The 10q25.3-26.1 G protein-coupled receptor gene *GPR26* is epigenetically silenced in human gliomas

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Abstract. Loss of heterozygosity (LOH) of the entire chromosome 10 is the most frequent genetic alteration in human glioblastoma (GBM). In addition to *PTEN/MMAC1* on 10q23.3, clustering of partial deletion break-points on 10q25.3-26.1 points to a second suppressor locus. The proposed target gene *DMBT1* was not confirmed. By somatic deletion mapping of this region, we identified the complementary DNA encoding the human homologue of rat orphan G protein-coupled receptor GPR26. *GPR26* is highly expressed in fetal and adult brain, but frequently reduced or absent in glioma cells and biopsies, due to *de novo* methylation of its 5' CpG island. Silencing of *GPR26* was reversed with 5-aza-deoxycytidine and the histone deacetylase inhibitor trichostatin A. Furthermore, overexpression of *GPR26* in HEK and in U87 glioma cells increased intracellular cAMP concentration which is considered to induce astrocytic differentiation. Interestingly, we observed concomitant silencing of *GPR26* with *O⁶-methylguanine-DNA methyl transferase (MGMT)*, a DNA repair gene co-localized on 10q25.3-26.1 (p=0.0001). We conclude that epigenetic silencing is a common mechanism in malignant gliomas that simultaneously inactivates *MGMT* and *GPR26*. The 10q25.3-26.1 region may contain an important epigenetic pathway in brain tumorigenesis.

Introduction

Loss of heterozygosity (LOH) of the entire chromosome 10 is the most frequent genetic alteration observed in human glioblastoma (GBM). Initial deletion studies on this chromosome led to the identification of the *PTEN/MMAC1* suppressor gene, located on chromosome band 10q23.3. This gene was not only mutated in glioblastomas, but also in a wide range of human cancers with 10q loss (1-6).

Further clustering of partial chromosome 10q deletion break-points by somatic deletion mapping in malignant gliomas suggested the existence of a second more telomeric tumor suppressor locus in the region 10q25.3-26.1 (7-11) Although a gene designated *Deleted in Malignant Brain Tumor 1 (DMBT1)* had been identified at this locus (12,13), its function in tumor suppression of glioma has remained speculative (14).

More distally, the region 10q25.3-26.1 encodes the gene for the DNA repair protein O⁶-methylguanine-DNA methyl transferase (MGMT). Presence of MGMT prevents carcinogenesis by alkylating agents (15). Allelic variants of the *MGMT* gene have been associated with gliomagenesis (16). In fact, the *MGMT* gene promoter is dominantly inactivated by frequent 10q25.3-q26.1 DNA methylation in glioma, and *MGMT* epigenetic silencing turned out to be relevant for patient survival providing a survival advantage when treated with alkylating agents (17-19). However, there is no evidence for a tumor suppressor pathway in which MGMT may be involved, and its gene is located further telomeric to the defined minimally lost area (3,9).

The minimally lost area commonly deletes the gene encoding the homologue of the rat orphan G protein-coupled receptor GPR26 (20) that is further inactivated by epigenetic silencing. Restoration of *GPR26* expression resulted in increased intracellular cAMP levels, a suppressor of tumor progression. This defines *GPR26* as a novel target for 10q25.3-q26.1 epigenetic silencing in glioma.

Materials and methods

Primary tumors and cell lines. BS series are primary tumor tissues obtained from patients diagnosed with primary CNS tumors, were classified according to the World Health Organization (WHO) grading system. Secondary GBM (GBMII) were distinguished from primary GBM (GBMI) when a record of earlier stage of glioma was available (21). All biopsies showed allelic loss of entire 10q or at least of the 10q25.3-26.1 area (9). Normal brain tissue used as template for microarray was obtained from samples of brain surgery for non-neoplastic disease. Human brain tumor cell lines

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LN18, LN71, LN215, LN235, LN308, LN319, LN340, LN401, LN405, LN427, LN428, U87, U343, derived from adult patients with *de novo* glioblastoma were selected for this study (22). The LN cell lines were kindly provided by Erwin van Meir and Nicolas de Tribolet, Lausanne, Switzerland. Cell lines U87 and U343 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). All cell lines, showed loss of heterozygosity at 10q25.3-26.1 (3,9) and the genetic status of *p53, p16, p14^{ARF}, PTEN* tumor suppressor genes has been reported (3,22). Cells were cultured using DMEM supplemented with 10% fetal calf serum and standard antibiotics. Cells were transfected by $CaCl_2$ precipitation. Stably transfected cells were selected by addition of 50 μg/ml Geneticin® into the culture medium for 15-20 days.

Expressed sequence tag markers. Following expressed sequence tags (EST) comprised between 10q25.3-26.1 STS markers D10S221 and D10S575 (Fig. 1) were selected for further analyses: stSG45253, stSG30245, A006D08, WI-17804, WI-16905, Bdy77b02, WI-14638, SGC33199, sts-N35985, SGC35172, WI-16392, WI-17828, WI-15113, WI-18655, stSG29862, stSG3812, WI-6602, stSG1556, A005K22, stSG8848, KIAA0140, stSG13277, SGC38118, SGC30414, A008K16, WI-17472.

RNA extraction and reverse-transcription. Total RNA from biopsies and cell lines was extracted using TRIzol (Life Technologies, Gaithersburg, USA) and reverse-transcribed with *Superscript RNaseH-reverse transcriptase* according to the manufacturer's instructions (Life Technologies). Human fetal brain RNA and cDNA were purchased from Clontech (Palo Alto, CA, USA).

Genomic and transcript analyses of ESTs. Genomic and complementary DNAs were amplified by PCR using the EST markers described above. Multiplex PCR was performed using GAPDH to control PCR amplification.

Extension of EST WI-6602. The phagemid clone 21465 containing the WI-6602 sequence was obtained from the *Image* consortium library (UK HGMP Resource Centre, Hinxton, Cambridge, UK) and sequence was completed using the ABI sequencing system (Perkin-Elmer, Foster City, CA, USA). The 5'-end of the corresponding phagemid was extended by rapid amplification cDNA ends (RACE) from human fetal brain cDNA (Clontech), with primers gga gattgaaccgtttggagaattagggaa and cccctccctcagaaaggtgcca. Confirmation that Hs.278719 and extended Hs.1275 are part of the same transcription unit was provided by RT-PCR on whole fetal brain cDNA template with primers gccac caagaagatcagcaccttca and gagacgaggtgccatgttagatc. Open reading frame (ORF) and sequence homologies were searched through the web resources at the US National Center for Biotechnology Information (NCBI): http://www.ncbi.nlm.nih.gov/BLAST and http://www.ncbi.nlm.nih.gov/gorf/gorf.html, respectively. Multiple sequence alignment was performed with the T-Coffee program available on-line at the Swiss EMBnet node server thttp://www.ch.embnet.org/index.html.

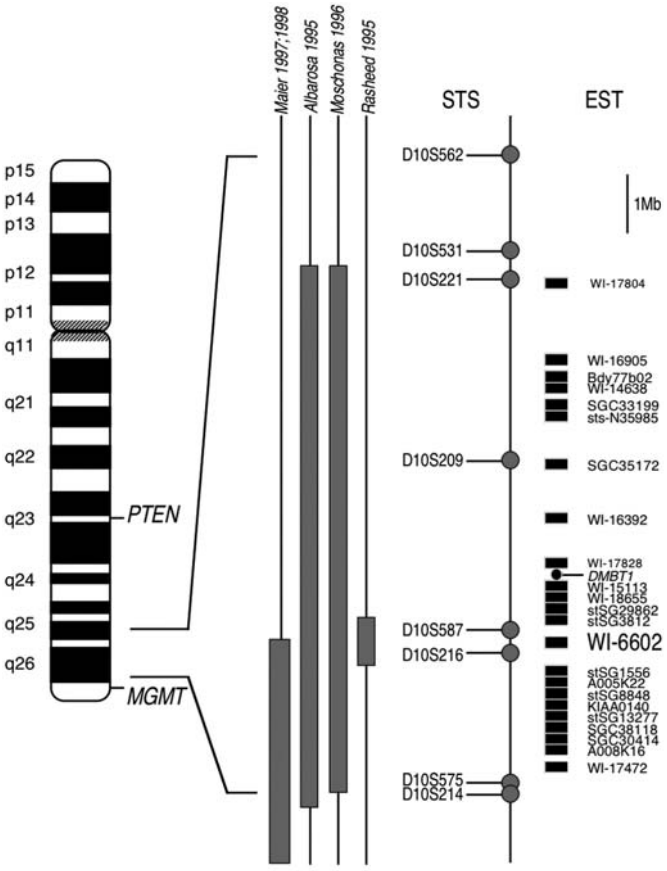

Figure 1. Compilation of somatic deletion mapping studies on 10q25.3-26.1. Chromosome 10 pictogram (left part) with position of *PTEN* and *MGMT* genes and enlargement of the 10q25.3-26.1 area between markers D10S562 and D10S214 (right part). Minimally lost regions (3, 7-11) are shown by grey bars (middle), together with sequence tagged site (STS) and expressed sequence tag (EST) maps (right). Scale bar represents 1 megabase (Mb).

Construction of expression plasmids. *GPR26* cDNA obtained by RT-PCR was cloned into the expression plasmid pcDNA3.1 and sequenced. Open reading frames for enhanced green fluorescent protein (EGFP) and MYC tags were fused in-frame to the 3' end of the *GPR26* coding sequence devoid of stop codon.

Micro-array analysis of glioma mRNA. Total RNA from 12 GBMI, 3 GBMII, and 8 astrocytomas (AS) was amplified and labelled using the Affymetrix 2-cycle amplification protocol as per manufacturer's instructions (Affymetrix). Samples were hybridized to Affymetrix U133v2.0 GeneChips and scanned using an Affymetrix GeneChip scanner as per manufacturer's instructions. Expression values were estimated using the GC-RMA implementation found in the Genedata Refiner 4.1 (Genedata, Basel, Switzerland) package. Data-mining and visualization was performed using the Genedata Analyst 4.1 package. All samples were quantile normalized and median scaled to correct for minor variations in their expression distributions.

Search for somatic GPR26 mutations. Coding regions of exons 1, 2 and 3 were sequenced from amplified genomic DNA of cell lines LN18, LN235, LNz308, LN401, LN427, LN428, U87, U343 and primary tumors BS6T, BS13T and BS30 using the following primers. Exon 1: cctgagcgccggcgc

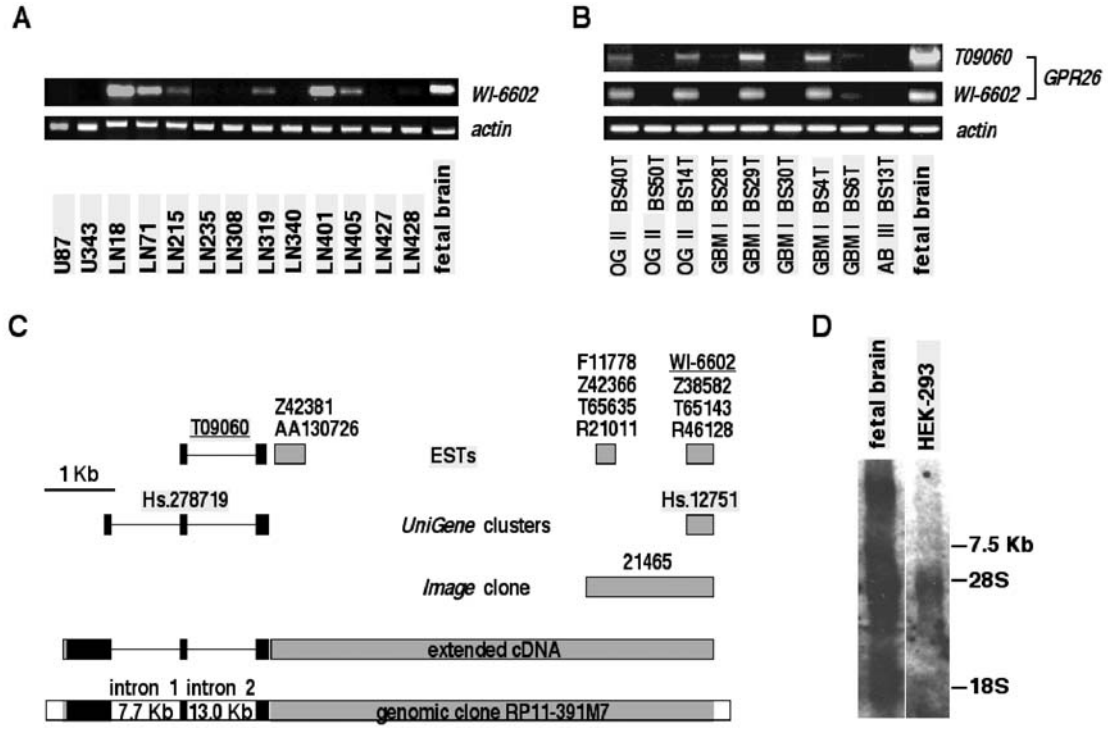

Figure 2. EST WI-6602 is part of the *GPR26* transcription unit. (A) WI-6602 (upper panel) and actin (lower panel) cDNA detection in glioma cell lines by PCR and ethidium bromide staining of agarose gel; (B) T09060 (upper panel), WI-6602 (middle panel) and actin (lower panel) cDNA expression in primary gliomas. WHO tumor grades (II-IV) and differentiation of primary gliomas are indicated. AB, astroblastoma; AS astrocytoma; GBMI, primary glioblastoma; GBMII, secondary glioblastoma; (C) Top, expressed sequence tags (ESTs) and *UniGene* clusters of overlapping ESTs. Middle, 5' extended cDNA. The sequence of phagemid *Image* clone 21465 was completed and 5' extended, resulting in a 6.6 kb cDNA, with a 100% continuous homology with the human chromosome 10 genomic clone RP11-391M7 and overlap at its 5'-end with the infant brain-specific *UniGene* cluster Hs.278719. Confirmation that both Hs.278719 and extended Hs.12751 transcribed are part of the same transcription unit was provided by RT-PCR. Bottom, more 5'-end sequences were identified based on a 92% sequence homology between the *UniGene* cluster Hs.278719 and the rat orphan G protein-coupled receptor GPR26 (20). *GPR26* coding region is indicated by filled boxes, introns are not to scale; (D) Size of the WI-6602 transcripts. Northern blotting of 15 μg total RNAs from fetal brain and from the embryonic kidney cell line HEK293 probed with cDNA clone 21465 containing WI-6602.

ggggcgcg and ctcccactcccgccgagatcccgcgaggct; exon 2: gcccaaggtcaatagcta and gacacacctgccaatcaggc; exon 3: ggctagtggagctcttctccacggtg and gagacgaggtgccatgttagatc.

Restoration of GPR26 expression. Glioma cell lines were plated at low-density and incubated with 1 μM 5-aza-2'-deoxycytidine (AZA), and/or 1 μM trichostatin A (TSA). Optimal conditions were found to be a 48-h treatment with AZA, and TSA added 6 h before cells were harvested. *GPR26* expression was assessed using *GPR26* (gccaccaagaagatcagca ccttca and gagacgaggtgccatgttagatc) and *ACTB* (ggtgtaacgcaa ctaagtcatag and gcatggagtcctgtggcatccacg) primers as internal control on reverse-transcribed RNA. PCR products were separated on agarose gels. *GPR26* products were also subjected to Southern blotting.

DNA modification. Tumor genomic DNA was modified with bisulfite following the protocol previously described (23). Primers *CGGGTACC*TTTtTTtgggagTTatgg and *CGGGATC* caAcaAAcaAaAcaAcacc were used to directly amplifiy and sequence modified DNA from biopsies, as described in Fig. 4.

Intracellular cAMP measurement. Analysis was performed with the commercially available kit cAMP Direct Biotrak (Amersham, Piscataway, NJ, USA) following manufacturer's instruction. Standard deviations were calculated based on 2 measurements.

Cell proliferation assay. Bromodeoxyuridine (BrdU) was added to the medium 1 h before cell harvesting at 10 μmol/l concentration. Cells were labeled with an anti-BrdU antibody and DNA staining dye (7-AAD). Fluorescence-activated cell sorting (FACS) analysis was done according to the manufacturer's instructions (Becton Dickinson, Franklin Lakes, NJ).

GenBank accession numbers. The sequence of the human *GPR26* mRNA was deposited under the accession number AJ505757 (EMBL), and the deduced peptide sequence under the accession number CAD44281 (Genbank) and Q8NDV2 (SwissProt).

Results

Identification of the GPR26 transcript. We selected from the region surrounding the 10q25.3-26.1 minimally lost area (3,7,9-11) 26 EST markers and tested them by PCR on genomic and complementary DNAs of glioblastoma cell lines and primary brain tumors of WHO grades II-IV (Fig. 1). No homozygous deletions were detected following amplification of genomic tumor-derived DNA. However, the EST marker WI-6602 showed expression levels comparable to fetal brain in only 3 out of 13 glioma cell lines (23%), with marked decrease or loss of expression in the other lines (Fig. 2A) and in 2/6 primary malignant gliomas with established allelic

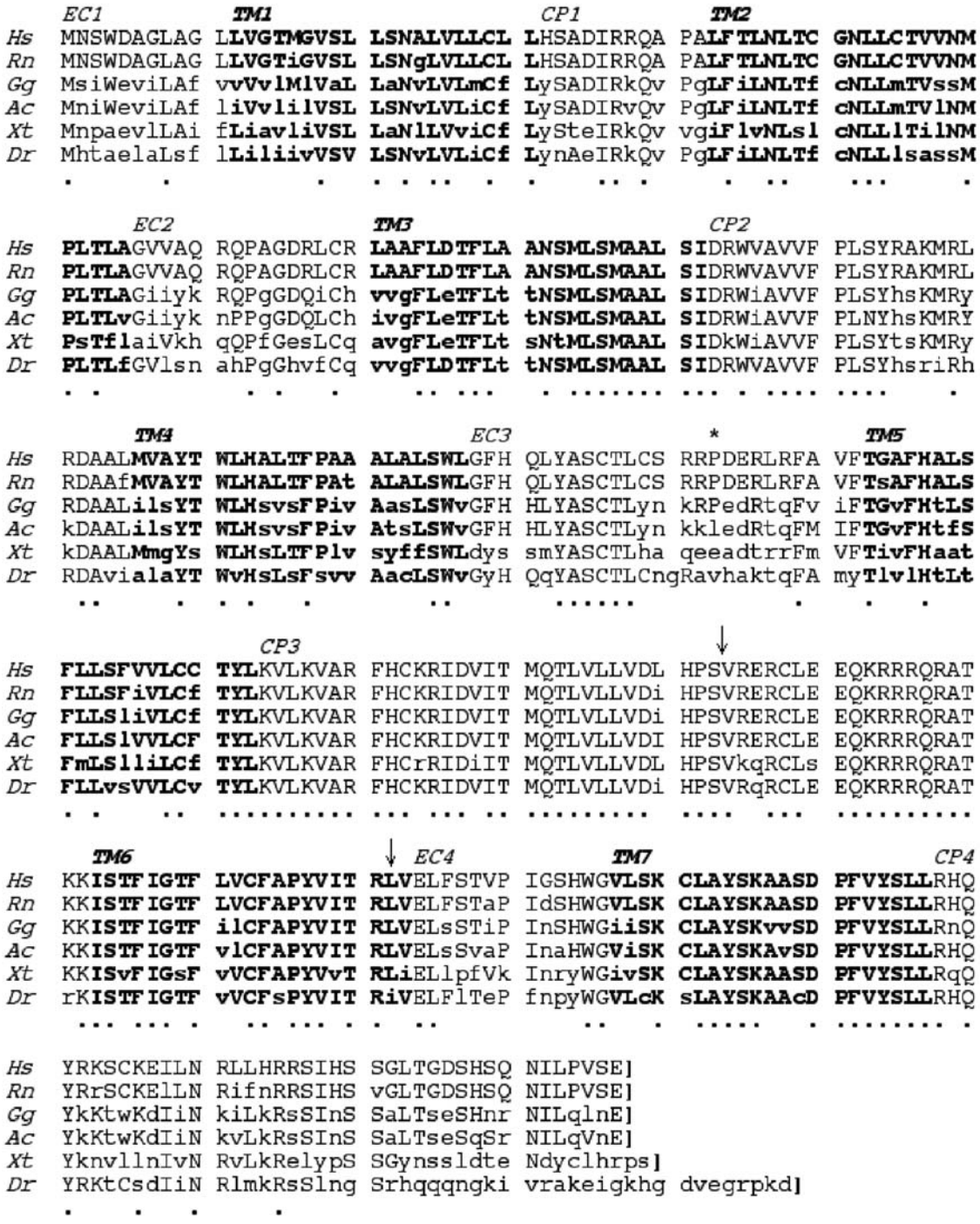

Figure 3. Sequence similarities between vertebrate GPR26 orthologues. Primary sequence alignment of human (*Hs*) CAD44281; rat (*Rn*) AAF21012; bird (*Gallus gallus, Gg*) XP_421809; reptilian (*Anolis carolinensis, Ac*); amphibian (*Xenopus tropicalis, Xt*) and bone fish (*Danio rerio, Dr*) XP_693338. Reptile GPR26 was reconstituted by tBlastn search on translated *Anolis carolinensis* genome with the human sequence CAD44281. Amino acid one-letter code used. Evolutionary conserved amino acids are uppercase and highlighted with dots; amino acids divergent from the human sequence are lowercase and grey; transmembrane domains (TMs) described by Lee *et al* (20) are bold; extra-cytoplasmic (EC) and cytoplasmic (CP) domains are plain. Arrows indicate codons S223 and R261 interrupted by exon boundaries. Asterisk indicates codon P163 in which the synonymous single nucleotide polymorphism CCG→CCA (rs12263344) was observed.

loss in the 10q25.3-26.1 area (9) (Fig. 2B, middle row). Genomic position of WI-6620 validates the compilation of the minimally areas of loss shown in Fig. 1.

WI-6602 is part of the *UniGene* cluster Hs.12751 (Fig. 2C, top) expressed in infant brain. 5' extension of this cDNA gave rise to a 7512-bp cDNA that also covered the infant brain-expressed *UniGene* cluster Hs.278719, sequence of which displays 92% nucleotide sequence homology with the cDNA encoding the rat orphan G protein-coupled receptor GPR26 (20). RT-PCR revealed also consistent expression profiles between *GPR26* EST markers WI-6602 and T09060 in primary human glial tumors (Fig. 2C). Definition of a single transcription unit that covers both *Unigene* clusters was in agreement with the 7.5 Kb-long transcript size detected by

Table I. Status of human gliomas at SNP rs12263344.

Glioma		SNP status
GBM cell lines		
U87		P163: CCG
U343		P163: CCA
LN18		P163: CCG
LN235		P163: CCA
LN308		P163: CCG
LN401		P163: CCA
LN427		P163: CCA
LN428		P163: CCG
Primary tumors	**Tumor type/grade**	**SNP status**
BS13T	AB III	P163: CCG
BS6T	GBM I	P163: CCA
BS30T	GBM I	P163: CCA

GBM I, primary gliobastoma; AB, astroblastoma WHO grade III.

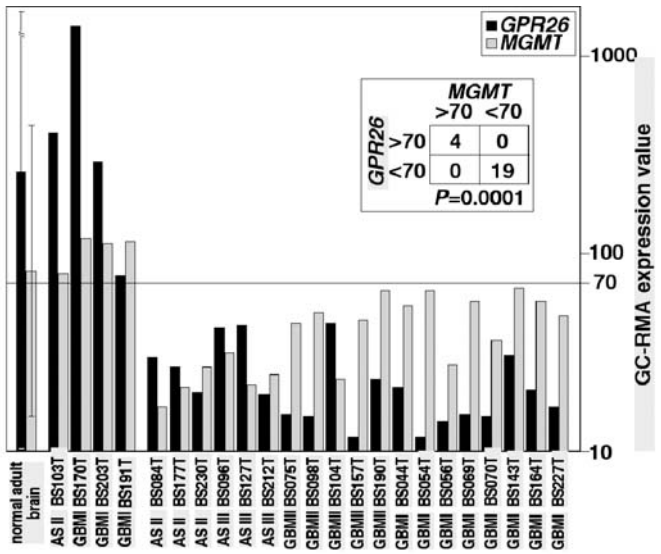

Figure 4. Co-expression of *GPR26* and *MGMT* in primary gliomas. Microarray expression analysis of *GPR26* (black) and *MGMT* (grey) on microarrays of 23 primary gliomas. GC-RMA expression values for *GPR26* (Affymetrix probeset ID 244617_at) and *MGMT* (Affymetrix probeset ID 204880_at) transcripts are shown on the y-axis. Significant co-expression (p=0.0001, Fisher's exact test) is shown by using a cut-off of 70 (thicker line) between low and high expression levels for both transcripts.

Northern blot analysis of fetal brain RNA probed with the *Image* cDNA clone 21465 (Fig. 2D).

GPR26 contains two introns that split the coding sequence of 1014 nucleotides into three parts of 668, 114 and 232 nucleotides respectively, at codons 223 and 321 (Fig. 3, arrows). The encoded GPR26 protein consists of 337 amino acids 96% identical with rat gpr26. Comparison with other verebrate orthologues shows the highest sequence conservation at the third cytoplasmic domain (Fig. 3). We sequenced the *GPR26* coding region from eight glioblastoma cell lines and three primary brain tumors with allelic loss in this area (3,9). No mutations were detected, except for the synonymous single nucleotide polymorphism rs12263344 at codon encoding proline 163 (CCG→CCA) detected in 6/11 of tumors as well as in corresponding germline lymphocyte DNA (Fig. 3, star; Table I). Given that the upstream region of the *GPR26* gene contains a dense CpG island (Fig. 4, top), (24) that the 10q25.3-q26.1 gene *MGMT* is frequently subject to frequent silencing by hypermethylation in gliomas (17) and that epigenetic silencing in cancer can cover chromosomal regions of several megabases, we hypothesized that *GPR26* was inactivated by epigenetic silencing.

Re-expression of GPR26 by 5-aza-deoxycytidine and/or trichostatin A. We analyzed RNA expression of *GPR26* and *MGMT* genes, both located within 6 megabases of the telomere of chromosome 10q on 23 primary glioma microarrays. Definition of a cut-off of 70 GC-RMA expression values for both transcripts showed highly significant (p=0.0001) co-expression of *GPR26* and *MGMT*, suggesting a common mechanism of down-regulation (Fig. 4). On the other hand, assessment of the methylation status of the 5' CpG island (23) of *GPR26* in 14 primary gliomas revealed a cluster of methylated CpG within 60 nucleotides surrounding the *GPR26* translation initiation codon (Fig. 5A). Moreover, comparison of the methylation status of this area with *GPR26* expression profiles in 9 glioma cells and biopsies indicates a correlation between complete methylation of the CpG doublet located 12 bp downstream of the ATG codon and little or no *GPR26* expression. In contrast, limited or no methylation is needed for *GPR26* expression as shown for LN18 and LN71 (Figs. 2A and 5B), but not sufficient, as seen in LN308. Addition of the methylation inhibitor 5-aza-deoxycytidine (AZA) into culture medium restored *GPR26* expression in U87 cells, while the histone deacetylase inhibitor trichostatin A (TSA) was required in U343 cells (Fig. 5C), suggesting that histone deacetylation is an additional contributor of *GPR26* epigenetic silencing.

Re-expression of GPR26 increases cAMP levels. G protein-coupled receptor signaling is mediated via cAMP increase. Initial transfection of the GPR26-negative HEK293 cells, routinely used for cAMP studies (25), with the human *GPR26* cDNA resulted in a 7-fold increase of intracellular cAMP levels (Fig. 6A). In U87 cells, that do not express *GPR26* mRNA either, intracellular cAMP levels were elevated by a factor of 2 following transfection with a GPR26 expression vector. A previous observation suggested a link between high intracellular cAMP levels and lower proliferative activity in HEK293 cells (20). However, comparison of HEK293 stably transfected to express GPR26 with control HEK293 cells did not show evidence for changes in the distribution of cell cycle phases G0/G1, G2/M and S (Fig. 6B). Since it has been established in rat C6 glioma cells that high intracellular cAMP levels are associated with astrocytic differentiation (26) our data support that GPR26/cAMP signaling may rather play a role in differentiation of human glioma.

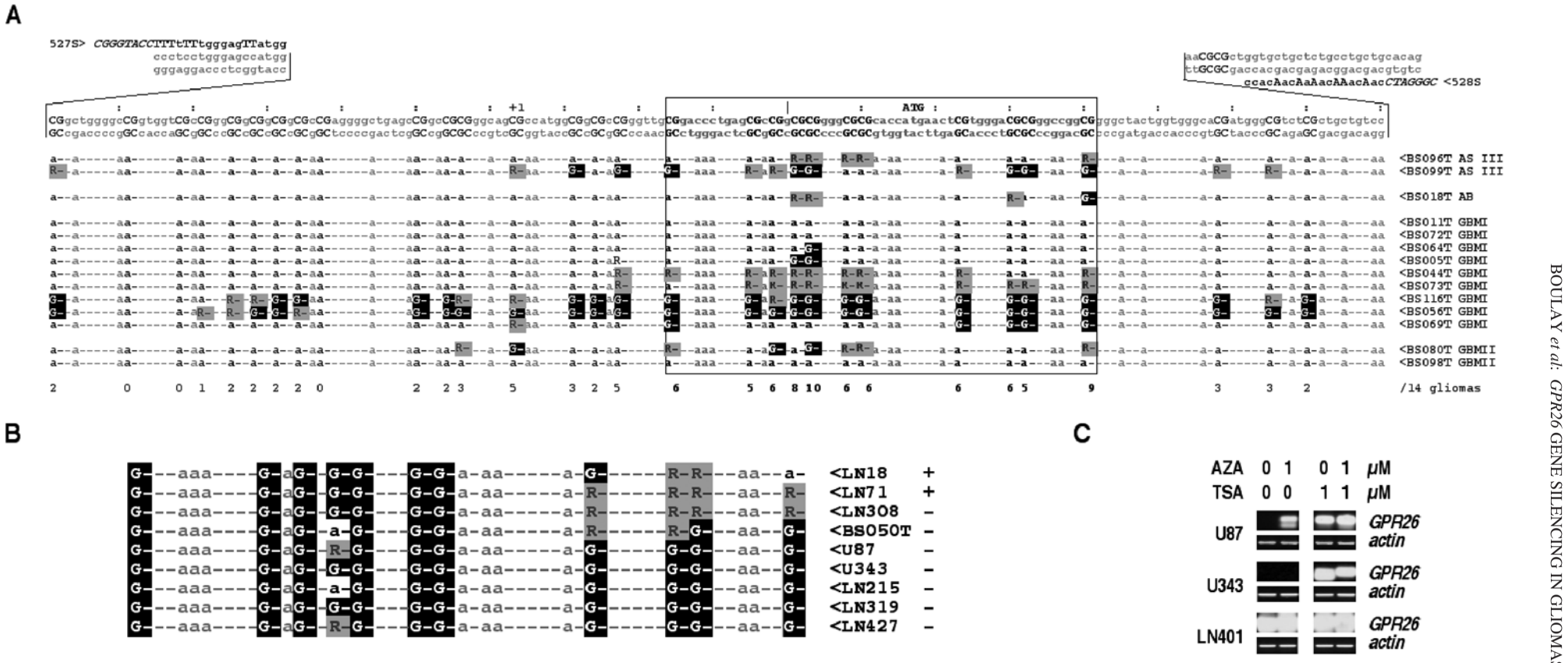

Figure 5. Reversion of *GPR26* gene silencing. (A) Top, *GPR26* genomic sequence (shown double stranded) surrounding the *GPR26* transcription (+1) and translation (ATG) initiation sites. CpG doublets are uppercase. Primers 527S and 528S used to amplifiy modified DNA are aligned with wild-type DNA. Nucleotides shown uppercase and in italics in primers 527S and 528S represent modified and stabilizing nucleotides a 5'-ends, respectively. Middle, methylation status of *GPR26* in primary gliomas. G associates with a C protected from modification by methylation; A recognizes a modified C as a T; a mixed population of G and A is shown as a 'R' (purine). Bottom, number of occurrence of methylated CpG among 14 primary gliomas. The most frequently methylated area is boxed; (B) *GPR26* methylation status in gliomas. '+' stands for high *GPR26* expression and '-' for low or no expression from the data shown in Fig. 2A and B; (C) Restoration of *GPR26* expression in glioma cells. Cell lines U87, U343 and LN401 were grown in the presence or not of 5-aza-deoxycytidine (AZA) and/or trichostatin (TSA) and assayed for RNA expression of *GPR26* (top) and *actin* (bottom) by RT-PCR. Actin, ethidium bromide staining of agarose gel. *GPR26*, inverted image from Southern blot analysis.

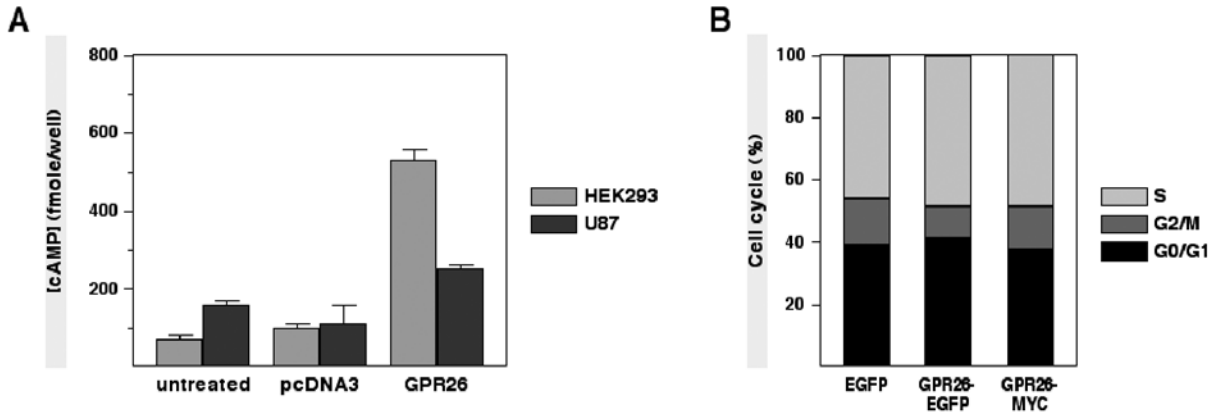

Figure 6. (A) Induction of intracellular cAMP upon GPR26 transfection. cAMP levels in HEK293 and in U87 are shown in light grey and dark grey, respectively; (B) Cell cycle analysis of HEK293 expressing GPR26. Phase distribution of HEK293 cells transfected with a plasmid expressing control enhanced green fluorescent protein (EGFP, left); a plasmid expressing GPR26 tagged by 3' fusion with EGFP (GPR26-EGFP, middle) or a plasmid expressing GPR26 tagged by 3' fusion with MYC (GPR26-MYC, right). Data based on two independent experiments.

Discussion

Earlier studies delimited the *Deleted in Malignant Brain Tumor 1 (DMBT1)* locus (10) and the corresponding gene between markers D10S209 and D10S587. However, *DMBT1* has been reported not to be specifically targeted in primary gliomas (14), and corresponding transcripts were mainly isolated from tissue of the gastro-intestinal tract and from lung, but, surprisingly not from brain tissue (12). This is also consistent with the two independent studies (3,9,11) that genetically excluded *DMBT1* as a suppressor gene. More telomeric, *MGMT* has been identified as a main target gene for DNA methylation-based inactivation with high prevalence in low-grade astrocytomas (18,19). Since MGMT prevents carcinogenesis by alkylating agents (15) and its gene frequently inactivated in gliomas, MGMT is a critical 10q25.3-26.1 tumor suppressor operative in brain.

GPR26 is located within the smallest interval consistent with all the somatic deletion studies reported on distal 10q in gliomas (Fig. 1). On the other hand, 10q25.3-26.1 epigenetic silencing appears to potentially inactivate a large array of genes in brain tumors. Moreover, EST markers contained within the *GPR26* transcript were exclusively isolated from the infant brain cDNA library (*UniGene* Library No. 37), and are highly expressed in human adult brain as well as in rat adult brain (20). Thus, *GPR26* expression profile in normal brain and frequent absence of expression in gliomas would also be compatible with a tumor suppression function specific of the glial lineage. In the particular case of GPR26, hemizygous loss in conjunction with epigenetic silencing of the 10q25.3-26.1 area of the remaining copy would be needed for gene inactivation in brain tumors.

Gene silencing by aberrant promoter methylation has been extensively reported in various tumor types, including glioma (27-31). It has recently been shown that CpG methylation in colorectal cancer can span several megabases, thereby silencing expression of genes located in a same region (24). On the other hand, TSA inhibits histone deacetylase, a process required for chromatin decondensation and initiation of gene transcription (32). Interestingly, histone deacetylase is a promising drug target for cancer treatment (33). In fact, both epigenetic mechanisms have been linked to the transcriptional repressor MeCP2 (34). It is not clear whether TSA alone can restore expression of methylated genes, or whether synergy with 5-aza-deoxycytidine is required (35). Our data show that, for example in glioblastoma cell lines U87 and U343, TSA alone can be sufficient to induce re-expression of *GPR26*.

Whether GBM occur *de novo* or derive from a lower grade astrocytoma has suggested the clinical distinction between primary and secondary GBM, respectively. This classification has been further supported by distinct prevalences of altered glioma pathways (21). Based on the prevalence of *PTEN* (10q23) loss in primary GBM, *GPR26* (10q25-26) expression has recently been proposed as a recognition marker between primary and secondary GBM, based on the observation that expression of this gene is reduced with increasing age (36). Among our samples, 25/32 (78%) of primary GBM and 2/3 (67%) of secondary GBM had 10q loss (9). We also observed parallel expression between *GPR26* and *MGMT* (p=0.0001) (Fig. 4), while *MGMT* (10q26) low expression and *TP53* mutation are frequently associated in secondary GBM (21). However, we found *GPR26* expression to be reduced or lost in all glioblastoma samples whether they were primary and secondary GBMs (9,21). Likewise, 10q deletion and epigenetic silencing was found in both primary and secondary GBM (21). We therefore believe that definition of the *GPR26* status in a larger number of GBM may help to clarify this issue and possibly to define a molecular subset of GBM.

Upon GRP26 transfection, HEK293 cells showed a marked increase of intracellular cAMP levels while elevation of cAMP levels was 2-fold in U87 glioblastoma cells. The attenuated response in U87 cells may be due to the low transfection efficiency of glioma cells, or the absence of putative GPR26 ligand. Although the cognate ligand for the orphan G-protein-coupled GPR26 remains to be identified, the fact that GPR26 has close homology to the serotonin receptor 5-HT5A identifies GPR26 as a member of the amine-like receptor subfamily of G protein-coupled receptors. In addition, full activation of this signaling pathway may need additional components that can also be lacking in glioblastoma cells. Indeed, as

described for other G protein-coupled receptors that are parts of multi-chain receptor complexes, GPR26 forms a hetero-oligomer with the serotonin receptor 5-HT1A (37,38).

Possible roles of GPR26/cAMP signaling in tumorigenesis have been suggested. A single study has showed that expression of rat *gpr26* reduced the growth rate and induces morphological changes in HEK293 cells (20). However, in our hands, GPR26 expression in HEK293 cells did not alter distribution of cell cycle phases. Other observations established that high intracellular cAMP levels are associated with astrocytic differentiation in rat cortical precursor cells (26). In addition, defects in cAMP pathway were shown to potentially initiate carcinogenesis in cells of the central nervous system (39). Our data show that *GPR26* together with *MGMT* belong to a region on 10q25.3-26.1 that is frequently inactivated by hemizygous deletion and epigenetic silencing of the residual alleles. This region may therefore represent an important epigenetic pathway in brain tumorigenesis.

Acknowledgements

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