24**: 401-6.
- 8 Kouvaris JR, Miliadou A, Kouloulis VE, Kolokouris D, Balafouta MJ, Papacharalampous XN, *et al.* Phase II study of temozolomide and concomitant

- whole-brain radiotherapy in patients with brain metastases from solid tumors. *Onkologie*. 2007; **30**: 361-6.
- 9 Lim S, Shahinian H, Maya MM, Yong W, Heaney AP. Temozolomide: a novel treatment for pituitary carcinoma. *Lancet Oncol*. 2006; **7**: 518-20.
 - 10 Fadul CE, Kominsky AL, Meyer LP, Kingman LS, Kinlaw WB, Rhodes CH, *et al*. Long-term response of pituitary carcinoma to temozolomide. Report of two cases. *J Neurosurg*. 2006; **105**: 621-6.
 - 11 Mollemann M, Wolter M, Felsberg J, Collins VP, Reifenberger G. Frequent promoter hypermethylation and low expression of the MGMT gene in oligodendroglial tumors. *Int J Cancer*. 2005; **113**: 379-85.
 - 12 Levin N, Lavon I, Zelikovitsh B, Fuchs D, Bokstein F, Fellig Y, *et al*. Progressive low-grade oligodendrogliomas: response to temozolomide and correlation between genetic profile and O6-methylguanine DNA methyltransferase protein expression. *Cancer*. 2006; **106**: 1759-65.
 - 13 Chinot OL, Barrie M, Fuentes S, Eudes N, Lancelot S, Metellus P, *et al*. Correlation between O6-methylguanine-DNA methyltransferase and survival in inoperable newly diagnosed glioblastoma patients treated with neoadjuvant temozolomide. *J Clin Oncol*. 2007; **25**: 1470-5.
 - 14 Tsumanuma I, Tanaka R, Washiyama K. Clinicopathological study of pineal parenchymal tumors: correlation between histopathological features, proliferative potential, and prognosis. *Brain Tumor Pathol*. 1999; **16**: 61-8.
 - 15 Numoto RT. Pineal parenchymal tumors: cell differentiation and prognosis. *J Cancer Res Clin Oncol*. 1994; **120**: 683-90.
 - 16 Schild SE, Scheithauer BW, Haddock MG, Wong WW, Lyons MK, Marks LB, *et al*.

- Histologically confirmed pineal tumors and other germ cell tumors of the brain. *Cancer*. 1996; **78**: 2564-71.
- 17 Fauchon F, Jouvét A, Paquis P, Saint-Pierre G, Mottolese C, Ben Hassel M, *et al*. Parenchymal pineal tumors: a clinicopathological study of 76 cases. *Int J Radiat Oncol Biol Phys*. 2000; **46**: 959-68.
 - 18 Stoiber EM, Schaible B, Herfarth K, Schulz-Ertner D, Huber PE, Debus J, *et al*. Long term outcome of adolescent and adult patients with pineal parenchymal tumors treated with fractionated radiotherapy between 1982 and 2003--a single institution's experience. *Radiat Oncol*. 2010; **5**: 122.
 - 19 Lau Q, Scheithauer B, Kovacs K, Horvath E, Syro LV, Lloyd R. MGMT immunoexpression in aggressive pituitary adenoma and carcinoma. *Pituitary*. 2010; **13**: 367-79.
 - 20 Widhalm G, Wolfsberger S, Preusser M, Woehrer A, Kotter MR, Czech T, *et al*. O(6)-methylguanine DNA methyltransferase immunoexpression in nonfunctioning pituitary adenomas: are progressive tumors potential candidates for temozolomide treatment? *Cancer*. 2009; **115**: 1070-80.
 - 21 McCormack AI, McDonald KL, Gill AJ, Clark SJ, Burt MG, Campbell KA, *et al*. Low O6-methylguanine-DNA methyltransferase (MGMT) expression and response to temozolomide in aggressive pituitary tumours. *Clin Endocrinol (Oxf)*. 2009; **71**: 226-33.
 - 22 Kovacs K, Scheithauer BW, Lombardero M, McLendon RE, Syro LV, Uribe H, *et al*. MGMT immunoexpression predicts responsiveness of pituitary tumors to temozolomide therapy. *Acta Neuropathol*. 2008; **115**: 261-2.
 - 23 Gerson SL. Clinical relevance of MGMT in the treatment of cancer. *J Clin Oncol*. 2002; **20**: 2388-99.

- 24 Gerson SL. MGMT: its role in cancer aetiology and cancer therapeutics. *Nat Rev Cancer*. 2004; **4**: 296-307.
- 25 Jacinto FV, Esteller M. MGMT hypermethylation: a prognostic foe, a predictive friend. *DNA Repair (Amst)*. 2007; **6**: 1155-60.
- 26 Rickert CH, Simon R, Bergmann M, Dockhorn-Dworniczak B, Paulus W. Comparative genomic hybridization in pineal parenchymal tumors. *Genes Chromosomes Cancer*. 2001; **30**: 99-104.
- 27 Tsumanuma I, Sato M, Okazaki H, Tanaka R, Washiyama K, Kawasaki T, *et al*. The analysis of p53 tumor suppressor gene in pineal parenchymal tumors. *Noshuyo Byori*. 1995; **12**: 39-43.
- 28 Sarnat HB, Nochlin D, Born DE. Neuronal nuclear antigen (NeuN): a marker of neuronal maturation in early human fetal nervous system. *Brain Dev*. 1998; **20**: 88-94.
- 29 Englund C, Alvord EC, Jr., Folkerth RD, Silbergeld D, Born DE, Small R, *et al*. NeuN expression correlates with reduced mitotic index of neoplastic cells in central neurocytomas. *Neuropathol Appl Neurobiol*. 2005; **31**: 429-38.
- 30 Lind D, Franken S, Kappler J, Jankowski J, Schilling K. Characterization of the neuronal marker NeuN as a multiply phosphorylated antigen with discrete subcellular localization. *Journal of neuroscience research*. 2005; **79**: 295-302.

Figure 1 Case 2

Figure 2

Case 4

Figure 3

Case 10

Table 1. Summary of antibodies

Antibody	Clone	Type	Dilution	Antigen retrieval	Company
synaptophysin	SY38	mouse	1:100	Pressure cook (PC)	Dako
Neurofilament	2F11	mouse	1:50	none	Dako
NeuN	A60	mouse	1:100	PC	CHEMICON
p53	DO-7	mouse	1:100	PC	Dako
Ki-67	MIB-1	mouse	1:100	PC	Dako
MGMT	MT3.1	mouse	1:200	PC	CHEMICON

Table 2. Patient characteristics and histological findings

Case No.	Age (yr)	Sex	Histology	WHO grade	Mitotic index (/HPF)	necrosis	Immunoreactivity					
							MGMT	Synapto-physin	NF	NeuN	p53	MIB-1 Index (%)
1	24	F	Pineocytoma	I	0	-	0	3+	3+	0	0	0
2	59	F	Pineocytoma	I	0	-	1+	3+	2+	0	0	0
3	70	F	Pineocytoma	I	0	-	2+	3+	1+	0	0	0
4	32	M	PPTID	II	0	-	3+	3+	2+	2+	0	3.7
5	67	F	PPTID	III	0	-	3+	3+	0	1+	0	0.7
6	67	F	PPTID	II	0	-	3+	3+	2+	0	0	5.3
7	68	F	PPTID	II	0	-	3+	3+	1+	0	0	1.1
8	69	F	PPTID	II	0	-	3+	3+	1+	2+	0	6.4
9	71	F	PPTID	II	0	-	3+	3+	2+	1+	0	4.0
10	30	M	Pineoblastoma	IV	0	-	3+	3+	1+	0	0	12.7
11	36	F	Pineoblastoma	IV	3	-	3+	3+	2+	3+	0	10.5
12	49	M	Pineoblastoma	IV	1	+	3+	1+	2+	0	0	8.4

PPTID: Pineal parencymal tumor of intermediated differentiation
MGMT: O⁶-methylguanine DNA methyltransferase, NF: neurofilament protein

Table 3. Recent immunohistochemical studies of pineal parenchymal tumors

Authors	Histology	Total (N)	Summary
Numoto R (1994)	5 PCs, 2 mixed PC/PBs and 4 PBs	11	The MIB-1 labeling index correlated with histological malignancy. NF and synaptophysin positivity correlated with seeding potentials.
Tsumanuma I et al (1995)	5 PCs and 4 PBs	9	All cases were immunonegative for p53.
Tsumanuma I et al (1999)	4 PCs, 4 PCs with anaplasia and 5 PBs	13	The mean MIB-1 index of PBs was significantly higher than that of other types of PPT. The mean MIB-1 index of NF-positive cases was significantly lower than that of negative cases.
Jouvet A et al (2000)	11 PCs, 39 PPTIDs and 16 PBs	66	A number of mitoses greater than 6 and the presence of necrosis were associated with a poorer outcome, while positive immunostaining for NF was associated with a better survival.
Fauchon F et al (2000)	19 PCs, 28 PPTIDs and 29 PBs	76	The findings of the report of Jouvet A et al were confirmed.
Rickert CH et al (2001)	3 PCs, 3 PPTIDs and 3 PBs	9	The average MIB-1 indices were 1.3% for PCs, 10.1% for PPTIDs, and 27.2% for PBs.
Yamane Y et al (2002)	4 PCs, 5 PPTIDs and 14 PBs	23	The NF showed significant differences of reactivity among PC, PPTID and PB.
Arivazhagan A et al (2008)	6 PCs, 10 PPTIDs, 17 PBs	33	The mean MIB-1 labeling index in PCs, PPTIDs and PBs were 1.58, 16.1 and 23.52. NF immunoreactivity indicated better prognosis.
Marcol W et al (2009)	15 PCs, 1 PPTIDs and 11 PBs	27	The immunoreactivity of tubulin and NSE was correlated with patients' survival type while Bcl-2, p53 and nestin correlated negatively with survival time.
Fèvre-Montange M (2011)	2 PC, 27 PPTID and 4 PB	33	The number of mitosis and the mean MIB-1 labeling index were significantly different among tumor grades. The mean MIB-1 labeling index in PCs, PPTID grade II, PPTID grade III and PB were 0, 5.2, 11.2 and 36.4.

NSE: Neuron specific enolase