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ORIGINAL ARTICLE

Low O^6-methylguanine-DNA methyltransferase (MGMT) expression and response to temozolomide in aggressive pituitary tumours

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Summary

Context Recent case reports detail the successful use of temozolomide in the management of aggressive pituitary tumours. O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that counteracts the effect of temozolomide.

Objective To study MGMT expression in pituitary tumours and consider whether MGMT expression is associated with response to temozolomide therapy in aggressive pituitary tumours.

Patients We report two patients with aggressive pituitary tumours treated with temozolomide, one who responded to temozolomide and the other who did not. MGMT expression was assessed in a further 88 archived pituitary tumour samples.

Design MGMT expression was assessed by immunohistochemistry. *MGMT* promoter methylation was studied by methylation-specific polymerase chain reaction (MSP), sequencing of *MGMT* was performed and loss of heterozygosity (LOH) analysis undertaken.

Results Low MGMT expression and *MGMT* promoter methylation were found in the pituitary tumour of the patient who responded to temozolomide. Conversely, high MGMT expression was seen in the patient demonstrating a poor response to temozolomide. Eleven out of 88 archived tumour samples (13%) had low MGMT expression. Prolactinomas were more likely to have low MGMT expression compared with other pituitary tumour subtypes ($P < 0{\cdot}001$). There was no significant difference in MGMT expression between invasive and noninvasive tumours, or between recurrent and nonrecurrent tumours. A significant inverse correlation was found between MGMT expression and promoter methylation ($P = 0{\cdot}012$).

Conclusion MGMT expression as assessed by immunohistochemistry may predict response to temozolomide therapy in patients with aggressive pituitary tumours. *MGMT* promoter methylation is likely to explain low MGMT expression in some, but not all, pituitary tumours.

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Introduction

Clinically significant pituitary tumours occur in approximately 1 in every 1000 individuals.[1] The majority of pituitary tumours are benign adenomas. However, between 35% and 55% of adenomas demonstrate invasion into bone, dura or adjacent structures such as the cavernous or sphenoid sinuses or brain.[2,3] Although it is a rare phenomenon, a subset of invasive adenomas display aggressive behaviour and become resistant to medical therapy, causing substantial morbidity; these tumours require multiple operations and radiotherapy in an attempt to control tumour growth. Pituitary carcinoma is defined by the presence of craniospinal and/or systemic metastases.[3] It occurs in only 0·2% of resected pituitary tumours and the average survival is less than 4 years.[3,4]

Various chemotherapeutic regimes have been tried in the management of pituitary carcinoma. Although occasional temporary responses are reported, the results are usually disappointing.[5] Recent case studies have successfully used temozolomide, an alkylating chemotherapeutic drug, in the management of pituitary carcinoma and aggressive pituitary tumours.[6–10] Temozolomide is widely used in the management of glioblastoma multiforme and is effective in

other neuro-oncological tumours as well as other neuroendocrine tumours.[11–14] Temozolomide is administered orally, readily crosses the blood–brain barrier and is not cell-cycle specific, advantageous when treating relatively slow-growing pituitary tumours.[6]

O^6-methylguanine-DNA methyltransferase (MGMT) is a DNA repair protein that reverses alkylation at the O^6 position of guanine. As such, MGMT counteracts the effect of temozolomide, which alkylates DNA at this position.[15] Low tumour MGMT expression has been shown in some studies to correlate with temozolomide response and increased survival in patients with brain tumours.[16–18] A commonly proposed mechanism of reduced MGMT expression is methylation of its promoter, although different tumour types vary widely in the frequency of methylation.[19] In general, studies of gliomas have shown a correlation between *MGMT* promoter methylation and temozolomide response, suggesting that, for this tumour type at least, methylation is a key event in down-regulating MGMT expression.[11,12] Kovacs *et al.*[20] recently assessed MGMT immunohistochemistry in two patients with aggressive pituitary tumours treated with temozolomide. Low expression was demonstrated in the patient who responded to temozolomide whereas high expression was seen in the patient with no response to this agent.[20]

We report two cases of aggressive pituitary tumours, one in which a positive clinical response to temozolomide was associated with low tumour MGMT expression and methylation of its gene promoter, and the other in which temozolomide resistance occurred with high tumour MGMT expression. We extended our findings to show, for the first time, the spectrum of MGMT immunostaining across a large cohort of human pituitary tumours. Low MGMT expression was significantly more common in prolactinomas than in other tumour subtypes but there was no association between MGMT expression and clinical parameters of invasiveness. We also found a significant correlation between *MGMT* promoter methylation and MGMT expression, although methylation status alone could not fully explain the variation in MGMT expression.

Patients and methods

Patients

The two patients with aggressive pituitary tumours were identified and treated with temozolomide at Flinders Medical Centre, Adelaide, Australia (case 1) and St Bartholomew's Hospital, London, UK (case 2), respectively. Paraffin-embedded tumour tissue was obtained to undertake MGMT immunohistochemistry. In addition to this, case 1 had DNA from both tumour and peripheral blood leucocytes available for methylation analysis, *MGMT* sequencing and loss of heterozygosity (LOH) analysis.

Pituitary tumour cohort

A further 121 patients who had undergone pituitary surgery between 1990 and 2006 and had sufficient archived paraffin tissue in the Department of Anatomical Pathology, Royal North Shore Hospital, Sydney, Australia were selected. All 121 patient tumour samples underwent histopathological review and MGMT immunohistochemistry. MGMT immunohistochemistry was considered informative only if a strong internal positive control was present (see below). On this basis, 22 noninformative cases were excluded from the statistical analysis. A further eight samples were excluded due to the presence of < 80% tumour tissue in the pathological tumour sample (minimizing contamination by normal tissue for the purposes of DNA studies) or lack of available clinical data. In three patients, histopathological review demonstrated only non-neoplastic anterior pituitary tissue in the archived tissue. These were then classified as 'normal pituitary'. In addition, four autopsy pituitary samples, confirmed normal by routine microscopy, were included as controls.

Thus, 88 patient tumour samples comprised the study cohort (Table 1). Both functional and nonfunctional tumour subtypes were represented: functional pituitary tumours had clinical and biochemical evidence of hormonal hypersecretion. The cohort also included both invasive and noninvasive tumours, and recurrent tumours. Invasive tumours were defined on the basis of sphenoid sinus and/or cavernous sinus invasion described on computed tomography (CT)/magnetic resonance imaging (MRI) reports or the operative report. Tumours were graded according to the modified Hardy classification.[21] Grade 1 tumours were microadenomas (< 1 cm diameter). Grade 2 tumours consisted of macroadenomas (> 1 cm diameter) with or without suprasellar extension. Grade 3 tumours demonstrated local invasion of sphenoid sinus and/or cavernous sinuses. Grade 4 tumours had evidence of central nervous system/extracranial spread on CT/MRI with or without distant metastases. Grade 3 and 4 tumours were classified as invasive. Based on this classification, there were 42 noninvasive pituitary tumours (10 Grade 1, 32 Grade 2) and 46 invasive tumours (44 Grade 3, 2 Grade 4). Tumour samples obtained from a repeat surgical treatment were classified as recurrent.

Methylation analysis of the *MGMT* promoter was performed on 63 tumour samples. All 11 tumours with low expression of MGMT by immunohistochemistry were selected for analysis and a random sample of other tumours was also studied.

Table 1. Pituitary tumour cohort ($n = 88$)

	Invasive ($n = 46$)	Noninvasive ($n = 42$)	Recurrent ($n = 12$)
Nonfunctioning adenomas ($n = 42$)	25	17	8
GH-secreting tumours ($n = 24$)	11	13	1
PRL-secreting tumours ($n = 14$)	7	7	2
ACTH-secreting tumours ($n = 6$)	1	5	1
FSH-secreting tumours ($n = 2$)	2	0	0

Sequencing of *MGMT* was performed in 21 tumours, including all 11 with low MGMT expression, and a representative sample of intermediate and high expressing tumours. LOH analysis of 10q26, encompassing *MGMT*, was performed in those tumours for which DNA from peripheral blood leucocytes was available, and comprised one patient with low MGMT expression and four other patients with intermediate or high MGMT expression.

The study was approved by the Northern Sydney Central Coast Area Health Human Research Ethics Committee.

Immunohistochemistry

Immunohistochemistry for MGMT was performed on formalin-fixed paraffin-embedded tissue using a mouse monoclonal antibody (Clone MT23·2, Cat: MA3-16537, Affinity Bioreagents, CO, USA). Slides were stained using the Vision Biosystems bondMax autostainer (Vision Biosystems, Mount Waverley, Victoria, Australia) using a biotin-free detection system in accordance with the manufacturer's protocol (further details available from the authors upon request).

External positive and negative controls (tonsillar tissue with areas of known positive and negative staining) were examined with each batch of stains. In addition, endothelial cells and lymphocytes acted as internal positive controls. Only slides in which these internal positive controls stained positive were considered informative. Twenty-two cases were excluded in which these internal controls and tumour cells consistently stained negatively despite repeated staining and variations in antigen retrieval and incubation protocols. Slides were examined by a single observer (A.G.) in conjunction with a haematoxylin and eosin stained section. The observer was blinded as to the clinical and molecular data including the hormone production status. Cytoplasmic staining was considered nonspecific and only nuclear staining was evaluated. Slides were scored semiquantitatively. Low MGMT expression was defined as absent or focal (< 10%) staining of tumour nuclei, intermediate expression as 10–90% of tumour nuclei positive and high expression as diffuse positive staining of more than 90% of tumour nuclei regardless of intensity.

DNA extraction

DNA was extracted from the same tumour block used to cut slides for MGMT immunohistochemistry. Paraffin-embedded tumour tissue was subjected to prolonged proteinase K digestion (4 days) as described previously.[22] For LOH analysis, DNA was extracted from fresh-frozen pituitary tumour tissue (15–50 mg) using TRIzol reagent according to the manufacturer's protocol (Invitrogen, California, USA). Only paraffin tumour tissue was available for LOH analysis from case 1. DNA from peripheral blood leucocytes was obtained for purposes of comparison in the LOH studies and was extracted using commercially available reagents (Gentra Puregene DNA Purification Kit, Qiagen, Victoria, Australia).

Methylation analysis

Methylation of the *MGMT* promoter was determined by the method of methylation-specific polymerase chain reaction (MSP), using previously described primers in a nested, two-stage PCR approach.[19,23] Tumour DNA (500 ng) was first subjected to bisulfite modification (EZ-96 DNA Methylation Kit, Zymo Research, California, USA). We determined that the sensitivity of this method was at least one methylated allele in every 100 unmethylated alleles by studying serial dilutions of commercially available methylated and unmethylated controls (methylated control, Chemicon, USA; unmethylated control, Roche Diagnostics, Australia). Normal human pituitary tissue was also subjected to methylation analysis. Reaction conditions available from authors upon request.

Mutation analysis

Exons 1–5 of *MGMT* were sequenced. Exon 1 is contained within the promoter region and is noncoding. Primer sequences and reaction details are available from authors upon request. Sequencing was performed by Sydney University Prince Alfred Macromolecular Analysis Centre (SUPAMAC) using the ABI PRISM 3700 platform (Applied Biosystems, Australia).

Detection of LOH

Five microsatellite markers were chosen at 10q26, spanning a region flanking *MGMT* (D10S2465, D10S1655, D10S217, D10S1651, D10S1483). PCR amplification was carried out on tumour and leucocyte DNA using fluorescent tagged primers. Reaction conditions are provided in supplemental material. Marker results were classified as noninformative if heterozygosity was not found in leucocyte DNA. LOH was identified by a reduction in allele band density of at least 50% or the absence of an expected allelic band, and confirmed by Density Subtraction-Scan (DS-Scan) in visually difficult cases.

Statistical analysis

Pearson χ^2 and linear-by-linear association tests (SPSS version 14·0; SPSS Inc., Chicago, IL, USA) were used to examine the association between: (1) MGMT expression assessed by immunohistochemistry and either pituitary tumour subtype or invasiveness; and (2) MGMT expression and MSP results.

Results

Case report 1

A 42-year-old man presented in 1985 with bitemporal visual field loss, hypogonadotrophic hypogonadism and hyperprolactinaemia with a pituitary macroadenoma demonstrated on CT scanning. Bromocriptine was given in high doses but prolactin levels remained elevated despite radiological evidence of tumour regression and normalization of visual fields. In 1995, transsphenoidal hypophysectomy and postoperative radiotherapy was performed because of visual deterioration and headaches with marked tumour growth, chiasmal compression and evidence of cavernous sinus invasion on MRI. Histology confirmed a pituitary adenoma with positive immunohistochemical staining for prolactin and a few mitotic figures were noted. Over the next 5 years there was some tumour

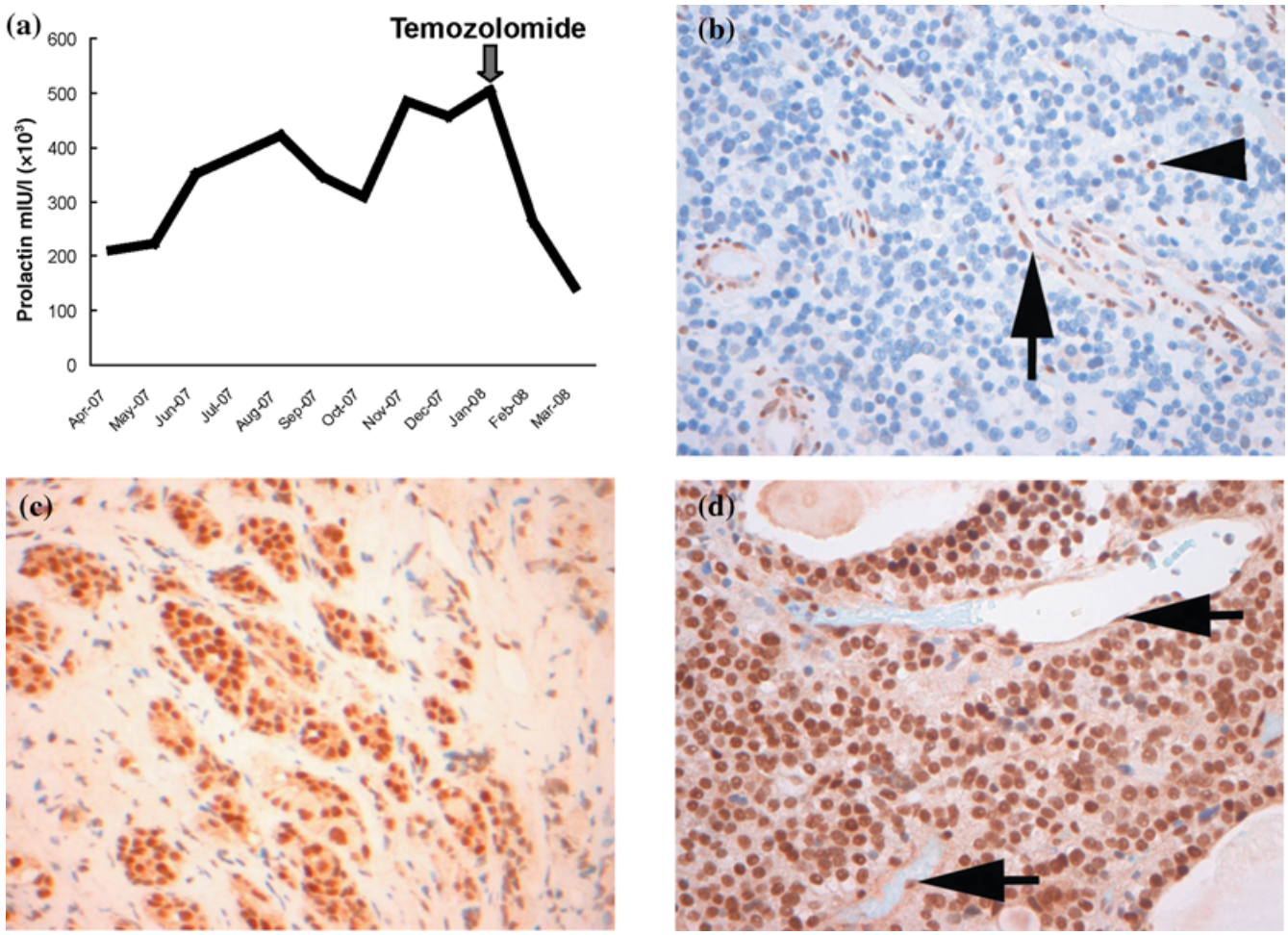

Fig. 1 (a) Serum prolactin concentrations in case 1 before and after commencement of temozolomide therapy (arrowed). (b) MGMT immunohistochemistry (IHC): case 1 pituitary tumour shows completely negative nuclear staining for MGMT. The endothelial cells (arrow) and lymphocytes (arrowhead) act as internal positive controls (original magnification 400×). (c) MGMT IHC: case 1 adjacent non-neoplastic pituitary tissue shows strong nuclear staining for MGMT. Cytoplasmic staining is considered nonspecific (original magnification 400×). (d) MGMT IHC: case 2 pituitary tumour shows diffuse strong nuclear staining for MGMT with positive staining in endothelial cells (arrows) (original magnification 400×).

regression and hormonal response. However, in 2000 a metastatic lesion in the cerebellum was noted on MRI (later resected and confirmed histologically) and pituitary tumour growth was apparent. Pituitary carcinoma was therefore diagnosed. By 2007, several other intracranial metastatic deposits had developed as well as multiple vertebral and pelvic bony metastases. There was marked clinical deterioration with severe headaches, progressive visual loss and gait disturbance. Between 2002 and 2007, five surgeries, repeated radiotherapy and the use of cabergoline therapy each produced only transient responses. Octreotide was trailed briefly without effect. Serum prolactin concentrations increased to a peak of 504 602 mIU/l (normal range < 360 mIU/l) in early 2008 (Fig. 1a). In January 2008, temozolomide was commenced at 200 mg/m^2 daily for five consecutive days every 28 days, and was well tolerated. Prolactin levels decreased progressively (Fig. 1a), and last measured 90 863 mIU/l. Furthermore, significant tumour shrinkage was demonstrated on an MRI performed 4 months after commencing temozolomide together with marked clinical improvement, including some reversal of visual field loss and a return to independent living.

We performed MGMT immunohistochemistry on tissue obtained from pituitary resection in 2007. This revealed absent nuclear staining in tumour tissue but strong nuclear staining in endothelial cells, which acted as a positive internal control (Fig. 1b). Strong nuclear staining was also seen in adjacent non-neoplastic tissue (Fig. 1c).

Case report 2

A 48-year-old man presented in 2000 with acromegaly associated with a large pituitary tumour. He initially underwent transcranial hypophysectomy because of massive extrasellar extension. However, because of persisting tumour and active acromegaly, he subsequently underwent a further two transsphenoidal operations followed by external beam radiotherapy in 2002 and medical therapy with octreotide Long-Acting Release (LAR) 30 mg monthly. In early 2004 he developed a sudden left temporal visual field loss associated with massive tumour regrowth, and despite further surgery he lost all vision in the left eye. In 2006, he was referred to St Bartholomew's

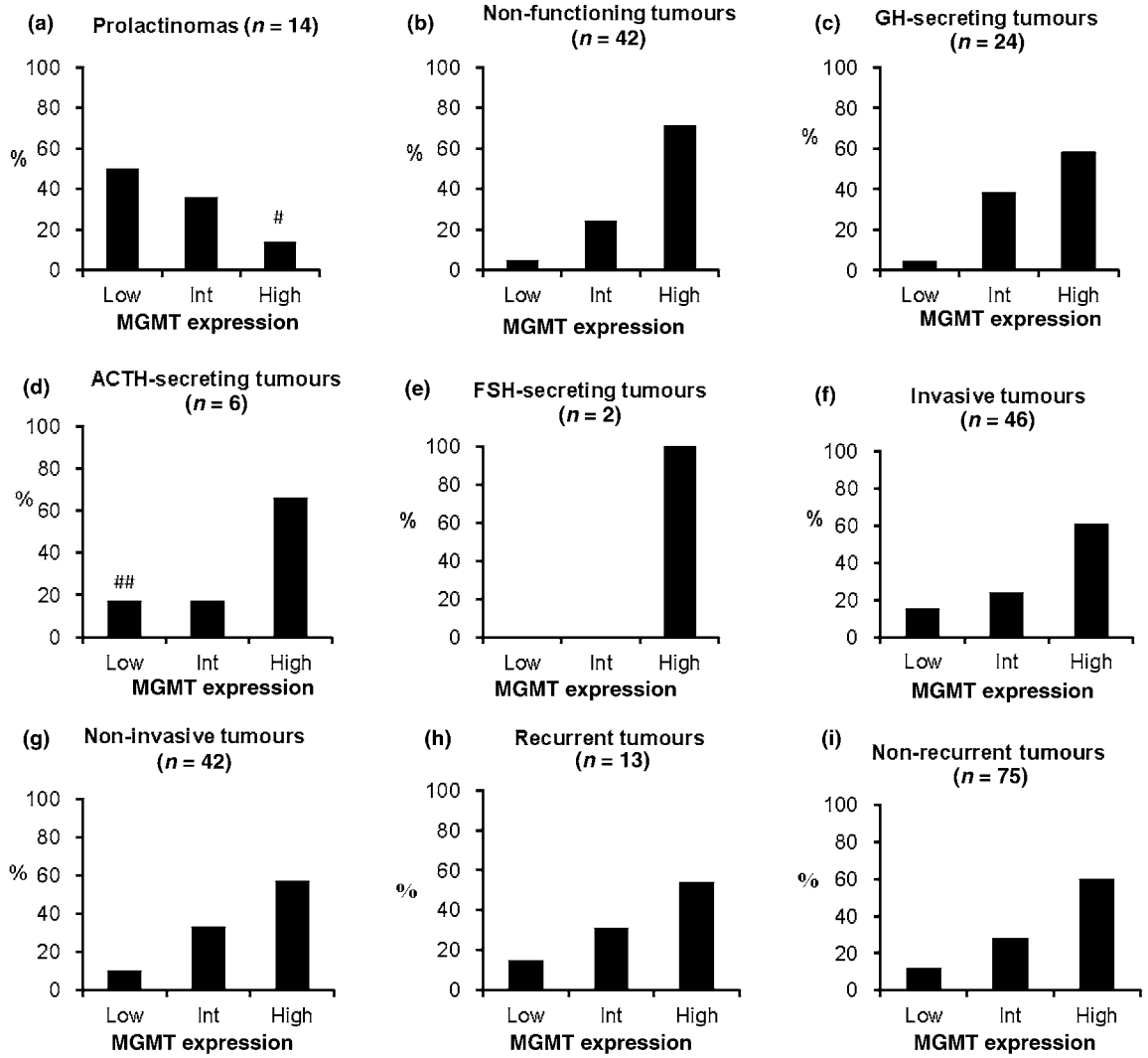

Fig. 2 MGMT immunohistochemistry: (a) prolactinomas; (b) non-functioning pituitary tumours; (c) GH-secreting tumours; (d) ACTH-secreting tumours; (e) FSH-secreting tumours; (f) invasive tumours; (g) noninvasive tumours; (h) recurrent tumours; (i) nonrecurrent tumours. Prolactinomas *vs.* other pituitary tumour subtypes, $P < 0{\cdot}001$; invasive *vs.* noninvasive tumours, $P = 0{\cdot}553$; recurrent *vs.* nonrecurrent tumours, $P = 0{\cdot}75$. # Includes a malignant prolactinoma. ## Includes a malignant ACTH-secreting tumour.

Hospital for consideration of further therapy, at which time he had severe headaches. Radiologically, tumour was now seen extending into the left orbital cavity and the left infra-temporal fossa. Further transfrontal surgery together with left orbital decompression and combined treatment with cabergoline and octreotide LAR 30 mg monthly failed to halt tumour progression. He proceeded to trans-glabellar craniotomy and tumour was found encasing the optic nerve and anterior cerebral artery. At this point he was given three cycles of temozolomide 150 mg/m^2 daily, which, although well tolerated, did not reduce GH or IGF-1 levels or produce radiological change in tumour size. He became increasingly confused and was transferred to a hospice where he subsequently died.

We performed MGMT immunohistochemistry on tissue obtained from pituitary tumour resection in 2006. This revealed strong nuclear staining in tumour tissue as well as the positive internal controls (Fig. 1d).

MGMT protein expression

The spectrum of MGMT expression was determined by MGMT immunohistochemistry in a large pituitary tumour cohort. The 22 noninformative samples (18%) excluded from statistical analysis because of poor internal controls did not differ from the study cohort in terms of subtype representation or relative proportions of invasive, noninvasive or recurrent tumours.

The results of MGMT expression by tumour subtype, invasiveness and recurrence are presented in Fig. 2. Eleven tumour samples (13%) demonstrated low MGMT staining, and comprised seven prolactinomas, two nonfunctioning adenomas, one GH-secreting adenoma and one ACTH-secreting carcinoma. Twenty-five (28%) and 52 (59%) tumour samples demonstrated intermediate and high MGMT expression, respectively. Prolactinomas were more likely to have low MGMT expression than other pituitary tumour subtypes ($P < 0{\cdot}001$) (Fig. 2a–e).

Table 2. Correlation of *MGMT* promoter methylation and *MGMT* expression

	IHC– (Low)	IHC+ (Int/High)
MSP– (U)	8/11	34/35
MSP+ (M)	3/11*	1/35
	$P = 0{\cdot}012$	

MGMT promoter methylation status assessed by methylation-specific PCR (MSP): unmethylated result (U) shown as negative (–); methylated result (M) shown as positive (+).
MGMT expression assessed by immunohistochemistry (IHC): low expression shown as negative (–); intermediate (Int) or high expression shown as (+).
*Includes case 1.

Table 3. *MGMT* polymorphisms among pituitary tumours sequenced

Polymorphism	Low	Int/High
–56C>T	1/12	2/10
I143V	3/12	2/10
R178K	3/12	2/10
L53L	0/12	3/10
L84F	0/12	2/10

Polymorphisms are shown based on low, intermediate (Int) or high *MGMT* expression.

There was no significant difference in MGMT expression between invasive and noninvasive tumours, or between recurrent and non-recurrent tumours ($P = 0{\cdot}553$, $0{\cdot}75$, respectively) (Fig. 2f–i). Three 'normal pituitary' samples, obtained from patients with pituitary disease in which no tumour remained in the paraffin block, demonstrated high MGMT expression, as did all non-neoplastic pituitary tissue that was adjacent to evaluated pituitary tumours. All four normal pituitaries obtained from autopsy were considered noninformative according to the immunohistochemistry criteria noted above.

Methylation analysis

To explore the mechanism of MGMT loss in our cohort of pituitary tumours, we next examined *MGMT* promoter methylation. MGMT methylation status could be determined by MSP in 46 out of 64 tumours (72%), an amplification success rate using bisulfite-treated paraffin-embedded tumour tissue that is consistent with the landmark study of Hegi *et al*. in glioblastoma.[24]

MGMT promoter methylation was demonstrated in case 1 and in three archived tumour samples (9%). Forty-two of 46 tumours were unmethylated (91%), including eight tumours with low MGMT expression as assessed by immunohistochemistry. There was a significant inverse correlation between MGMT expression and methylation of its promoter (Table 2, $P = 0{\cdot}012$). Two 'normal pituitary' samples were unmethylated, the third could not be amplified. A normal pituitary sample obtained from autopsy was unmethylated.

LOH analysis

LOH has previously been reported to occur in pituitary tumours at 10q26, which encompasses *MGMT*.[25] We therefore tested whether LOH at 10q26 was associated with MGMT expression in our cohort, although paired peripheral blood and tumour DNA was only available from six cases, including case 1. Four out of the five markers analysed in each case were informative and no LOH was detected (data not shown).

MGMT sequencing

We sequenced *MGMT* from a representative sample of our cohort to exclude gene mutation as a cause of low MGMT expression. No *MGMT* coding sequence mutation was identified in case 1 or in 21 other tumour samples. We found several well-described MGMT polymorphisms (Table 3) occurring with equal frequency among high, intermediate and low MGMT-expressing tumours, although the L53L/L84F polymorphisms were only present in tumours expressing MGMT.

Discussion

With the addition of our two cases, there are now eight published cases describing the use of temozolomide in pituitary carcinoma and invasive, aggressive pituitary adenomas: six cases showed a positive response to temozolomide. Five of these six cases were prolactinomas, three were carcinomas (including our case 1) and two were aggressive adenomas; the sixth case was an LH-secreting pituitary carcinoma.[6,7,9,10] We have described the first case of an aggressive GH-secreting pituitary adenoma treated with temozolomide (case 2). Case 2 becomes the second reported case of a pituitary tumour nonresponsive to temozolomide, the other case being an aggressive silent corticotroph adenoma.[20] Our work suggests that low MGMT protein expression, as assessed by immunohistochemistry, may be associated with a clinical response to temozolomide in aggressive pituitary tumours and supports the brief report of Kovacs *et al*.[20]

We have now also provided, for the first time, results of MGMT immunostaining across a large cohort of pituitary tumours. We consider that a comment is needed regarding the interpretation of this particular technique. Immunohistochemical stains may be prone to false negative staining for a variety of reasons, including differences in preservation, fixation, processing or length of time in paraffin. Given the potential clinical significance of negative MGMT immunostaining, we have used endothelial cells as a reliable internal positive control because they are scattered throughout all pituitary tumours and have been shown to be consistently positive in other organs in published reports[26,27] and in our own optimization studies. We required a negative case to contain these positive internal controls, and excluded from consideration those cases in which internal controls were absent. Using this criterion, 18% of our tumour cohort were excluded. Although we may have underestimated the frequency of tumours with low expression of MGMT on this basis, our approach is supported by studies of other immunohistochemical stains that are best performed with strict attention to internal positive controls.[28,29]

We found 13% of pituitary tumours demonstrated true low expression of MGMT. Prolactinomas accounted for more than 60% of this group. This is of particular interest given that prolactinomas are the predominant subtype in which success with temozolomide has been reported. Low expression of MGMT may not be a feature of all prolactinomas, in that those requiring surgery are most often large, invasive tumours or tumours that have shown resistance to dopamine agonist therapy and may be biologically distinct from small or dopamine-sensitive prolactinomas.

We did not find any significant difference in MGMT expression between invasive and noninvasive pituitary tumours, or between recurrent and nonrecurrent tumours. Low expression of MGMT was demonstrated in 15% of invasive and 10% of noninvasive tumours, and in 15% of recurrent and 12% of nonrecurrent tumours. Our work suggests that temozolomide might be effective in benign as well as clearly malignant pituitary tumours, in a manner analogous to the use of temozolomide in both low- and high-grade gliomas.[12,30] Furthermore, Syro *et al.*[8] reported histological findings of increased differentiation, decreased mitoses and Ki-67 index in a case of an invasive prolactinoma where repeat surgery was undertaken after successful use of temozolomide. The tumour was also more easily resected at this subsequent surgery.[8] Temozolomide has also been reported to enhance radiation response in MGMT-negative human glioblastoma cell lines.[31] Taken together, these findings may widen the clinical applications of temozolomide use in aggressive pituitary tumours.

We found an inverse correlation between MGMT protein expression and *MGMT* promoter methylation, which is in agreement with other studies.[16,19,32] Loss of MGMT protein expression has been strongly associated with *MGMT* promoter methylation, particularly in gliomas, lymphomas, nonsmall cell lung carcinoma and colorectal carcinoma.[16,19,33] These tumours appear to have higher frequencies of *MGMT* promoter methylation than other tumour types, with gliomas reported to contain methylation in up to 88% of cases.[16] Our results suggest that pituitary tumours have a lower frequency of methylation (9%), consistent with the findings of Bello *et al.*, in which 23% of pituitary tumours contained *MGMT* promoter methylation, although they did not analyse MGMT protein expression.[34] In our study, all four methylated tumours had both methylated and unmethylated alleles detected, not an uncommon finding in human tissue samples, and is explained either by tumour heterogeneity or contamination by normal tissue.[19] The technique of MSP is very sensitive and may, on occasion, detect low-level tumour methylation at which protein expression is not affected; indeed, this may explain why one of the methylated tumours in our study exhibited high MGMT expression.[16,35] Conversely, eight tumours with low MGMT expression on immunohistochemistry were unmethylated on MSP. Although we chose primers validated in multiple MGMT studies, where methylation of this *MGMT* promoter region correlated with MGMT expression, we cannot exclude the possibility that methylation in other sites of the *MGMT* promoter may be relevant to protein expression in pituitary tumours.[11,12,16,19]

Alternatively, *MGMT* methylation may not be the sole reason for loss of MGMT expression in pituitary tumours. For instance, Bates *et al.* reported LOH at 10q26, a region that includes the *MGMT* gene, in 15% of invasive pituitary adenomas.[25] Although we could not detect LOH in this region in two tumours with low MGMT expression, our assessment was very limited because of the lack of available complementary blood DNA. Further assessment of LOH in this region and its relationship to MGMT expression is warranted.

Loss of expression is not commonly due to deletion, mutation or rearrangement of *MGMT*, or related to mRNA instability in tumour cell lines that lack MGMT activity.[19] However, Halford *et al.* reported that 5% of a large cohort of colorectal carcinomas contained *MGMT* mutations, at least 50% of which were associated with reduced protein expression.[27] *MGMT* polymorphisms have also been suggested to cause subtle changes in protein function.[36] More recently, a strong association has been reported to occur between a germline *MGMT* promoter polymorphism (c.–56C>T) and gene silencing, probably through increased susceptibility to promoter methylation.[37] We found no *MGMT* mutations, but we did find a number of common *MGMT* polymorphisms in both low and high MGMT expressing pituitary tumours.

MGMT immunohistochemistry is a promising technique that warrants further prospective studies to determine its full utility in predicting response to temozolomide therapy in patients with aggressive pituitary tumours. Further work is also required to determine whether other molecular events occur in pituitary tumours, such as codeletion of chromosome 1p/19q or loss of other DNA mismatch repair proteins, that could modulate the response to temozolomide therapy.[38,39] It is possible that future therapies targeted to down-regulate MGMT expression may be of benefit to those patients who do not respond to temozolomide.[40–42] Knowledge of the MGMT status of a tumour can thus guide more effective use of temozolomide in patients with aggressive pituitary tumours.

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