Evaluation of 06-methylguanine-DNA methyltransferase expression in children and adolescent pituitary adenoma

Xueming Shen

Capital Medical University Sanbo Brain Hospital

Yakun Yang

Capital Medical University Sanbo Brain Hospital

Zuocheng Yang

Capital Medical University Sanbo Brain Hospital

Ning Liu

Capital Medical University Sanbo Brain Hospital

Xueling Qi

Capital Medical University Sanbo Brain Hospital

Hongqi Xu

Department of Neurosurgery, The People's Hospital of Anyang City

Changxiang Yan

Capital Medical University Sanbo Brain Hospital

Song Han (✉ 13581776844@163.com)

Capital Medical University Sanbo Brain Hospital <https://orcid.org/0000-0001-7538-5185>

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Abstract

Background

Temozolomide can be used in the treatment of pituitary adenoma. Its efficacy can be evaluated by the expression of O6-methylguanine-DNA methyltransferase (MGMT). However, the previous study population was mainly adult. This study was the first to evaluate the expression of MGMT in children and adolescents with pituitary adenomas.

Methods

The clinical features and biological characteristics of 38 cases of pituitary adenoma in children and adolescents were analyzed retrospectively. The expression of MGMT, Ki-67, p53 was marked by immunohistochemical staining.

Results

In this cohort, the expression of MGMT protein is 16/38 (42.11%). The low expression rate of MGMT in children and adolescent somatotroph adenomas was only 11.11%, which was much lower than that of adults. Ki-67 expression range of 1% -10% [mean $4.24 \pm 2.71\%$]. The positive rate of p53 was 22/38 (57.89%). The statistical analysis showed that the expression of MGMT was related to the diameter of the tumor ($P=0.01$), the diameter of the tumor was large, and the expression of MGMT was high. The expression of MGMT was not associated with age, sex, tumor type, invasiveness, texture, apoplexy, recurrence, and expression of p53 and Ki-67.

Conclusion

Somatotroph adenomas, large-diameter children and adolescent pituitary adenomas may be not suitable for temozolomide treatment. To determine whether temozolomide responds to salvage therapy in children and adolescents with pituitary adenomas, MGMT expression should be assessed in all pituitary adenomas, the time span between specimen collection and temozolomide treatment needs to be considered because of the instability of MGMT protein expression.

Background

Pituitary adenomas (PA) are uncommon in children and adolescents, accounting for < 3% of childhood supratentorial tumors and between 3–6% of all surgically treated adenomas [1]. PA has a serious impact on the growth, development, vision, and future fertility of the patient, and may be secondary to cardiovascular events [2–5]. Like adults, pituitary adenomas in children and adolescents are also treated differently depending on the type of tumor. For example, lactotroph adenomas are preferentially treated with dopamine agonists [6, 7]; corticotroph adenomas and somatotroph adenomas are treated surgically [3, 8, 9]. Compared with adults, invasive PAs are more common in children and adolescents, furthermore, the incidence of apoplexy is also higher [4, 10]. Although a significant progress has been made in the

medical management of pituitary adenomas over the past decades, the invasive tumors are difficult to manage and are associated with poor prognosis and fatality, the treatment of these tumors still remains challenging, as conventional chemotherapy exhibits low response rates, whereas radiotherapy fails to control tumor growth and may be associated with considerable side effects[11, 12].

Temozolomide (TMZ), a routine chemotherapy for glioblastoma (GBM), is an imidazotetrazine derivative, oral cytotoxic chemotherapeutic agent that inhibits DNA replication. For aggressive PA, pituitary carcinoma and atypical pituitary adenomas (APA) (WHO 2004 classification, the WHO 2017 classification delete diagnosis[13, 14]) treatment in adults could choose temozolomide[15–18]. This drug has currently become a therapeutic option for aggressive PA, which has been shown to reduce tumor volume and reduce the pituitary hormone levels. The cytotoxic activity of temozolomide depends on the expression of O-6 methylguanine DNA transferase (MGMT), a DNA repair enzyme present in tumor cells that could lead to the inactivation of TMZ. The lower expression of MGMT is reportedly predictive of responsiveness to TMZ[19, 20]. And several studies report that MGMT expression is associated with PA patients' prognoses [21].

However, the use of temozolomide has not been reported in children and adolescents. The efficacy of temozolomide can be evaluated using MGMT protein expression levels [22, 23]. In addition, the expression of MGMT has a positive effect on prognosis [12, 24]. Therefore, this study detected the expression of MGMT protein in PA tissue samples of children and adolescents, combined with some biological characteristics and molecular indicators for a comprehensive evaluation.

Methods

This study was a single-center study of 38 patients with surgically-treated PA, admitted to the Sanbo Brain Hospital, Capital Medical University, from June 2012 to September 2017. The age range of reported PA in adolescents is 18–21 years [2, 9, 25–27]. Patients 20 years or younger with PA were included in this study. The ethics committee of Sanbo Brain Hospital approved the study. Written informed consents were obtained from all the patients involved in the study.

Immunohistochemistry (IHC)

Tumor specimens were fixed in 10% formaldehyde and embedded in paraffin for histological sectioning. Paraffin-embedded sections were deparaffinized in xylene and dehydrated in graded alcohol. Immunohistochemistry studies for the MGMT protein were performed using a mouse monoclonal antibody (1:10) (clone UMAB56; ZSGB-BIO). In addition, we screened for other molecular markers, including Ki-67 (1:200) (clone Mib1; ZSGB-BIO) and p53 (1:300) (clone BP53.12; ZSGB-BIO). Antigen retrieval was performed in citrate buffer at pH 9.0. The sections were incubated overnight at 4°C with the primary antibody. Following this, the sections were rinsed with 1X phosphate buffered saline (PBS) and incubated with the horseradish peroxidase conjugated secondary antibody, followed by a rinse in PBS, incubation with 3,3'-diaminobenzidine staining and counterstaining with hematoxylin blue. The negative

control sections were treated using the same parameters except that PBS without antibody was used for the incubation steps.

Brown-yellow staining of nuclei was defined as MGMT positive expression. Five cell-dense high-power fields (200X magnification) were imaged using Leica microscope, and the number of MGMT positive cells of a total of 500 tumor cells were counted. Using semi-quantitative method, MGMT immunoexpression was scored using a 4-tiered scale (1 = negative or limited to < 10%, 2 = 10–25%, 3 = 25–50%, 4 = > 50%). Scores 1 and 2 were combined to form the category of low level MGMT expression; scores 3 and 4 represented intermediate and high MGMT expression, respectively [12, 28]. The Ki-67 index was defined as the proportion of MIB-1 positive tumor nuclei out of 500 total cells. The p53 labeling index was determined by the percentage of positively stained nuclei.

Tumor Invasive Capacity and Texture

The invasion of suprasellar and saddle base was determined according to the Hardy classification. Invasive PA was determined by III, IV or C, D, E on the Hardy scale [29]. Parasellar invasion was determined using the Knosp grading and grades Ⅲ and Ⅳ were defined as invasive PA [30]. Based on the Mahmoud classification method [31], the tumors were divided into three groups including a. soft texture group: a tumor that is easily suctioned with an aspirator, b. medium texture group: a tumor that is hard to remove with an aspirator, and c. tough texture group: a tumor that cannot be suctioned with an aspirator, and for which bipolar electrocoagulation or sharp segmentation resection is needed.

Statistical Analysis

SPSS 21.0 was used to analyze the data. The measurement data were expressed as the mean $\pm$ SD (standard deviation). The Student's t-test was used to compare the mean difference between two measurement data. The relationship between MGMT expression and age, sex, tumor diameter, type, texture, p53, Ki-67, apoplexy and recurrence were analyzed using the chi square test or logistic regression. Bilateral $p < 0.05$ for the difference was statistically significant.

Results

Sample Characteristics

The patient cohort included 38 cases, aged 12–20 years (17.67 ± 1.63 years), of which 23 were males and 15 were females, accounting for 3.5% of the 1073 surgically treated pituitary patients in the same period. The clinical manifestations are: headache, menstrual disorder, visual impairment, accelerated development, galactorrhea, growth retardation, gynaecomastia, etc. (Table 1). Of these, 13 were invasive PA cases and 12 were cases of apoplexy (31.58%). The texture of the tumor was soft in 30 cases (78.95%), moderate in three cases (7.89%), and tough in five cases (13.16%).

Table 1
Summary of the clinical characteristics of 38 patients

Characteristics	number	%
Age		
Mean	17.67 ± 1.63	/
Group		
12–14	5	13.16
15–20	33	86.84
Gender		
Female	15	39.47
Male	23	60.53
Presenting symptom		
Headache	17	44.74
Menstrual disorders	12	31.58
Visual impairment	11	28.95
Accelerated development	4	10.53
Galactorrhea	4	10.53
Growth retardation	3	7.89
Gynaecomastia	2	5.26
Max tumor dimension		
Microadenoma	4	10.53
Macroadenoma	15	39.47
Giant adenoma	19	50.00
Extent of adenoma		
Purely intrasellar	9	23.68
Suprasellar extension	18	47.37
Cavernous sinus invasion	8	21.05
Third ventricle invasion	2	5.26
Anterior cranial fossa extension	1	2.63

Immunohistochemical Analysis

Of the 38 specimens, 15 were negative for MGMT expression, one was positive for expression less than 10%, three were positive for 25–50%, and 19 were showed an expression greater than 50%. Therefore, 16 specimens (42.11%) displayed a low expression of MGMT. Ki-67 expression ranged between 1% and 10% (mean $4.24 \pm 2.71\%$). Considering a Ki-67 expression greater than 3% as the diagnostic criteria of the original atypical pituitary adenoma, the tumors were divided into low and high expression groups. Low MGMT expression was observed in 5/18 (27.78%) and 11/20 (55.00%) specimens in the Ki-67 high and low expression groups, respectively. Alternately, low MGMT expression was observed in 5/12 (41.67%) and 6/13 (46.15%) specimens in the apoplexy and the invasive group, respectively. In the recurrent group, 2/6 (33.33%) specimens showed low MGMT expression. p53 positive tumors were found in 22/38 (57.89%) patients.

MGMT Expression Instability

Two patients in this cohort were treated with two surgeries each in our center, and MGMT protein expression was detected in the specimens. In these two patients, one of them was a 12-year-old boy with growth hormone pituitary adenoma. The first transsphenoidal surgery failed to completely remove the tumor, so the tumor recurred 11 months after the surgery, and the tumor was finally completely removed by pterional approach. However, MGMT expression was 95% (high expression), Ki-67 was 4% and p53 was negative in the sample in first operation (Fig. 1C) and 10% (low expression), Ki-67 was 2% and p53 also was negative in the second sample in the second surgery (Fig. 1F). In the other patient with nonfunctioning pituitary adenoma, after the first transsphenoidal subtotal resection of pituitary adenoma, the second transsphenoidal resection was performed 10 months later due to tumor recurrence. The first specimen was negative and the second specimen was 60% positive for MGMT (high expression). Ki-67 and p53 were expressed relatively stably (Fig. 1A, B, D, **and E**).

MGMT and Tumor Biological and Molecular Characterization

The Chi-square test or logistic regression were used to analyze the relationship between the expression of MGMT and age, gender, tumor biological characteristics such as diameter, invasiveness, texture, apoplexy, Ki-67, and p53 expression. A significant correlation was identified between the tumor diameter and MGMT expression ($B = 2.56$, $SE = 0.99$, $P = 0.01$, $\text{Exp}(B) = 12.89$). The larger the tumor diameter, the higher the MGMT expression. The remaining indicators did not show a correlation (Table 2).

Table 2
Correlation of MGMT expression with clinical and biological characteristics.

Variables	MGMT immuno-expression		
	low expression	high expression	p value
Age (mean $\pm$ SD) (years)	17.50 $\pm$ 1.79	16.77 $\pm$ 2.56	
Maximum diameter (mean $\pm$ SD) (mm)	24.88 $\pm$ 14.32	29.64 $\pm$ 15.16	
Age group (years)			0.37
12–14	1	4	
15–20	15	18	
Gender			0.92
Male	9	12	
Female	7	10	
Diameter(mm)			0.01
≤10 mm	4	0	
10–40 mm	10	15	
≥40 mm	2	7	
Invasiveness			0.72
Yes	6	7	
No	10	15	
Type			0.09
Silent adenoma	3	3	
Lactotroph adenomas	11	10	
Corticotroph adenomas	0	1	
Somatotroph adenomas	1	8	
Plurihormonal adenomas	1	0	
Texture			0.48
Soft	14	16	
Medium	1	2	
Tough	1	4	

Variables	MGMT immuno-expression	
P53	0.58	
Positive	8	13
Negative	8	9
Ki-67	0.09	
>3%	5	13
≤3%	11	9
Apoplexy	0.97	
Yes	5	7
No	11	15
Recurrence	0.98	
Yes	2	4
No	14	18

MGMT and Prognosis

The cohort was followed up from 3 months to 65 months (mean 34.84 ± 20.81 months). Among them, eight cases relapsed and the recurrence time ranged from 10 months to 52 months (mean 25.25 ± 17.09 months). Among the recurrence cases, four showed low MGMT expression (50.00%). In the non-relapsed cases, 12/30 (40.00%) showed a low MGMT expression. Statistical analysis did not show a correlation between tumor recurrence and MGMT expression ($p = 0.87$).

Discussion

PAs are very rare in children and account for about 3% of intracranial tumors [1]. The incidence of PA increases in adolescence, but is still a relatively rare tumor [32]. In this group of 38 cases, five were children and 33 were adolescents, suggesting that PA incidence may be related to changes in adolescent hormone levels. Biological characteristics of PAs in children and adolescents include: high rates of functional PAs [25], high rates of invasive PAs [33], and high rates of apoplexy [10]. In adults, temozolomide may be used for aggressive PA, PCs, and APAs [15–18]. In addition, bromocriptine resistance in patients with temozolomide treatment is equally effective [34, 35]. The efficacy of temozolomide can be evaluated using MGMT protein expression levels [22, 23]. In addition, the expression of MGMT has a positive effect on prognosis [12, 24]. The assessment of MGMT expression in PAs in children and adolescents has not previously been studied.

Immunohistochemical detection of MGMT protein expression is relatively easy in paraffin-embedded tissue. Low MGMT expression predicts positive response (tumor inhibition) to temozolomide [23]. In this study, the expression of MGMT protein was detected in 38 cases of PA in children and adolescents, and 16/38 (42.11%) of these showed low MGMT expression. A previous study reported low MGMT expression in 26% of functional PA in a cohort of adult-dominated patients [24]. Low MGMT expression was also reported in prolactinoma (78-85.71%) [36, 37], corticotroph adenomas (62.5-81.48%) [37, 38], and somatotroph adenomas (90.0-91.7%) [37, 39]. In the current study, the functional PAs were 32/38 (84.21%), of which 13/32 (34.21%) showed low MGMT expression. Low MGMT expression was seen in 11/22 (50.00%) prolactinomas, 1/9 (11.11%) somatotroph adenomas, and only one case of corticotroph adenoma. Overall, low MGMT expressing somatotroph adenomas are not as common in children and adolescents compared to adults. Therefore, this type of pituitary adenoma may not be suitable for temozolomide treatment.

The proportion of invasive PAs in this cohort was 13/38 (34.21%). Although there was no correlation between MGMT expression and invasiveness ($p > 0.05$), 6/13 (46.15%) showed low MGMT expression and may be suitable candidates for treatment with temozolomide. In adult-dominated PAs, the correlation between MGMT expression and invasiveness is reported inconsistently. Some studies suggest a correlation between low to moderate MGMT and increased invasiveness [24], while others do not support the same correlation [37, 40]. To predict the likelihood of temozolomide response, all invasive pituitary tumors should be evaluated for MGMT expression. Interestingly, the study found that the diameter of the tumor was related to the expression of MGMT ($p = 0.01$). The larger the tumor, the higher would be the MGMT expression, 100% of microadenomas showed low MGMT expression. However, only 25% and 22.2% of macro and giant adenomas showed low MGMT expression, respectively. These results suggest that macroadenoma and giant adenoma are not suitable for temozolomide treatment.

Ki-67, a nuclear antigen, is a proliferation marker in different neoplastic lesions. In this cohort, there was no correlation between MGMT expression and Ki-67 expression ($p = 0.09$). Inactivation of p53 is known to be involved in aberrant cell proliferation. p53 expression is a prognostic indicator of various primary human tumors. Therefore, immunohistochemistry was performed to detect the expression of p53 in pituitary adenomas. In this cohort, most of the samples were positive for p53 expression; however, there was no correlation between p53 expression and MGMT expression ($p = 0.58$).

The expression level of MGMT in pituitary tumor tissue may change over time, but Lau Q et al reported stable MGMT expression in most primary and recurrent tumor samples [28]. Radiation therapy has a significant impact on the level of MGMT expression in tumors. Among the two patients, one was a somatotroph adenoma, while the other was a non-functioning PA. No radiation therapy was applied. The two cases were treated with prednisone replacement therapy, and one was treated with desmopressin acetate tablets for diabetes insipidus. It remains unclear if the change of MGMT expression is related to surgery, drug treatment, or other unknown factors. Further studies are warranted to understand the differential expression of MGMT, particularly in cases undergoing surgical interventions.

Conclusions

In this article, the MGMT expression of PAs in children and adolescents were evaluated for the first time. Somatotroph adenomas and large-diameter PAs, both of which not have low MGMT expression, may not be suitable for temozolomide treatment. In the context of PAs in children and adolescents, the measurement of MGMT expression could serve as an indicator for choice of temozolomide as a treatment option.

Declarations

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved Ethics Committee of Sanbo Brain Hospital. The patients/participants provided their written informed consent to participate in this study. For participants aged under 16, their parents or legal guardians provided written informed consent for their participation in the study.

Consent for publication

Written, informed consent was obtained from the individual(s) AND/OR minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Availability of data and materials

The clinical datasets generated during and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Competing interests

The authors declared that they have no conflicts of interest to this work.

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Authors' contributions

CXY and SH conceived and designed the idea, XMS, YKY and ZCY did data collection and wrote the manuscript. YKY and ZCY did data collection and performed the data analysis. NL, XLQ and HQX did literature review and reviewed the manuscript. CXY, XMS, SH, ZCY, NL, XLQ and HQX contributed to the reviewing of the final manuscript. All authors approved the final format of the submitted manuscript.

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41. Fgure, Legend.

Figures

Figure 1

The patient was a male child with pituitary adenoma and his parents had written consent for the publication of potentially identifiable information. The first operation began at 12 years of age and was treated again at 1 year interval. All the surgical specimens were examined by MGMT, Ki-67 and p53. The results showed that Ki-67 was stably expressed in 3-5% (A, D); p53 was negatively expressed (B, E), but the expression of MGMT was not stability, 95% (C) for the first time and 10% (F) for the second time.