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MGMT expression and pituitary tumours: relationship to tumour biology

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Abstract Over the past half decade, temozolomide, an oral akylating chemotherapeutic agent, has been shown to have significant activity in the management of aggressive pituitary tumours. The expression of 06-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme, is an important predictor of response to therapy. Low MGMT expression has been reported with a higher frequency amongst more aggressive pituitary tumours, suggesting MGMT may play a role in pituitary tumour progression. In this study, we performed a microarray analysis to determine whether there was a distinct gene expression profile between tumours with low MGMT and high MGMT expression. Overall, 1,403 differentially expressed genes were identified with raw p values less than 0.05. Gene set enrichment analysis (GSEA) revealed significant differences in the gene expression profile between high and low MGMT expressing pituitary tumours. High MGMT expressing pituitary tumours were found to have upregulation of components of the FGFR family and downstream signaling cascades such as PI3 K/Akt and MAPK pathways. Activation of genes involved in the DNA damage response and DNA repair pathways, as well as genes involved in transcription, were identified in pituitary tumours with low MGMT expression. These results form the basis of our proposed model to describe the role of MGMT in pituitary tumorigenesis.

Keywords Pituitary tumour · Temozolomide · MGMT · Pituitary tumorigenesis

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Introduction

Pituitary tumours comprise 15 % of intracranial neoplasms, and clinically significant pituitary tumours occur with a frequency of approximately 1 in 1,000 people [1–3]. There is a wide spectrum of clinical behaviour observed amongst pituitary tumours. The majority are indolent, slow-growing neoplasms, but notwithstanding this, have the potential to cause devastating morbidity through effects of hormonal hypersecretion, or hyposecretion. More than half of pituitary tumours are invasive, demonstrating infiltration of structures surrounding the sella, including bone, dura and cavernous sinuses [4]. A small subset of invasive pituitary tumours behave aggressively, demonstrating progressive growth despite multimodal therapy.

The term pituitary carcinoma, accounting for 0.2 % of all pituitary tumours, is reserved for pituitary tumours with demonstrable craniospinal and/or systemic metastases [4].

Significant advances in our understanding of pituitary tumourigenesis have emerged in the past decade. The benign nature of the majority of pituitary tumours is thought to result from a process termed *oncogene-induced senescence (OIS)*. OIS is characterised by activation of tumorigenic pathways, initially resulting in a burst of tumour cell proliferation. However, subsequent DNA replicative stress and DNA damage response signals then activate senescence pathways resulting in cell cycle arrest. Ataxia telangiectasia mutated (ATM) upregulation, indicative of DNA damage signaling, has been demonstrated in human somatotropinomas [5]. Upregulation of key cell cycle regulator genes, *p53* and *p21,* are central components of the senescence response [6]. Activation of the oncogenic Raf/MEK/ERK and PI3 K/Akt/mTOR pathways have been recognised as key upstream drivers in the development of pituitary tumours [7, 8]. A recent study speculated that OIS is responsible for the attenuation in effect of downstream targets of these pathways [9].

It is now generally accepted that tumour cell proliferation and transformation into tumours with invasive properties, and occasionally malignant potential, occur as a result of an accumulation of genetic and epigenetic events, in combination with aberrant growth factor signaling and/or hormonal stimulation [10]. Several biological markers have been reported in association with invasive or recurrent pituitary tumours. These include increased levels of PTTG, VEGF, MMP9 and topoisomerase IIα, as well as decreased expression of E-cadherin and β-catenin [11–15].

Over the past 5 years, temozolomide, an oral alkylating chemotherapeutic agent, has been shown to have significant activity in the management of aggressive pituitary tumours [16–24]. The expression of 06-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme that directly removes the alkylating lesion induced by temozolomide, is an important predictor of response to temozolomide therapy ([25], for review). The loss of MGMT expression, through promoter methylation and other as yet unidentified routes, has been reported with a higher frequency amongst more aggressive pituitary tumours [26–30]. These observations suggest MGMT may play a role in pituitary tumour progression.

The primary objective of this study was to determine, using microarray analysis, whether a distinct gene expression profile is associated with pituitary tumours demonstrating low MGMT expression by immunohistochemistry. MGMT mRNA expression was also examined by qPCR to provide additional validation for immunohistochemical stratification, although we did recognise the possibility that posttranslational modifications of MGMT may explain differences between mRNA and protein expression results. It was anticipated that the identification of differentially expressed genes and/or performance of Gene Set Enrichment Analysis (GSEA) would discover novel mechanisms linking MGMT with pituitary tumorigenesis.

Methods

Pituitary tumour cohort

Pituitary tumour tissue was collected from patients undergoing pituitary surgery between 2006 and 2009 at Royal North Shore and North Shore Private Hospitals, Sydney, Australia. This study was approved by the Northern Sydney Health Human Ethics Committee and informed consent was obtained from patients for the use of tumour tissue in research. At the time of surgery, pituitary tumour tissue was snap-frozen in liquid nitrogen and stored at $-80°$ in the Neuroendocrine Tumour and Tissue Bank of the Cancer Genetics Unit, Kolling Institute of Medical Research, Sydney, Australia. In addition, paraffin-embedded pituitary tumour tissue from the same surgery was obtained from the Department of Anatomical Pathology, Royal North Shore Hospital.

Twenty-one patients were selected for inclusion in this study and two normal pituitary RNA samples were purchased commercially (Ambion Inc., USA) to serve as controls. For the purposes of the microarray analysis, 19 tumours were selected as they demonstrated low or high MGMT expression by immunohistochemistry, according to our previously described scoring method [21]. An additional two tumours with intermediate MGMT expression were included in the Real-Time PCR experiments. Clinical details and tumour characteristics are shown in Table 1. A tumour was classified as atypical if an elevated mitotic index, Ki67 > 3 % and extensive nuclear staining for p53 was seen, as defined by the WHO classification [31].

MGMT immunohistochemistry

Immunohistochemistry for MGMT was performed on formalin-fixed paraffin-embedded tissue using a mouse monoclonal antibody (Clone MT23.2, Cat: MA3016537, Affinity Bioreagents, CO, USA), as described previously [21]. In brief, 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. External positive and negative controls (tonsillar tissue with areas of known positive and negative staining) were included. In addition, endothelial cells and lymphocytes acted as internal positive controls. Slides were examined by

Table 1 Clinical details, hormone and MGMT expression profile of the pituitary tumour cohort

Tumour	Age	Sex	Clinical subtype	Hardy gradeb	Recurrent tumour	Atypical tumour	Hormone IHC	MGMT IHC
1	36	F	PRL	1	N	N	PRL	Low
2	59	F	ACTH	2	N	N	ACTH	Low
3	41	M	NF	3	N	N	ACTH	Low
4	24	M	NF	2	N	N	–	Low
5	79	M	NF	3	N	N	–	Low
6a	67	M	NF	4	Y	Y	–	Low
7	39	M	NF	2	N	N	TSH, LH	Low
8	71	M	ACTH	1	N	N	ACTH	Int
9a	66	M	NF	2	N	N	–	Int
10	61	M	NF	2	N	N	GH, PRL	High
11	34	M	GH	2	N	Y	GH, TSH, FSH	High
12	39	M	GH	3	N	N	GH	High
13	60	F	NF	3	N	N	–	High
14	56	M	NF	3	N	N	–	High
15	62	F	NF	3	Y	N	TSH	High
16	58	M	NF	3	N	N	LH	High
17	65	M	NF	2	N	N	–	High
18	65	M	NF	2	N	N	–	High
19	66	M	NF	2	N	N	TSH, LH	High
20	60	F	NF	3	N	N	TSH	High
21	55	F	NF	2	N	N	–	High

[a] Primary and recurrent tumour from same patient

[b] Hardy grades: 1 = microadenoma, 2 = macroadenoma, 3 = macroadenoma with cavernous sinus or sphenoid sinus invasion, 4 = carcinoma; *IHC* immunohistochemistry, Low = <10 % of tumour cells demonstrating nuclear staining of MGMT; Int (intermediate) = 10–90 % of tumour nuclei positive; High = diffuse positive staining of >90 % of tumour nuclei regardless of intensity; *PRL* prolactin, *ACTH* adrenocorticotrophin, *NF* non-functioning, *GH* growth hormone, *TSH* thyroid stimulating hormone, *LH* luteinising hormone, *FSH* follicle-stimulating hormone

a single observer (A.G.) who was blinded to clinical and molecular data. MGMT expression was scored semiquantitatively. Low MGMT expression was defined as less than 10 % of tumour cells demonstrating nuclear staining of MGMT, 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, as described previously [21].

RNA extraction and preparation

Total RNA was extracted from fresh-frozen pituitary tumours using TRI Reagent according to the manufacturer's protocol (Invitrogen, San Diego, CA). Purification of RNA was performed using 7.5 M lithium chloride (Ambion Inc., Texas, USA). RNA concentration and purity was determined by measuring UV absorbance at 260/280 nm (NanoDrop Spectrophotometer ND-1,000; Nanodrop Technologies, Wilmington, DE, USA). The RNA Integrity Number (RIN) was derived with the Agilent Bioanalyzer 2100 (Agilent Technologies Inc., Santa Clara, CA, USA) to ensure RNA quality. DNA contamination of tumour RNA was excluded by (1) performing PCR of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using intron-spanning primers, and (2) inclusion of a no reverse transcriptase control in the qPCR experiments.

Real-time PCR

Total RNA from each tumour and normal tissue was reverse-transcribed into cDNA using random hexamers and the Superscript III first-strand synthesis system (Invitrogen). Quantitative PCR was performed using a 5′nuclease technique with a specific *MGMT* Taqman® Gene Expression Assay and Taqman® Gene Expression Master Mix (Applied Biosystems, Foster City, CA, USA). The assay contains both forward and reverse primers and one FAM dye-labelled Taqman® Minor groove binder probe in a final concentration of 250 nM. The *MGMT* assay was run as a singleplex reaction alongside a duplicate singleplex reaction containing VIC-labelled *18S* as the housekeeping gene (Applied Biosystems, Foster City, CA, USA). The reaction efficiencies of each probe were determined to be 100 % by

performance of a standard curve. All qPCR was performed using the 7900HT Fast Real Time PCR System and cycled according to the ABI recommended cycling conditions, using different detectors for the FAM and VIC-labelled probes (Applied Biosystems, Foster City, CA, USA). Samples were run in triplicate and experiments repeated twice. Analysis of the qPCR results was performed using the Sequence Detection System (SDS) software tool developed for the 7900HT Fast Real Time PCR System. Relative *MGMT* mRNA expression was determined from tumour samples using mRNA expression from a normal pituitary sample as calibrator.

Microarray

RNA labeling, hybridisation to the Affymetrix Gene 1.0 ST Array, scanning and quality control assessment was performed by the Ramaciotti Centre for Gene Function Analysis (University of New South Wales, Sydney, Australia). Raw CEL files were obtained from the Ramaciotti Centre and subsequent analysis performed using Gene Pattern software available through the Peter Wills Bioinformatics Centre at the Garvan Institute of Medical Research, Sydney, Australia. Normalisation of all samples was initially performed. Differential gene expression was compared between tumours stratified according to MGMT expression by immunohistochemistry (high vs low). Comparative gene expression analysis was performed using Limma analysis.

Gene set analysis was undertaken using Gene Signature Enrichment Analysis (GSEA). GSEA software was available through the Broad Institute (www.broadinstitute.org/gsea) [32, 33]. Gene expression profiles were interrogated against gene sets contained with the Molecular Signatures Database v3.0. Initial analysis was performed using the curated gene set collection (assembled from online pathway databases and publications on PubMed). Further analysis utilised the Gene Ontology (GO) gene set collection also available through the Molecular Signatures Database.

GSEA was developed to evaluate microarray data at the level of gene sets. It may lead to identification of important biological processes that are not evident by performing single-gene analysis. This is because there may be only modest changes occurring in individual genes but significant changes occurring in a collection of genes comprising a relevant biological pathway. GSEA is performed by using the ranked list of genes generated from the microarray analysis and determining whether an a priori set of genes are randomly distributed throughout the gene list or primarily found at the top or bottom. The process is repeated for numerous gene sets comprised within a database. An enrichment score reflects the degree to which a gene set is overrepresented at the top or bottom of the gene list and is calculated as the maximum deviation from zero. Genes occurring at the very extremes of the list are weighted more heavily compared with genes occurring in the middle of the gene list. Statistical significance is denoted by the *p* value and the q value is then calculated to account for multiple hypothesis testing and represents a false discovery rate [32].

Results

MGMT expression: RT-PCR versus immunohistochemistry

MGMT expression was determined in 21 pituitary tumours by both immunohistochemistry (MGMT protein) and RT-PCR (*MGMT* mRNA). There were 7 pituitary tumours with low MGMT expression, 2 with intermediate and 12 with high MGMT expression on immunostaining. Table 2 illustrates the relative mRNA expression levels of the 21 tumour samples compared with normal pituitary gland *MGMT*

Table 2 MGMT expression results—RT-PCR (mRNA) versus Immunohistochemistry (protein)

Tumour	MGMT RT-PCR (ΔΔ CT[a])	MGMT IHC
1	−0.944	Low
2	−0.719	Low
3	−2.436	Low
4	−2.835	Low
5	−2.498	Low
6	−0.550	Low
7	−1.681	Low
8	0.332	Int
9	−3.277	Int
10	0.803	High
11	−2.667	High
12	1.245	High
13	−0.108	High
14	0.341	High
15	0.852	High
16	−0.044	High
17	−0.401	High
18	0.309	High
19	0.001	High
20	0.632	High
21	0.430	High

[a] ΔΔ CT: relative tumour *MGMT* mRNA expression levels compared with normal pituitary gland *MGMT* mRNA expression

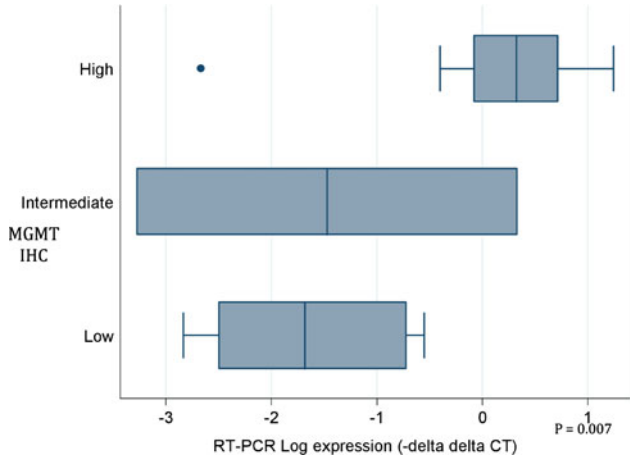

Fig. 1 Correlation between MGMT Expression by Immunohistochemistry and RT-PCR

expression; these results are presented alongside MGMT expression levels according to immunohistochemistry.

All 7 pituitary tumours with low MGMT expression by immunohistochemistry also demonstrated decreased *MGMT* mRNA expression by RT-PCR (ΔΔ CT range −0.55 to −2.835). Eleven of 12 pituitary tumours with high MGMT expression by immunohistochemistry demonstrated greater *MGMT* mRNA expression levels by RT-PCR compared with low MGMT-expressing pituitary tumours (ΔΔ CT range −0.401 to +1.245). There was one pituitary tumour with high MGMT expression by immunohistochemistry that showed low *MGMT* mRNA expression (ΔΔ CT −2.667). The two intermediate-expressing pituitary tumours according to immunohistochemistry displayed widely different *MGMT* mRNA expression (ΔΔ CT −3.277 and +0.332). Overall, there was a strong correlation between *MGMT* mRNA expression by RT-PCR and protein expression by immunohistochemistry as illustrated in Fig. 1. Higher *MGMT* mRNA expression was significantly correlated with high protein expression, and conversely low MGMT protein expression was associated with low *MGMT* mRNA expression ($p = 0.007$).

Gene expression profiles: high versus low MGMT expressing pituitary tumours

The gene expression profile of the 7 pituitary tumours with low MGMT expression was compared with that of the 12 pituitary tumours with high expression on MGMT immunostaining. The two tumours with intermediate MGMT expression were not included in this analysis. Utilising a p value threshold of less than 0.05, there were 1,403 genes with differential expression between low and high MGMT expressing tumours (708 upregulated in high expressing tumours, 695 upregulated in low expressing tumours).

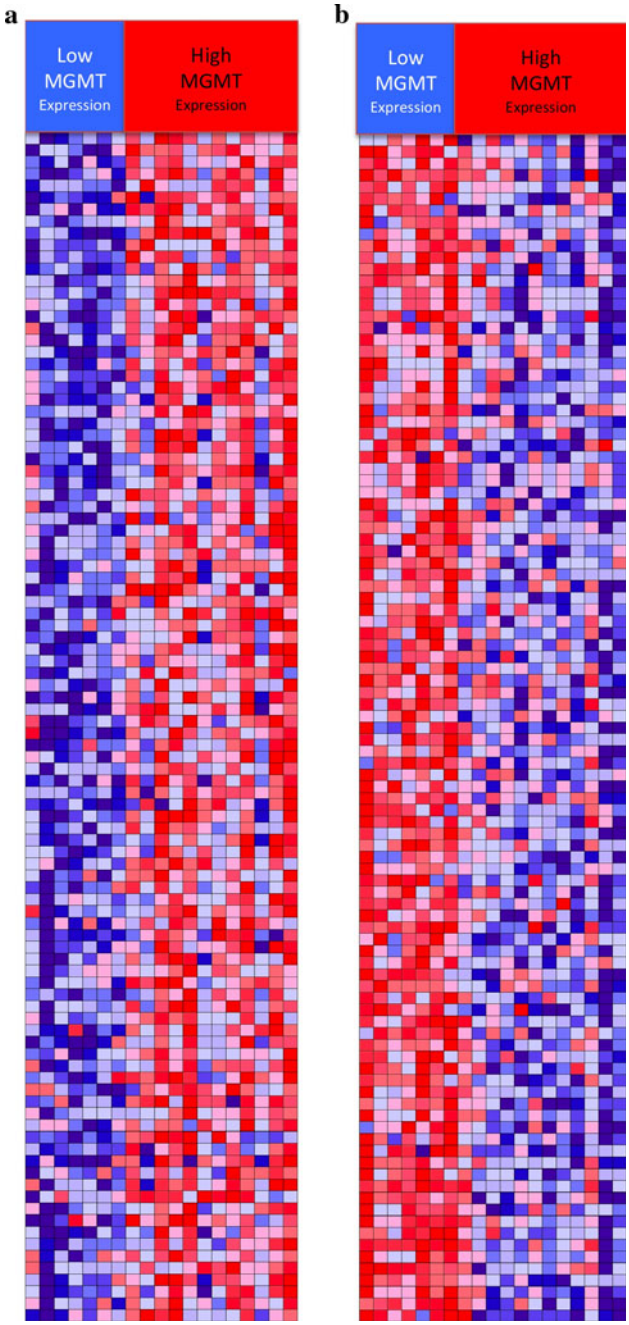

Fig. 2 Microarray heatmaps of top 100 upregulated genes (**a**) in high MGMT expressing tumours, and (**b**) in low MGMT expressing tumours. Each gene is represented as a row of *coloured* boxes. Tumour samples are represented in columns and are divided into 2 groups. Low MGMT-expressing tumours, as determined by immunohistochemistry, are denoted by the *blue bar* at the top of the columns encompassing the first 7 tumours. High MGMT-expressing tumours are denoted by the *red bar* at the top of the columns and encompass the second 12 tumours. Blue boxes represent a downregulated gene, red boxes an upregulated gene

Figure 2 illustrates the heatmap for the top 100 differentially expressed genes across all tumour samples: (a) upregulated in high MGMT expressing pituitary tumours compared with

low MGMT expressing tumours; and (b) upregulated in low MGMT expressing tumours compared with high MGMT expressing tumours. In an attempt to identify the most significantly differentially expressed genes between the 2 tumour groups and decrease the false positive discovery rate, multiple hypotheses testing was performed (q value < 0.05). However, no significantly differentially expressed genes were then identified.

Amongst the most upregulated genes in pituitary tumours with high MGMT expression were a number of transcription factors including *POU3F1*, *FOXD3* and *IKZF3*. Fibroblast growth factor 19 appears along with calpain 11, each with roles in integrin and growth factor signalling. In addition, high-mobility group box 1 (HMGB1) proteins, calpain family members, and microRNA-223 via downregulation of RhoB, have been implicated in chemoresistance [34–36].

In contrast, a number of genes with roles in the cellular DNA damage response pathways were identified amongst the most upregulated genes in low MGMT expressing tumours compared with high MGMT expressing tumours. These included *ERCC6*, *XRCC1*, *PER3*, *RIPK1*, *CASP9* and *CDK7* [37–40]. In addition, low MGMT expressing pituitary tumours exhibited upregulation of multiple genes involved in RNA metabolism, transcriptional regulation, cell cycle control, nucleocytoplasmic transport and intracellular trafficking (*DDX50*, *DDX46*, *PER3*, *NUP188*, *BTAF1*, *KIF16B*, *BICD1*, *CDK7*) [38, 40–45]. Several zinc finger proteins also appear amongst the most upregulated genes in low MGMT expressing tumours.

Gene set enrichment analysis (GSEA): high versus low MGMT expressing pituitary tumours

GSEA was performed on the gene expression profiles of the low and high MGMT expressing pituitary tumours. Interrogation of both curated gene sets and Gene Ontology gene sets was performed.

Amongst the pituitary tumours with high MGMT expression, 115 of 2,517 curated gene sets were significantly upregulated ($p < 0.01$). The 20 most upregulated gene sets are shown in Table 3. Enrichment of ribosomal proteins predominates amongst the first 6 gene sets. There is enrichment of receptors involved in hormone and neurotransmitter transduction, and upregulation of components of the FGFR family and downstream signalling cascades such as PI3 K/Akt and MAP kinase pathways. Examples of enrichment plots from these gene sets are shown in Fig. 3a. Gene Ontology Gene Set analysis revealed 30 of 584 gene sets significantly enriched in high MGMT expressing pituitary tumours ($p = 0.01$) (data not shown). Gene sets involved in epidermis and ectodermal development are highly upregulated, and include genes such as keratins, collagens and follistatin. Also featured amongst the

Table 3 Top 20 upregulated gene sets (curated gene set analysis)—high MGMT expressing pituitary tumours

Gene set	Number of genes with core enrichment	Enrichment score	*P* value	*Q* value
Reactome regulation of beta cell development	67/112	0.51	<0.001	<0.001
Reactome regulation of gene expression in beta cells	58/100	0.50	<0.001	<0.001
Reactome viral mRNA translation	48/84	0.50	<0.001	<0.001
KEGG Ribosome	51/87	0.51	<0.001	<0.001
Reactome formation of a pool of free 40S subunits	51/94	0.46	<0.001	<0.001
Reactome peptide chain elongation	48/84	0.49	<0.001	<0.001
Reactome GPCR ligand binding	185/385	0.40	<0.001	0.001
Reactome class A1 Rhodopsin Like Receptors	136/285	0.40	<0.001	0.004
Reactome peptide ligand binding receptors	84/169	0.41	<0.001	0.008
West adrenocortical tumour markers DN	8/10	0.60	<0.001	0.010
Reactome downstream signaling of activated FGFR	15/42	0.49	<0.001	0.012
Biocarta cardiacegf pathway	8/18	0.66	<0.001	0.011
Huper breast basal vs luminal up	29/54	0.47	<0.001	0.011
Nakayama soft tissue tumours PCA2 DN	36/80	0.45	<0.001	0.019
Rickman head and neck cancer E	40/86	0.42	<0.001	0.021
KEGG complement and coagulation cascades	36/68	0.44	<0.001	0.022
Reactome FRS2mediated cascade	12/27	0.58	<0.001	0.023
Reactome amine ligand binding receptors	27/42	0.51	<0.001	0.024
HSIAO liver specific genes	117/236	0.36	<0.001	0.026
Reactome phospholipase C mediated cascade	12/23	0.62	<0.001	0.026

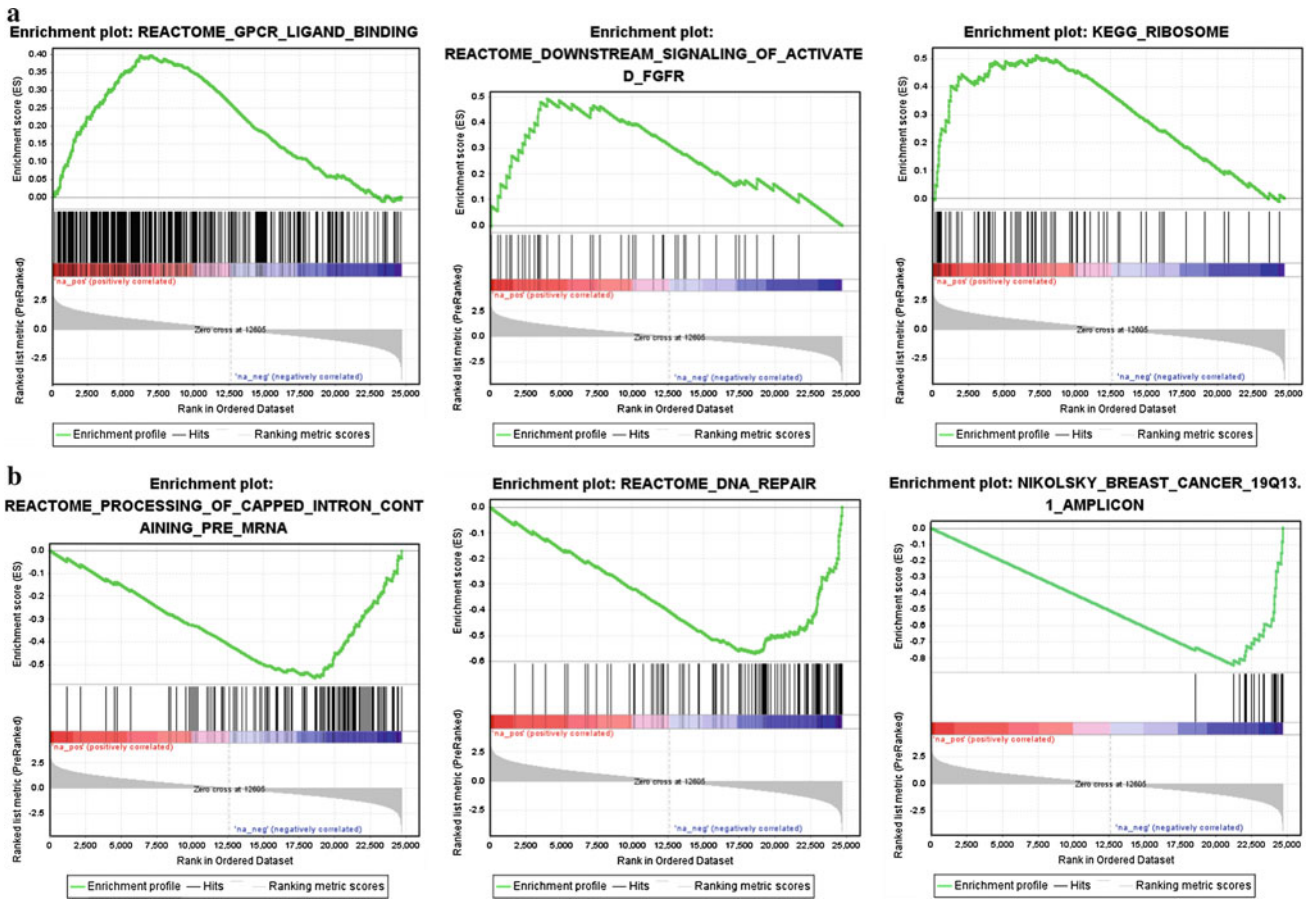

Fig. 3 Gene set enrichment plots from upregulated gene sets in (**a**) high MGMT expressing pituitary tumours, and (**b**) low MGMT expressing pituitary tumours. Along the horizontal axis the maximal gene list is shown in ranked order with upregulated genes (in high MGMT expressing pituitary tumours) represented by the *red bar* and upregulated genes (in low MGMT expressing tumours) by the blue bar. Each time a member of the gene set is found within the gene list it is represented by a vertical black line. The *bottom graph* (in *grey*) illustrates the weighting given to genes depending on where they occur in the ranked gene list, and this is used to formulate the enrichment plot shown in *green* in the *top graph*. The enrichment score is calculated as the maximal deviation from zero from the enrichment plot

upregulated gene sets are genes involved in G protein coupled receptor and phosphoinositide mediated signaling pathways.

Low MGMT expressing pituitary tumours demonstrated significant enrichment of 352 of 2,517 curated gene sets ($p < 0.01$). Table 4 lists the 20 most upregulated gene sets amongst the tumours with low MGMT expression. Eleven of these gene sets include genes involved in transcription, mRNA processing and transport. DNA repair gene sets are also a notable feature of this list. Nikolsky breast cancer 19q13.1 amplicon gene set is composed entirely of zinc finger proteins. In addition, published cancer gene sets involving copy number changes appear on the list. Examples of enrichment plots from these gene sets are shown in Fig. 3b. Gene Ontology Gene Set analysis revealed 77 of 584 significantly enriched gene sets amongst the pituitary tumours with low MGMT expression ($p < 0.01$) (data not shown). Supporting the curated gene set analysis, gene sets involving DNA repair, mRNA transcription and processing predominate in low MGMT expressing pituitary tumours.

Discussion

There have now been multiple publications addressing the relationship between MGMT status and temozolomide response in aggressive pituitary tumours, recently extensively reviewed [25]. A few studies have examined MGMT expression by immunohistochemistry amongst larger archived cohorts of pituitary tumours [21, 29]. In these studies and the current study, no appreciable difference has been found in MGMT expression when comparing invasive and non-invasive adenomas. However, not all invasive adenomas display aggressive behaviour. Other studies have

Table 4 Top 20 upregulated gene sets (curated gene set analysis)—low MGMT expressing pituitary tumours

Gene set	Number of genes with core enrichment	Enrichment score	*P* value	q value
Reactome processing of capped intron containing premRNA	77/137	−0.56	<0.001	<0.001
Reactome late phase of HIV life cycle	61/89	-0.61	<0.001	<0.001
Nikolsky breast cancer 19q13.1 amplicon	21/22	-0.84	<0.001	<0.001
Reactome HIV infection	110/182	-0.53	<0.001	<0.001
Reactome transport of mature mRNA derived from an intron containing transcript	36/50	−0.61	<0.001	<0.001
Reactome DNA repair	58/104	−0.57	<0.001	<0.001
Reactome transport of ribonucleoproteins into the host nucleus	23/28	−0.70	<0.001	<0.001
Reactome SNRNP assembly	34/48	−0.64	<0.001	<0.001
Reactome HIV Life Cycle	65/102	−0.57	<0.001	<0.001
Reactome Transcription Coupled NER	28/44	−0.64	<0.001	<0.001
Moreaux multiple myeloma by Taci DN	68/131	−0.52	<0.001	<0.001
Lindgren bladder cancer with LOH in Chr9q	67/116	−0.56	<0.001	<0.001
Lastowska neuroblastoma copy number up	83/171	−0.52	<0.001	<0.001
Reactome regulation of glucokinase by glucokinase regulatory protein	22/28	−0.69	<0.001	<0.001
Reactome nucleotide excision repair	31/49	−0.63	<0.001	<0.001
Reactome transport of the sibp independent mature mRNA	25/31	−0.70	<0.001	<0.001
Reactome RNA Pol II CTD phosphorylation and interaction with CE	21/26	−0.72	<0.001	<0.001
Reactome elongation and processing of capped transcripts	67/134	−0.52	<0.001	<0.001
KEGG nucleotide excision repair	24/44	−0.60	<0.001	<0.001
KEGG aminoacyl tRNA biosynthesis	29/41	−0.63	<0.001	<0.001

reported a higher incidence of low MGMT expression amongst more aggressive subtypes of pituitary tumours, suggesting MGMT may be involved in pituitary tumorigenesis [27–29, 46]. In one small study, 92 % of 12 silent subtype 3 adenomas (associated with an increased risk of aggressive behaviour) and 50 % of 10 pituitary carcinomas demonstrated low MGMT expression [29]. Low MGMT expression is particularly common amongst invasive Crooke's cell adenomas and tumours of patients with Nelson's syndrome [28, 46]. Furthermore, low MGMT expression was seen more commonly in non-functioning pituitary tumours that recurred on follow-up compared with nonrecurrent tumours [27]. Our study is the first to examine in detail, the relationship between MGMT and pituitary tumour gene expression. In fact, the microarray experiment reported herein is the first genome-wide gene expression profiling to directly compare low versus high MGMT expression in any tumour type.

MGMT expression as determined by immunohistochemistry, has met with some controversy as a clinical tool in predicting response to temozolomide in pituitary tumours [22, 23]. Furthermore, several groups have shown no correlation between promoter methylation and MGMT expression by immunohistochemistry in pituitary tumours [22, 23, 29]. Whilst loss of MGMT expression may be associated with promoter methylation in a proportion of cases, the techniques used to assess promoter methylation are highly sensitive and can detect minute amounts of methylation that may not translate into effects on protein expression. Furthermore, it is likely that there exist other, as yet unknown, mechanisms driving low MGMT expression. Therefore, we deemed it important to ascertain the relationship between MGMT protein expression and *MGMT* mRNA expression. This would allow us to determine the biological validity of using differential MGMT protein expression by immunohistochemistry as a means to assess for differences in tumour biology. We did establish a strong correlation between *MGMT* mRNA expression by RT-PCR and protein expression by immunohistochemistry. There was one tumour with high MGMT expression and another with intermediate expression by immunohistochemistry, which both demonstrated reduced mRNA expression by RT-PCR. Interestingly, the intermediate tumour with markedly reduced mRNA expression progressed to a carcinoma the subsequent year and MGMT expression (by immunohistochemistry) in the carcinoma was low. It is possible these disparate results relate to tumour heterogeneity, given fresh frozen tumour tissue is used to perform RT-PCR and a different section of tumour is paraffin-embedded and used for immunohistochemistry. This is the first study in pituitary tumours to examine *MGMT* mRNA expression. In the wider literature, there are only a handful of studies examining

MGMT expression at the mRNA level. Others have reported a good correlation between MGMT protein activity and mRNA levels in MGMT deficient (Mer−) and MGMT proficient (Mer+) tumour cell lines, and also amongst human tumour samples [47–49].

The primary objective of this study was to determine whether there were any gene expression differences, at the whole genome level, between tumours with low MGMT and high MGMT protein expression. Overall, we identified 1,403 differentially expressed genes with raw p-values less than 0.05. However, significance of these results did not withstand adjustment for multiple hypotheses consistent with low statistical power on the basis of small sample size. Given the lack of highly significant differentially expressed genes at the single-gene level, validation of individual genes was not performed. The performance of GSEA does, however, allow identification of differences in gene expression that are occurring across whole biologically associated gene sets. These differences may provide some valuable biological information. With this in mind, the GSEA results generated from this microarray study did reveal significant differences in the gene expression profile between high and low MGMT expressing pituitary tumours.

Results of the GSEA suggest that high MGMT expressing pituitary tumours are associated with upregulation of components of the FGFR family and downstream signaling cascades such as PI3 K/Akt and MAP kinase pathways. This association could be one of a "chemoresistant" phenotype, however the literature supports a more direct connection. Activator protein (AP)-1 transcription factor has been demonstrated as the downstream target of protein kinase C-mediated signaling involved in *MGMT* promoter activation [50]. AP-1 levels are also increased by activation of the growth factor signal transduction pathways [51]. Overexpression of basic fibroblast growth factor has been shown to increase MGMT expression by demethylation of the *MGMT* promoter [52]. EGFR signalling via Akt and ERK activation has been demonstrated to enhance DNA double strand break repair, whilst nuclear factor kappa β mediates DNA damage repair by positively regulating MGMT expression [53, 54]. In addition, there is evidence to support a positive feedback loop involving Akt activation, consequent cyclin D1 overexpression and activation of the DNA damage response [55]. The forced cell cycle progression caused by cyclin D1 overexpression induces DNA double strand breaks that activate DNA-dependent protein kinase (DNA-PK), which in turn activates Akt [55]. DNA-PK has been demonstrated to interact with MGMT [56].

There was a distinctly different gene expression profile characterising pituitary tumours with low MGMT expression. GSEA identified activation of genes involved in the DNA damage response and DNA repair pathways. In addition, genes involved in transcription were found to be upregulated. The DNA damage response and transcriptional activity are tightly linked. Transcription-coupled NER is a dedicated mechanism whereby the DNA damage response can be prioritised to cells with high transcriptional activity [57]. RNA polymerase II functions as a lesion sensor by stalling at sites of DNA damage and triggers preferential repair of the transcribed strand of active genes [57]. Thus, the finding of genes involved in transcriptional regulation amongst the highly upregulated genes in low MGMT expressing tumours is perhaps not surprising. Interestingly, a number of unidentified zinc finger proteins were listed amongst the most highly upregulated genes in low MGMT expressing tumours. Zinc finger proteins are recognised as important transcription factors, regulating gene expression through DNA-binding domains [58]. Many DNA repair proteins contain zinc finger motifs, which facilitate protein-nucleic acid and protein–protein interactions [59].

The exploration of a differential gene expression between human tumours with high and low MGMT protein expression, as presented here, is unique. Chahal et al. [60] in a recent study examined the gene expression profile in MGMT positive and negative glioblastoma cell lines. They reported a decreased angiogenic profile in MGMT positive cells associated with an increased sVEGFR-1/VEGFA ratio and demonstrated reduced tumorigenic potential of the MGMT positive cell line. Interestingly, they found more than 3,000 differentially expressed genes between MGMT positive and negative cell lines, including upregulation of fibroblast growth factors, integrins and forkhead box transcription factors. The gene ontology analysis suggested involvement of the MGMT protein in several pathways not yet described in relation to MGMT function, including the identification of many zinc finger transcription factor pathways. They speculated MGMT may be a regulator of key functional pathways.

In summary, pituitary tumours with high MGMT expression are enriched for gene sets of the fibroblast growth factor family and the downstream PI3 K/Akt and MAP kinase signalling pathways. On the other hand, low MGMT expressing pituitary tumours demonstrate upregulation of gene sets involved in DNA repair and transcription. These results in concert with the suggestion of a higher incidence of low MGMT expression amongst more aggressive subtypes of pituitary tumours, has led us to propose an integrated role for MGMT in pituitary tumorigenesis (Fig. 4). In this model, early pituitary tumour growth is characterised by activated growth factor signaling and upregulation of PI3 K/Akt/mTOR pathways. This results in a transient burst of cellular proliferation and consequent genomic instability and DNA damage. Activation of DNA damage response

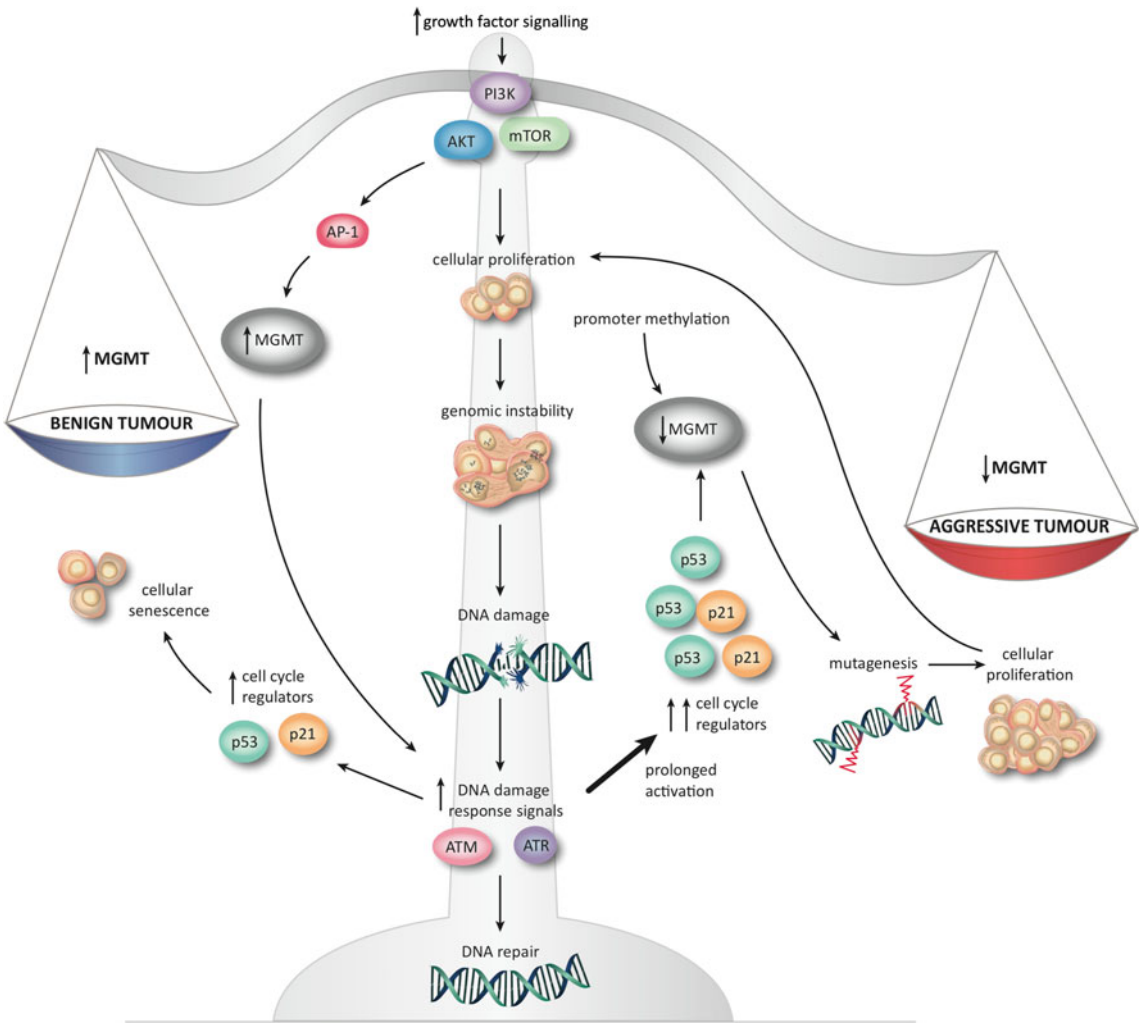

Fig. 4 Proposed role of MGMT in pituitary tumorigenesis

signals, involving ATM and ATR (ataxia-telangiectasia and Rad3-related) genes aims to effect DNA repair. DNA repair is facilitated by increased activation of cell cycle regulators, in particular p53 and p21, via DNA damage response signals. In early stages of pituitary tumour growth this results in oncogene-induced cellular senescence. The activated PI3 K/Akt/mTOR pathway also results in activation of transcription factors, such as AP-1, which are known to increase MGMT expression. It is possible that MGMT forms part of a positive feedback loop, acting to reinforce DNA damage signals. However, prolonged DNA damage signaling leads to increasing upregulation of p53 and preferential down-regulation of MGMT expression. More aggressive tumours are often associated with p53 overexpression and also may be subject to widespread promoter methylation silencing of genes, including *MGMT*. Low MGMT expression may then result in increased mutagenesis, which further drives the tumorigenic process and increases cellular proliferation. This feeds back into the cycle of genomic instability and further DNA damage.

In conclusion we report, for the first time, differences in the gene expression profiles between pituitary tumours with high and low MGMT expression. These results support the available literature suggesting the tumour expression of MGMT serves a wider biological relevance than simply a biomarker of response to alkylating chemotherapy.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A (2006) High prevalence of pituitary adenomas: a

cross-sectional study in the province of Liege, Belgium. J Clin Endocrinol Metab 91(12):4769–4775
2. Fernandez A, Karavitaki N, Wass JA (2010) Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). Clin Endocrinol (Oxf) 72(3): 377–382. doi:10.1111/j.1365-2265.2009.03667.x
3. Melmed S (2011) Pathogenesis of pituitary tumors. Nat Rev Endocrinol 7(5):257–266. doi:10.1038/nrendo.2011.40
4. Pernicone PJ, Scheithauer BW, Sebo TJ, Kovacs KT, Horvath E, Young WF Jr, Lloyd RV, Davis DH, Guthrie BL, Schoene WC (1997) Pituitary carcinoma: a clinicopathologic study of 15 cases. Cancer 79(4):804–812
5. Chesnokova V, Zonis S, Kovacs K, Ben-Shlomo A, Wawrowsky K, Bannykh S, Melmed S (2008) p21(Cip1) restrains pituitary tumor growth. Proc Natl Acad Sci USA 105(45):17498–17503
6. Chesnokova V, Melmed S (2010) Pituitary senescence: the evolving role of Pttg. Mol Cell Endocrinol 326(1–2):55–59. doi: 10.1016/j.mce.2010.02.012
7. Vandeva S, Tichomirowa MA, Zacharieva S, Daly AF, Beckers A (2010) Genetic factors in the development of pituitary adenomas. Endocr Dev 17:121–133. doi:10.1159/000262534
8. Gueorguiev M, Grossman AB (2009) Pituitary gland and beta-catenin signaling: from ontogeny to oncogenesis. Pituitary 12(3):245–255. doi:10.1007/s11102-008-0147-x
9. Dworakowska D, Grossman AB (2009) The pathophysiology of pituitary adenomas. Best Pract Res Clin Endocrinol Metab 23(5): 525–541
10. Asa SL, Ezzat S (2009) The pathogenesis of pituitary tumors. Annu Rev Pathol 4:97–126
11. Vidal S, Kovacs K, Horvath E, Rotondo F, Kuroki T, Lloyd RV, Scheithauer BW (2002) Topoisomerase IIalpha expression in pituitary adenomas and carcinomas: relationship to tumor behavior. Mod Pathol 15(11):1205–1212
12. Pan LX, Chen ZP, Liu YS, Zhao JH (2005) Magnetic resonance imaging and biological markers in pituitary adenomas with invasion of the cavernous sinus space. J Neurooncol 74(1):71–76
13. Qian ZR, Li CC, Yamasaki H, Mizusawa N, Yoshimoto K, Yamada S, Tashiro T, Horiguchi H, Wakatsuki S, Hirokawa M, Sano T (2002) Role of E-cadherin, alpha-, beta-, and gamma-catenins, and p120 (cell adhesion molecules) in prolactinoma behavior. Mod Pathol 15(12):1357–1365
14. Filippella M, Galland F, Kujas M, Young J, Faggiano A, Lombardi G, Colao A, Meduri G, Chanson P (2006) Pituitary tumour transforming gene (PTTG) expression correlates with the proliferative activity and recurrence status of pituitary adenomas: a clinical and immunohistochemical study. Clin Endocrinol (Oxf) 65(4):536–543
15. McCabe CJ, Khaira JS, Boelaert K, Heaney AP, Tannahill LA, Hussain S, Mitchell R, Olliff J, Sheppard MC, Franklyn JA, Gittoes NJ (2003) Expression of pituitary tumour transforming gene (PTTG) and fibroblast growth factor-2 (FGF-2) in human pituitary adenomas: relationships to clinical tumour behaviour. Clin Endocrinol (Oxf) 58(2):141–150
16. Fadul CE, Kominsky AL, Meyer LP, Kingman LS, Kinlaw WB, Rhodes CH, Eskey CJ, Simmons NE (2006) Long-term response of pituitary carcinoma to temozolomide, Report of two cases. J Neurosurg 105(4):621–626
17. Neff LM, Weil M, Cole A, Hedges TR, Shucart W, Lawrence D, Zhu JJ, Tischler AS, Lechan RM (2007) Temozolomide in the treatment of an invasive prolactinoma resistant to dopamine agonists. Pituitary 10(1):81–86
18. Syro LV, Uribe H, Penagos LC, Ortiz LD, Fadul CE, Horvath E, Kovacs K (2006) Antitumour effects of temozolomide in a man with a large, invasive prolactin-producing pituitary neoplasm. Clin Endocrinol (Oxf) 65(4):552–553
19. Moyes VJ, Alusi G, Sabin HI, Evanson J, Berney DM, Kovacs K, Monson JP, Plowman PN, Drake WM (2009) Treatment of Nelson's syndrome with temozolomide. Eur J Endocrinol 160(1):115–119
20. Mohammed S, Kovacs K, Mason W, Smyth H, Cusimano MD (2009) Use of temozolomide in aggressive pituitary tumors: case report. Neurosurgery 64(4):E773–E774 (discussion E774)
21. McCormack AI, McDonald KL, Gill AJ, Clark SJ, Burt MG, Campbell KA, Braund WJ, Little NS, Cook RJ, Grossman AB, Robinson BG, Clifton-Bligh RJ (2009) Low O6-methylguanine-DNA methyltransferase (MGMT) expression and response to temozolomide in aggressive pituitary tumours. Clin Endocrinol (Oxf) 71(2):226–233
22. Raverot G, Sturm N, de Fraipont F, Muller M, Salenave S, Caron P, Chabre O, Chanson P, Cortet-Rudelli C, Assaker R, Dufour H, Gaillard S, Francois P, Jouanneau E, Passagia JG, Bernier M, Cornelius A, Figarella-Branger D, Trouillas J, Borson-Chazot F, Brue T (2010) Temozolomide Treatment in Aggressive Pituitary Tumors and Pituitary Carcinomas: a French Multicenter Experience. J Clin Endocrinol Metab 95(10):4592–4599. doi:10.1210/jc.2010-0644
23. Bush ZM, Longtine JA, Cunningham T, Schiff D, Jane JA Jr, Vance ML, Thorner MO, Laws ER Jr, Lopes MB (2010) Temozolomide treatment for aggressive pituitary tumors: correlation of clinical outcome with O6-methylguanine methyltransferase (MGMT) promoter methylation and expression. J Clin Endocrinol Metab 95(11):E280–E290. doi:10.1210/jc.2010-0441
24. Losa M, Mazza E, Terreni MR, McCormack A, Gill AJ, Motta M, Cangi MG, Talarico A, Mortini P, Reni M (2010) Salvage therapy with Temozolomide in patients with aggressive or metastatic pituitary adenomas: experience in six cases. Eur J Endocrinol 163(6):843-851. doi:10.1530/EJE-10-0629
25. McCormack AI, Wass JA, Grossman AB (2011) Aggressive pituitary tumours: the role of temozolomide and the assessment of MGMT status. Eur J Clin Invest 41(10):1133–1148. doi: 10.1111/j.1365-2362.2011.02520.x
26. Lau Q, Scheithauer B, Kovacs K, Horvath E, Syro LV, Lloyd R (2010) MGMT immunoexpression in aggressive pituitary adenoma and carcinoma. Pituitary 13(4):367–379. doi:10.1007/s11102-010-0249-0
27. Widhalm G, Wolfsberger S, Preusser M, Woehrer A, Kotter MR, Czech T, Marosi C, Knosp E (2009) O(6)-methylguanine DNA methyltransferase immunoexpression in nonfunctioning pituitary adenomas: are progressive tumors potential candidates for temozolomide treatment? Cancer 115(5):1070–1080
28. Takeshita A, Inoshita N, Taguchi M, Okuda C, Fukuhara N, Oyama K, Ohashi K, Sano T, Takeuchi Y, Yamada S (2009) High incidence of low O6-methylguanine DNA methyltransferase (MGMT) expression in invasive macroadenomas of Cushing's disease. Eur J Endocrinol 161(4):553–559
29. Salehi F, Scheithauer BW, Kros JM, Lau Q, Fealey M, Erickson D, Kovacs K, Horvath E, Lloyd RV (2011) MGMT promoter methylation and immunoexpression in aggressive pituitary adenomas and carcinomas. J Neurooncol 104(3):647–657. doi: 10.1007/s11060-011-0532-6
30. Salehi F, Scheithauer BW, Kovacs K, Horvath E, Syro LV, Sharma S, Manoranjan B, Cusimano M (2012) O-6-methylguanine-DNA methyltransferase (MGMT) immunohistochemical expression in pituitary corticotroph adenomas. Neurosurgery 70(2):491–496. doi:10.1227/NEU.0b013e318230ac63
31. Lloyd RV, Kovacs K, Young WF (2004) Pituitary tumors. WHO classification of tumours of the endocrine organs. IARC Press, Lyon
32. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP (2005) Gene set enrichment analysis: a knowledge-

based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 102(43):15545–15550. doi:10.1073/pnas.0506580102
33. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC (2003) PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34(3):267–273. doi:10.1038/ng1180
34. Liu L, Yang M, Kang R, Wang Z, Zhao Y, Yu Y, Xie M, Yin X, Livesey KM, Loze MT, Tang D, Cao L (2011) DAMP-mediated autophagy contributes to drug resistance. Autophagy 7(1):112–114
35. Mlynarczuk-Bialy I, Roeckmann H, Kuckelkorn U, Schmidt B, Umbreen S, Golab J, Ludwig A, Montag C, Wiebusch L, Hagemeier C, Schadendorf D, Kloetzel PM, Seifert U (2006) Combined effect of proteasome and calpain inhibition on cisplatin-resistant human melanoma cells. Cancer Res 66(15):7598–7605. doi:10.1158/0008-5472.CAN-05-2614
36. Srougi MC, Burridge K (2011) The nuclear guanine nucleotide exchange factors Ect2 and Net1 regulate RhoB-mediated cell death after DNA damage. PLoS One 6(2):e17108. doi:10.1371/journal.pone.0017108
37. Warmerdam DO, Kanaar R (2010) Dealing with DNA damage: relationships between checkpoint and repair pathways. Mutat Res 704(1–3):2–11. doi:10.1016/j.mrrev.2009.12.001
38. Im JS, Jung BH, Kim SE, Lee KH, Lee JK (2010) Per3, a circadian gene, is required for Chk2 activation in human cells. FEBS Lett 584(23):4731–4734. doi:10.1016/j.febslet.2010.11.003
39. Kaina B (2003) DNA damage-triggered apoptosis: critical role of DNA repair, double-strand breaks, cell proliferation and signaling. Biochem Pharmacol 66(8):1547–1554. doi:S0006295203005100
40. Egly JM, Coin F (2011) A history of TFIIH: two decades of molecular biology on a pivotal transcription/repair factor. DNA Repair (Amst). doi:10.1016/j.dnarep.2011.04.021
41. Cordin O, Banroques J, Tanner NK, Linder P (2006) The DEAD-box protein family of RNA helicases. Gene 367:17–37. doi:10.1016/j.gene.2005.10.019
42. Kohler A, Hurt E (2010) Gene regulation by nucleoporins and links to cancer. Mol Cell 38(1):6–15. doi:10.1016/j.molcel.2010.01.040
43. Pereira LA, Klejman MP, Timmers HT (2003) Roles for BTAF1 and Mot1p in dynamics of TATA-binding protein and regulation of RNA polymerase II transcription. Gene 315:1–13. doi:S0378111903007145
44. Yu Y, Feng YM (2010) The role of kinesin family proteins in tumorigenesis and progression: potential biomarkers and molecular targets for cancer therapy. Cancer 116(22):5150–5160. doi:10.1002/cncr.25461
45. Fumoto K, Hoogenraad CC, Kikuchi A (2006) GSK-3beta-regulated interaction of BICD with dynein is involved in microtubule anchorage at centrosome. EMBO J 25(24):5670–5682. doi:10.1038/sj.emboj.7601459
46. Salehi F, Scheithauer BW, Moyes VJ, Drake WM, Syro LV, Manoranjan B, Sharma S, Horvath E, Kovacs K (2010) Low Immunohistochemical Expression of MGMT in ACTH Secreting Pituitary Tumors of Patients with Nelson Syndrome. Endocr Pathol 21(4):227–229. doi:10.1007/s12022-010-9138-3
47. Mineura K, Watanabe K, Yanagisawa T, Kowada M (1996) Quantification of O6-methylguanine-DNA methyltransferase mRNA in human brain tumors. Biochim Biophys Acta 1289(1):105–109
48. Citron M, Graver M, Schoenhaus M, Chen S, Decker R, Kleynerman L, Kahn LB, White A, Fornace AJ Jr, Yarosh D (1992) Detection of messenger RNA from O6-methylguanine-DNA methyltransferase gene MGMT in human normal and tumor tissues. J Natl Cancer Inst 84(5):337–340
49. Fornace AJ Jr, Papathanasiou MA, Hollander MC, Yarosh DB (1990) Expression of the O6-methylguanine-DNA methyltransferase gene MGMT in MER + and MER- human tumor cells. Cancer Res 50(24):7908–7911
50. Boldogh I, Ramana CV, Chen Z, Biswas T, Hazra TK, Grosch S, Grombacher T, Mitra S, Kaina B (1998) Regulation of expression of the DNA repair gene O6-methylguanine-DNA methyltransferase via protein kinase C-mediated signaling. Cancer Res 58(17):3950–3956
51. Ozanne BW, McGarry L, Spence HJ, Johnston I, Winnie J, Meagher L, Stapleton G (2000) Transcriptional regulation of cell invasion: AP-1 regulation of a multigenic invasion programme. Eur J Cancer 36(13 Spec No):1640–1648. doi:S0959-8049(00)00175-1
52. Fontijn D, Adema AD, Bhakat KK, Pinedo HM, Peters GJ, Boven E (2007) O6-methylguanine-DNA-methyltransferase promoter demethylation is involved in basic fibroblast growth factor induced resistance against temozolomide in human melanoma cells. Mol Cancer Ther 6(10):2807–2815
53. Golding SE, Morgan RN, Adams BR, Hawkins AJ, Povirk LF, Valerie K (2009) Pro-survival AKT and ERK signaling from EGFR and mutant EGFRvIII enhances DNA double-strand break repair in human glioma cells. Cancer Biol Ther 8(8):730–738
54. Lavon I, Fuchs D, Zrihan D, Efroni G, Zelikovitch B, Fellig Y, Siegal T (2007) Novel mechanism whereby nuclear factor kappaB mediates DNA damage repair through regulation of O(6)-methylguanine-DNA-methyltransferase. Cancer Res 67(18):8952–8959
55. Shimura T, Kakuda S, Ochiai Y, Nakagawa H, Kuwahara Y, Takai Y, Kobayashi J, Komatsu K, Fukumoto M (2010) Acquired radioresistance of human tumor cells by DNA-PK/AKT/GSK3beta-mediated cyclin D1 overexpression. Oncogene 29(34):4826–4837. doi:10.1038/onc.2010.238
56. Niture SK, Doneanu CE, Velu CS, Bailey NI, Srivenugopal KS (2005) Proteomic analysis of human O6-methylguanine-DNA methyltransferase by affinity chromatography and tandem mass spectrometry. Biochem Biophys Res Commun 337(4):1176–1184. doi:10.1016/j.bbrc.2005.09.177
57. Nouspikel T (2009) DNA repair in mammalian cells : nucleotide excision repair: variations on versatility. Cell Mol Life Sci 66(6):994–1009. doi:10.1007/s00018-009-8737-y
58. Gommans WM, Haisma HJ, Rots MG (2005) Engineering zinc finger protein transcription factors: the therapeutic relevance of switching endogenous gene expression on or off at command. J Mol Biol 354(3):507–519. doi:10.1016/j.jmb.2005.06.082
59. Witkiewicz-Kucharczyk A, Bal W (2006) Damage of zinc fingers in DNA repair proteins, a novel molecular mechanism in carcinogenesis. Toxicol Lett 162(1):29–42. doi:10.1016/j.toxlet.2005.10.018
60. Chahal M, Xu Y, Lesniak D, Graham K, Famulski K, Christensen JG, Aghi M, Jacques A, Murray D, Sabri S, Abdulkarim B (2010) MGMT modulates glioblastoma angiogenesis and response to the tyrosine kinase inhibitor sunitinib. Neuro Oncol 12(8):822–833. doi:10.1093/neuonc/noq017