Anaplastic oligodendrogliomas with 1p19q codeletion have a proneural gene expression profile

François Ducray¹, Ahmed Idbaih¹, Aurélien de Reyniès², Ivan Bièche³, Joëlle Thillet¹, Karima Mokhtari¹, Séverine Lair⁴, Yannick Marie¹, Sophie Paris¹, Michel Vidaud³, Khê Hoang-Xuan¹, Olivier Delattre⁴, Jean-Yves Delattre¹ and Marc Sanson*¹

Address: ¹Unité INSERM U711, Université Paris VI, 47-83 Boulevard de l’Hôpital, 75013 Paris, France, ²Programme Cartes d’Identité des Tumeurs (CIT), Ligue Nationale Contre le Cancer, Service de Bioinformatique, 14 rue Corvisart, 75014 Paris, France, ³Unité INSERM U745, Faculté des Sciences Pharmaceutiques et Biologiques, Université René Descartes – Paris V, 4 avenue de l’Observatoire 75270 Paris, France and ⁴Unité INSERM U830, Unité de Génétique Somatique et Service de Bio-Informatique, Institut Curie, 26 rue d’Ulm, 75006 Paris, France

Email: François Ducray - francoisducray@yahoo.fr; Ahmed Idbaih - ahmed.idbaih@gmail.com; Aurélien de Reyniès - reyniesA@ligue-cancer.net; Ivan Bieche - ivan.bieche@univ-paris5.fr; Joelle Thillet - thillet@ext.jussieu.fr; Karima Mokhtari - karima.mokhtari@psl.aphp.fr; Sèverine Lair - severine.lair@curie.fr; Yannick Marie - yamarie@ccr.jussieu.fr; Sophie Paris - sophie.paris@ccr.jussieu.fr; Michel Vidaud - michel.vidaud@univ-paris5.fr; Khê Hoang-Xuan - khe.hoang-xuan@psl.aphp.fr; Olivier Delattre - olivier.delattre@curie.fr; Jean-Yves Delattre - jean-yves.delattre@psl.aphp.fr; Marc Sanson* - marc.sanson@psl.aphp.fr

* Corresponding author

Abstract

Background: In high grade gliomas, 1p19q codeletion and EGFR amplification are mutually exclusive and predictive of dramatically different outcomes. We performed a microarray gene expression study of four high grade gliomas with 1p19q codeletion and nine with EGFR amplification, identified by CGH-array.

Results: The two groups of gliomas exhibited very different gene expression profiles and were consistently distinguished by unsupervised clustering analysis. One of the most striking differences was the expression of normal brain genes by oligodendrogliomas with 1p19q codeletion. These gliomas harbored a gene expression profile that partially resembled the gene expression of normal brain samples, whereas gliomas with EGFR amplification expressed many genes in common with glioblastoma cancer stem cells. The differences between the two types of gliomas and the expression of neuronal genes in gliomas with 1p19q codeletion were both validated in an independent series of 16 gliomas using real-time RT-PCR with a set of 22 genes differentiating the two groups of gliomas (AKR1C3, ATOH8, BMP2, C20orf42, CCNB1, CDK2, CHI3L1, CTNNBP2, DCX, EGFR, GALNT13, GBP1, IGFBP2, IQGAP1, LICAM, NCAM1, NOG, OLIG2, PDPN, PLAT, POSTN, RNF135). Immunohistochemical study of the most differentially expressed neuronal gene, alpha-internexin, clearly differentiated the two groups of gliomas, with 1p19q codeletion gliomas showing specific staining in tumor cells.

Conclusion: These findings provide evidence for neuronal differentiation in oligodendrogliomas with 1p19q codeletion and support the hypothesis that the cell of origin for gliomas with 1p19q codeletion could be a bi-potential progenitor cell, able to give rise to both neurons and oligodendrocytes.

Published: 20 May 2008

Molecular Cancer 2008, 7:41 doi:10.1186/1476-4598-7-41

Received: 15 December 2007

Accepted: 20 May 2008

This article is available from: http://www.molecular-cancer.com/content/7/1/41

© 2008 Ducray et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Background
The 1p19q codeletion and EGFR amplification are mutually exclusive and related to dramatically different outcomes in high grade gliomas. The 1p19q codeletion is strongly associated with an oligodendrogial phenotype and favorable prognosis [1]. It has recently been shown to be mediated by a specific t(1;19)(q10;p10) translocation [2]. To date the efforts performed to identify the genes specifically involved in the breakpoint have failed, mostly because both 1p and 19q centromeric regions contain highly repeated sequences. As a consequence the molecular mechanisms underlying the particular phenotype and the favorable outcome of this subset of gliomas remain completely unknown. Reliable detection of 1p19q codeletion requires an appropriate technique, such as CGH-array. Indeed, the most widely used LOH studies may not distinguish this signature from partial distal 1p and 19q deletion or gain, which have radically different prognostic implications [1]. On the other hand, EGFR amplification is tightly associated with chromosome 10 loss and gain of chromosome 7, representing another characteristic genomic signature [3]. EGFR amplification is more frequent in glioblastomas, but it is also found in a subset of anaplastic oligodendrogliomas and, in this setting, is predictive of extremely poor prognosis [4]. Recently, malignant gliomas have been separated into three expression profiles with distinct outcomes and histological correlations: 1) the proneural profile with a better prognosis, mostly corresponding to anaplastic gliomas (oligodendrogliomas and astrocytomas); 2) the proliferative and 3) mesenchymal profiles, corresponding mainly to glioblastomas [5]. However, correlation with 1p19q codeletion is still missing. Based on a set of gliomas analyzed by CGH-array [3], we selected tumors displaying one of these two characteristics and mutually exclusive patterns -1p19q codeletion or EGFR amplification- and compared their gene expression profiles.

Methods

Samples
The microarray study was done on 13 gliomas selected from the Salpêtrière database, based on the following criteria: 1) CGH-array profile showing either whole 1p19q codeletion or EGFR amplification, 2) high quality RNA availability. The samples were provided as snap-frozen sections of areas immediately adjacent to the region used for the histopathological diagnosis according to the World Health Organization Classification (WHO 2000). This set included 4 grade III oligodendrogliomas with complete 1p19q codeletion and 9 gliomas with EGFR amplification (5 glioblastomas (GBM), 3 grade III oligodendrogliomas, 1 grade III oligoastrocytoma (OAI)). Genomic characterization was performed using CGH array as previously described [1]. Among the 9 tumors with EGFR amplification, 8 out of 9 had chromosome 10q loss and chromosome 7 gain; 4 had a gain of chromosome 1p, and 4 had a partial loss of chromosome 1p. Among the 4 tumors with complete 1p19q codeletion none had EGFR amplification, 10q loss or chromosome 7 gain, and 2 had complete chromosome 4 loss. In order to compare the gene expression profile of the gliomas with normal brain, we used the gene expression data of 5 samples of corpus callosum (GSM175855, GSM175856, GSM175857, GSM175858, GSM176050) and 5 samples of cortex (GSM176049, GSM176344, GSM176345, GSM176346, GSM176347), available in the Gene Expression Omnibus repository (GSE7307) [6]. To compare the gene expression profile with glioblastomas cancer stem cells (CSC), we used the data of Beier et al. (GSE18711) [7]. All raw and normalized data files for the microarray analysis have been deposited [8] at the European Bioinformatics Institute (Hinxton, UK), and are publicly available under accession number E-MEXP-1507.

RNA extraction and hybridization
Approximately 50 mg of tissue from each tumor were used for total RNA extraction using the RNeasy Lipid Tissue mini kit (Qiagen, CA), according to the manufacturer instructions. RNA quality was verified with the Bioanalyzer System (Agilent Technologies, Paolo Alto, CA), using the RNA Nano Chips. One and half micrograms of RNA were processed for hybridization on the Genechip Human Genome U133 Plus 2.0 Expression array (Affymetrix, CA), which contains over 54,000 probe sets analyzing the expression level of over 47,000 transcripts and variants, including 38,500 well-characterized human genes. The processing was done according to the recommendations of the manufacturer.

Data analysis
Except as indicated, all transcriptome analysis was carried out using either R-system software (version 2.4.1) packages including those of Bioconductor [9] or original R code (A. de R.). Normalization was performed using the RMA method [10]. Clustering analysis was performed as previously reported [11]. Class comparison using a univariate t-test was performed using BRB Array Tools developed by Dr. Richard Simon and Amy Peng Lam [12]. A p-value < 0.001 was used to define differentially expressed genes. Gene set enrichment analysis was performed using GSEA v2.0 software [13] as described by Subramanian et al. [14]. For enrichment analysis in specific gene ontology terms (GO terms), we used a hypergeometric test to measure the association between a gene (probe set) list and a GO term. To this end, we mapped both the gene list and GO term related proteins to non-redundant Entrez Gene identifiers. The mapping of a probe set list to Entrez Gene ids was done using the annotation file HG-U133_Plus_2.annot.csv [15]. For each GO term, we obtained the list of non-redundant related protein identi-
fiers – either directly associated with the GO term or with one of its descendants – and mapped it to a non-redundant list of Entrez Gene ids. GO terms and their relationships (parent/child) were downloaded from The Gene Ontology site [16]. The list of proteins associated with GO terms (table gene_association.goa_human) was downloaded from [17]. We designated a threshold significance level for the hypergeometric test of $p < 0.01$ and the criteria that a GO term was represented by at least 2 Entrez Gene identifiers. Enrichment analysis of genes located on specific chromosomes was performed using DAVID tools [18].

**Real-time RT-PCR**

Quantitative RT-PCR was performed as previously described [19]. TBP (Genbank accession no. NM_003194), which encodes the TATA box-binding protein (a component of the DNA-binding protein complex transcription factor II D), was selected as an endogenous control because the levels of its transcript did not change across various normal tissues and tumor samples. The expression of the following 22 genes was quantified: AKR1C3 (upper primer (UP) 5’-CGT ATC ACC GCC AGG TTA ATT TCT TCC T-3’, lower primer (LP) 5’-GGG GAG GAG GAT GCC CAC GAT-3’); C20orf42 (UP 5’-CGC AGG TTC CCA TGG TAT-3’, LP 5’-GGC ACA ACT TCG CAG CCT CTA-3’); BMP2 (UP 5’-GC ACG TTG ACC CAT GAA GAA-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); C20orf42 (UP 5’-AGG GAA CTG CCA GAA GAA ACT GAC-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); C20orf42 (UP 5’-AAG GAA CTT GAA CAA GGA GAA CCA-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); CDK2 (UP 5’-AGG GCC AGC TGG ATT TTA TCG CAA AT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); CDK2 (UP 5’-AGG GCC AGC TGG ATT TTA TCG CAA AT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); CHISL (UP 5’-GAC CAC AGG CCA TCA CAC TCC-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); CTTNP2 (UP 5’-GCC TCT CCA TCC TTT TTG AAC CAG T-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); DCX (UP 5’-AGG CAA GAG CCC TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); EGF (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); EGFR (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GALNT13 (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GBP1 (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GBP1 (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA (UP 5’-AGG GAA CTG CCA TGG TCC TAT-3’, LP 5’-GGG GAA CTG GCC CAA GAA ACT GAC-3’); GGA

**Results**

**Transcriptomic differences partly reflect the underlying genomic alterations**

To study the relationship between the differences in gene expression profile and the underlying genomic alterations, we looked at the genomic localization of the differentially expressed genes. Class comparison using a univariate t-test demonstrated that 4458 probe sets were differentially expressed between the two groups of gliomas with a p-value < 0.001 and a maximum false discovery rate of 1.2% (see Additional file 1). The set of overexpressed genes in gliomas with EGFR amplification was significantly enriched in genes located on chromosome 1 (305 genes, $p < 10^{-4}$), chromosome 19 (151 genes, $p < 10^{-4}$), chromosome 7 (109 genes, $p < 10^{-4}$) and chromosome 4 (85 genes, $p < 10^{-4}$). In contrast, the set of overexpressed genes in gliomas with 1p19q codeletion was significantly enriched in genes located on chromosome 10 (216 genes, $p < 10^{-4}$). Thus, gene expression and copy number variation dynamics were tightly correlated in both tumor groups. This was even more obvious when we plotted the differentially expressed probe sets localized on chromosome 1, 19 and 10 according to their genomic localization and the log2 ratio of their geometric mean in both tumor groups (Figure 1). Indeed, almost all differentially expressed genes localized on 1p and 19q were underexpressed in gliomas with 1p19q codeletion, whereas differentially expressed genes localized to 1q and 19p were equally distributed (Figure 1).
Genomic localization of the differentially expressed probe sets ($p < 0.001$) localized on chromosome 1, 19 and 10. Each probe set is represented by a dot. Probe sets are ordered along the x axis according to their genomic position (only probe sets with unambiguous genomic mapping on UCSC were used). For each chromosome, the telomere of the short arm is on the left, and the telomere of the long arm is on the right. The dashed vertical line represents the position of the centromere. The y axis corresponds to the log2 ratio of the geometric mean in the gliomas with complete 1p19q loss versus the gliomas with EGFR amplification. Almost all probe sets localized on 1p and 19q were underexpressed in gliomas with 1p19q codeletion, whereas most of the probe sets localized on chromosome 10 were overexpressed.
**Unsupervised analysis consistently distinguishes the two groups of gliomas**

In order to study the differences in gene expression profile, we performed unsupervised hierarchical clustering analysis of the 13 glioma samples. This analysis was done using the 1366 probe sets whose expression varied the most across samples (this corresponds to the probe sets with a robust coefficient of variation (rCV) superior to the 97.5th rCV percentile). As shown in Figure 2, gliomas with 1p19q codeletion and gliomas with \textit{EGFR} amplification segregated into two distinct groups. This clustering was extremely robust and was conserved across different gene

---

**Figure 2**

Unsupervised clustering of 4 oligodendrogliomas with 1p19q codeletion and 9 gliomas with \textit{EGFR} amplification. Unsupervised hierarchical clustering was performed using the 1366 probe sets whose expression varied the most across the 13 samples (probe sets with a robust coefficient of variation superior to the 97.5th percentile). Samples and genes were clustered using Ward’s linkage and 1-Pearson correlation coefficient. For each probe set, data were median-centered (white), with the lowest and highest intensity values in blue and red, respectively. 1p19q = 1p19q codeletion, \textit{EGFR} = \textit{EGFR} amplification. The gliomas were classified into 2 groups according to their genomic profile. Gliomas with \textit{EGFR} amplification were classified into one cluster irrespective of their histology (red = glioblastoma, green = grade III oligodendroglioma, blue = grade III oligoastrocytoma). Gene cluster A was enriched in genes involved in proliferation, extracellular matrix, immune response, embryonic development and angiogenesis. Gene cluster B was enriched in genes involved in synaptic transmission. Gene cluster C was enriched in genes involved in neurogenesis and synaptic transmission.
lists and clustering methods. The genes were classified into three clusters: one cluster of genes overexpressed in gliomas with EGFR amplification (gene cluster A, 698 genes), one cluster of genes overexpressed in oligodendrogliomas with 1p19q codeletion (gene cluster C, 488 genes) and a smaller cluster of genes expressed by some samples of both groups (gene cluster B, 180 genes). Enrichment analysis was performed on these three gene clusters. First, chromosome enrichment analysis demonstrated that these gene clusters did not simply reflect the underlying genomic alterations. Indeed, gene cluster A was enriched in genes located on chromosome 4 (53 genes, p < 10^-4) and on chromosome 7 (43 genes, p < 10^-2), but not in genes located on chromosome 1 or chromosome 19. Neither gene cluster C nor gene cluster B was enriched in genes located on chromosome 10. In contrast, the three gene clusters were enriched in genes with specific ontologic classes. Gene cluster A was most significantly enriched in genes involved in: immune response (55 genes, p < 10^-4), extracellular matrix (37 genes, p < 10^-4), proliferation (45 genes, p < 10^-4), blood vessel development (17 genes, p < 10^-4) and embryonic development (14 genes, p < 10^-4). This cluster contained several genes well-known to be overexpressed in glioblastomas (IGFBP2, CHI3L1) and markers of glioblastoma cancer stem cells as well (CD133, IQGAP1). In contrast, gene cluster C was significantly enriched in genes with different specific ontologic classes: nervous system development (42 genes, p < 10^-4), synaptic transmission (26 genes, p < 10^-4), proliferation (17 genes, p < 10^-4) and neurogenesis (13 genes, p < 10^-4). Actually, most of the genes in gene cluster C were either related to neuronal function or neuronal development or known to be highly expressed in normal brain. Gene cluster B was also enriched in genes involved in synaptic transmission (17 genes, p < 10^-4) and nervous system development (16 genes, p < 10^-4). However, two ontologic classes specifically found in this cluster (neurofilament (3 genes, p < 10^-4) and axon ensheathment (2 genes, p < 10^-2)) suggested that this cluster contained genes expressed in more differentiated neural cells than gene cluster C.

**Oligodendrogliomas with 1p19q codeletion express specific subsets of neuronal genes**

In order to better characterize the expression of neuronal genes in gliomas with complete 1p19q codeletion, we performed a new hierarchical clustering analysis with samples of normal brain (GSE7307), including grey matter (cortex) and white matter (corpus callosum). As glioblastomas expressed genes of neural cancer stem cells, we also included samples of glioblastoma cancer stem cells (GSE7181) [7] in this analysis. Still using the most differentially expressed 1366 probe sets, the samples clustered into two major groups: one containing 1p19q codeleted gliomas, normal white matter and normal grey matter, and the other containing EGFR amplified gliomas and cancer stem cells (Figure 3). Again this clustering was found to be extremely robust and was conserved across different gene lists and clustering methods. Gene ontology enrichment analysis was performed on the main gene clusters (A to J). Despite the clustering of 1p19q codeleted gliomas with normal brain, they showed substantial differences. First, the genes characteristics of the corpus callosum (gene cluster A), enriched in myelination genes (5 genes, p < 10^-4), were not overexpressed in gliomas with 1p19q codeletion. Second, gliomas with 1p19q only overexpressed one subset of the neuronal genes characteristic of the cortex samples (gene clusters B and C). For example, among the neurofilament genes (INA, NEFH, NEFM, NEFL) present in cluster C, alphainternexin (INA) was the only one overexpressed in both gliomas with 1p19q codeletion and cortex samples in comparison to gliomas with EGFR amplification. Third, 1p19q codeleted gliomas were characterized by one specific cluster (gene cluster D) enriched in genes involved in CNS development (6 genes, p < 10^-4), which also contained genes known to be specifically expressed in neuronal cells in the physiological state, e.g. DCX and GALNT1 [21,22]. Thus, gliomas with 1p19q codeletion had a specific gene expression pattern of neuronal genes different from the samples of normal brain. Gliomas with EGFR amplification and glioblastoma cancer stem cells were both characterized by a large gene cluster (G) most significantly enriched in genes involved in proliferation (34 genes, p < 10^-4) and in CNS development (16 genes, p < 10^-4). Gliomas with EGFR amplification segregated from the cancer stem cells by one main gene cluster (I) enriched in genes involved in immune response (11 genes, p < 10^-4), extracellular matrix (7 genes, p < 10^-4) and angiogenesis (4 genes, p < 10^-4).

**Most characteristic genes associated with oligodendrogliomas with 1p19q codeletion**

To find the genes most specifically associated with oligodendrogliomas with 1p19q codeletion, we selected the probe sets that were consistently (> 2-fold) and significantly (t test p < 0.001) overexpressed in these gliomas when they were independently compared to each of the 4 other samples groups (i.e., gliomas with EGFR amplification, cortex samples, corpus callosum samples and glioblastoma cancer stem cells). Eighty-six probe sets corresponding to 39 well-annotated genes met these criteria (Table 1 and see additional file 1). Several genes on this list are known to be highly expressed in normal brain (CSMD3, C20orf42, CTTNBP2), and one is known to be specifically expressed by neuronal cells (GALNT13) [22]. Two transcription factors that play a role in CNS development were also specifically overexpressed (ATOH8, NFIB). ATOH8 is a basic-helix-loop-helix transcription factor, whose homolog in mouse has been demonstrated to regulate neuronal versus glial fate [23]. NFIB plays a
role in brain development, and Nfib deficient mice exhibit callosal agenesis [24]. Finally, an intriguing feature was the specific overexpression of both BMP2, which promotes astroglial differentiation, and its antagonist NOG, which has been shown to promote both neuronal and oligodendroglial differentiation [25-27].

Figure 3
Unsupervised clustering of 4 gliomas with 1p19q codeletion, 9 gliomas with EGFR amplification, 6 glioblastoma cancer stem cells cell lines and 10 normal brain tissue samples. Unsupervised hierarchical clustering was performed using the 1366 probe sets whose expression varied the most across the 29 samples (probe sets with a robust coefficient of variation superior to the 97.5th percentile). Samples and genes were clustered using Ward's linkage and 1-Pearson correlation coefficient. For each probe set, data were median-centered (white), with the lowest and highest intensity values in blue and red, respectively. 1p19q = 1p19q codeletion, EGFR = EGFR amplification, CC = corpus callosum, Cx = cortex, CSC = cancer stem cells. The 29 gliomas were classified into 2 groups and 5 subgroups. Gliomas with EGFR amplification were classified with the cancer stem cell lines. Gliomas with 1p19q codeletion were classified with the normal brain samples, however their gene expression pattern was clearly different from the gene expression pattern of the white matter (corpus callosum) and grey matter (cortex) samples.

Oligodendrogliomas with 1p19q have a proneural gene expression profile
As gliomas with 1p19q codeletion expressed neuronal genes, we performed gene set enrichment analysis (GSEA) to study the relationship between the gene expression profile of these gliomas and the "proneural" gene expression signatures that have been described in high grade gliomas
| Probe set  | Title                                                                 | Gene Symbol | Gene ontology (biological process)                        | High expression in:                      | FD/EGFR | FD/Cx | FD/CC | FD/Stem cells |
|------------|------------------------------------------------------------------------|-------------|----------------------------------------------------------|------------------------------------------|---------|-------|-------|---------------|
| 206785_s_at | Killer cell lectin-like receptor subfamily C, member 2                | KLRC1//KLRC2| Cellular defense response                                 | Natural killer cells                     | 104.2   | 92.1  | 61.4  | 66.7          |
| 243779_s_at, 236536_s_at | UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylglactosaminyl transferase 13 (GalNAc-T13) | GALNT13 | Protein amino acid O-linked glycosylation | Specifically expressed in neuronal cells | 31.8    | 18.2  | 17.1  | 15.0          |
| 1558706_s_at, 228890_at | Atonal homolog B (Drosophila)                                     | ATOH8      | Regulation of transcription                               | ---                                      | 26.1    | 30.8  | 21.4  | 27.1          |
| 240228_at | CUB and Sushi multiple domains 3                                       | CSMD3      | Integral to membrane                                      | Brain                                   | 22.7    | 11.8  | 13.9  | 15.1          |
| 207723_s_at | Killer cell lectin-like receptor subfamily C, member 3                | KLRC3      | Cellular defense response                                 | Natural killer cells                     | 17.9    | 27.3  | 13.1  | 11.3          |
| 230826_at | Monocyte to macrophage differentiation-associated 2                    | MMD2       | Cytolysis                                                | ---                                      | 17.2    | 7.2   | 9.1   | 26.2          |
| 60474_at, 218796_at | Chromosome 20 open reading frame 42                                | C20orf42   | Cell adhesion                                            | Brain (among others)                     | 16.2    | 37.4  | 29.1  | 19.6          |
| 231798_at | Noggin                                                                | NOG        | Nervous system development                                | ---                                      | 11.2    | 9.2   | 9.5   | 13.6          |
| 1556599_s_at | Cyclic AMP-regulated phosphoprotein, 21 kD                           | ARPP-21    | ---                                                       | ---                                      | 11.1    | 14.0  | 16.8  | 13.9          |
| 227845_s_at | Src homology 2 domain containing transforming protein D               | SHD        | Intracellular signaling cascade                           | ---                                      | 10.3    | 10.6  | 16.5  | 7.6           |
| 205289_at, 205290_s_at | Bone morphogenetic protein 2                                         | BMP2       | Positive regulation of astrocyte differentiation          | Brain (among others)                     | 10.1    | 30.7  | 25.9  | 12.7          |
| 205330_at | Meningioma (disrupted in balanced translocation) I                   | MN1        | Negative regulation of progression through cell cycle    | Ubiquitously expressed                   | 8.4     | 5.8   | 9.5   | 15.9          |
| 219668_at | Ganglioside-induced differentiation-associated protein 1-like 1       | GDAP1L1    | ---                                                       | ---                                      | 8.4     | 4.2   | 9.1   | 7.9           |
| 204530_s_at | Thymus high mobility group box protein TOX                           | TOX        | Regulation of transcription                               | ---                                      | 8.3     | 6.6   | 12.5  | 6.0           |
| 228790_at, 221959_at | Chromosome 8 open reading frame 72                                  | C8orf72    | ---                                                       | ---                                      | 7.6     | 14.6  | 19.1  | 36.6          |
| 232136_s_at, 233136_at | Cortactin binding protein 2                                          | CTTNBP2    | ---                                                       | Brain                                    | 5.4     | 7.1   | 4.3   | 6.5           |
| 219093_at | Pol(A) binding protein, cytoplasmic 5                                 | PABPC5     | ---                                                       | Fetal brain                             | 5.4     | 4.6   | 4.6   | 4.9           |
| 205773_at | Phosphotyrosine interaction domain containing 1                      | PID1       | ---                                                       | Brain (among others)                     | 5.1     | 12.3  | 23.0  | 4.0           |
| 1560265_at | Glutamate receptor, ionotropic, kainate 2                            | GRIK2      | Regulation of synaptic transmission                       | Cerebellum, cerebral cortex             | 4.5     | 3.7   | 5.9   | 7.1           |
| 238526_at | RAB3A interacting protein (rabin3)                                    | RAB3IP     | Protein transport                                         | Brain (among others)                     | 4.4     | 5.9   | 7.2   | 3.2           |
| 213001_at, 219514_at | Angiopoietin-like 2                                                   | ANGPTL2    | Development                                              | Heart among others                       | 3.9     | 12.2  | 6.0   | 4.0           |
| 229590_at | Ribosomal protein L13                                                | RPL13      | Translation                                              | ---                                      | 3.8     | 3.7   | 3.8   | 2.8           |
| 206117_at | Tropomyosin 1 (alpha)                                                 | TPM1       | Cell motility                                            | Muscle among others                      | 3.6     | 4.1   | 4.2   | 3.3           |
| 202315_s_at, 217223_s_at | Breakpoint cluster region                                            | BCR        | Regulation of Rho protein signal transduction            | ---                                      | 3.3     | 4.4   | 7.6   | 4.5           |
Table 1: Most characteristic genes associated with oligodendrogliomas with 1p19q codeletion (Continued)

| Gene Symbol | Description | Expression | p-Value |
|-------------|-------------|------------|---------|
| SLC2A13     | Solute carrier family 2 (facilitated glucose transporter), member 13 | Carbohydrate transport | 3.1 |
| HDAC4       | Histone deacetylase 4 | Nervous system development | 3.1 |
| POLR2F      | Polymerase (RNA) II (DNA directed) polypeptide F | Regulation of transcription | 3.0 |
| NFIB        | Nuclear factor I/B | Regulation of transcription, DNA-dependent, Brain development | 3.0 |
| THRA        | Thyroid hormone receptor, alpha (erythroblast leukemia viral (v-erb-a) oncogene homolog, avian) | Negative regulation of transcription | 2.9 |
| WDR37       | WD repeat domain 37 | --- | 2.8 |
| COX4I1      | Cytochrome c oxidase subunit IV isoform 1 | Electron transport | 2.4 |
| TRIM8       | Tripartite motif-containing 8/11-tripartite motif-containing 8 | --- | 2.3 |
| GCNSL2      | GCN5 general control-of-amino-acid-synthesis 5-like 2 (yeast) | Regulation of transcription, DNA-dependent | 2.3 |
| DGCR2       | DiGeorge syndrome critical region gene 2 | Cell adhesion | 2.3 |
| C1orf32     | Chromosome 10 open reading frame 32 | --- | 2.2 |
| KLRC4       | Killer cell lectin-like receptor subfamily C, member 4 | Cellular defense response | 2.2 |
| TAF1C       | TATA box binding protein (TBP)-associated factor, RNA polymerase I, C, 110kDa | Transcription | 2.1 |
| C1orf2      | Chromosome 11 open reading frame2 | --- | 2.0 |

Next we used the 35 genes signature developed by Phillips et al. to distinguish the three groups of high grade gliomas (proneural, proliferative and mesenchymal) in order to perform unsupervised hierarchical clustering [5]. As shown in Figure 5, the gliomas with 1p19q codeletion were classified as proneural. Thus, there was a clear association between the "proneural" gene expression profile and 1p19q codeletion.

Real-time RT-PCR validation
To validate these findings, we studied the expression of 22 selected genes differentially expressed (11 up and 11 down) between the two groups of gliomas in an independent data set of 16 gliomas (8 gliomas with 1p19q codeletion and 8 gliomas with 1p19q codeletion in comparison to corpus callosum, FD/Stem cells = FD in gliomas with 1p19q codeletion in comparison to glioblastomas cancer stem cells [7]).

with a good prognosis (Figure 4) [5,14,28]. This test determines the over-representation of a gene set (list of genes) at the extremes (top or bottom) of the ordered, non-redundant dataset (list of all of the genes being used to compare two groups of samples). This analysis demonstrated that gliomas with 1p19q codeletion in comparison to gliomas with EGFR amplification were significantly enriched in the "proneural" gene set associated with good prognosis reported by Phillips et al. and in the good prognosis neurogenesis-related gene set reported by Freije et al. (HC1A gene set) [5,28]. They were also enriched in the HC1B gene set (neuronal genes) of Freije et al. In contrast, gliomas with EGFR amplification were enriched in gene sets associated with poor prognosis ("proliferation" and "mesenchymal" gene sets of Phillips et al., and HC2A (enriched in proliferation genes) and HC2B (enriched in extracellular matrix genes) gene sets of Freije et al.) [5,28].
GSEA Enrichment Score curves. Gene set enrichment analysis (GSEA) was performed with 6 different gene sets obtained from the studies of Phillips et al. and Freije et al. Phillips’ study gene sets: A: Proneural gene set (n = 220 genes), B: Proliferative gene set (n = 148 genes), C: Mesenchymal gene set (n = 126 genes). Freije’s study gene sets: D: HC1A neurogenesis related gene set (n = 73), E: HC2A proliferation related gene set (n = 66 genes), F: HC2B extracellular matrix related gene set (n = 239 genes) [5, 28]. "Signal-to-Noise" ratio (SNR) statistic was used to rank the genes according to their correlation with either the 1p19q codeletion phenotype (red) or EGFR amplification phenotype (blue). The graph on the bottom of each panel represents the ranked, ordered, non-redundant list of genes. Genes on the far left (red) correlated the most with 1p19q codeleted samples, and genes on the far right (blue) correlated the most with EGFR amplified samples. On each panel, the vertical black lines indicate the position of each of the genes of the studied gene set in the ordered, non-redundant data set. The green curve corresponds to the ES (enrichment score) curve, which is the running sum of the weighted enrichment score obtained from GSEA software. A and D show that gliomas with 1p19q codeletion were significantly enriched in the proneural and neurogenesis related (HC1A) gene sets. B, C, D and E show that gliomas with EGFR amplification were significantly enriched in the proliferation/HC2A and mesenchymal/HC2B gene sets.
Figure 6 and see additional file 2). Using a univariate t-test, 21 out of the 22 genes studied were shown to be differentially expressed between the two groups of gliomas with a p-value < 0.05 (only NCAM1 was not validated). This confirmed that gliomas with 1p19q codeletion overexpressed neuronal/normal brain genes (AKR1C3, C20orf42, CITNBP2, L1CAM, GALNT13) as well as genes implicated in gliogenesis and neurogenesis (OLIG2, BMP2, NOG, DCX, ATOH8). Except L1CAM and AKR1C3, all of these genes were also overexpressed in comparison to the normal brain samples, including two genes (DCX, GALNT13) known to be exclusively expressed in neuronal cells. BMP2, NOG, C20orf42, GALNT13 and OLIG2

Unsupervised clustering of the 4 gliomas with 1p19q codeletion and the 9 gliomas with EGFR amplification using 35 genes signature of Phillips et al. [5]. Samples and genes were clustered using Ward's linkage and 1-Pearson correlation coefficient. 1p19q = gliomas with 1p19q codeletion, EGFR = gliomas with EGFR amplification. Gliomas with 1p19q codeletion were classified as proneural, whereas gliomas with EGFR amplification had both a mesenchymal and proliferative profile. In red are the genes whose expression was studied in real-time RT-PCR in an independent data set.
belong to the list of proneural genes reported by Phillips [5].

In the gliomas with EGFR amplification, we confirmed the overexpression of genes implicated in proliferation (CCNB1, CDK2), extracellular matrix remodeling (PLAT, POSTN), immune response (GBP1), cancer stem cell signaling (IQGAP1) as well as several genes known to be highly expressed in glioblastomas (IGFBP2, CHI3L1, PDPN). CCNB1, CDK2 belong to the proliferative gene list, and CHI3L1 and PDPN, to the mesenchymal gene list of Phillips [5].

**Alpha-internexin immunohistochemistry**

Finally, to validate the expression of neuronal genes in gliomas with 1p19q codeletion at the protein level, we studied the expression of the internexin neuronal intermediate filament protein alpha (INA) which was one of the neuronal genes most overexpressed in these gliomas in comparison to gliomas with EGFR amplification (FC = 15, p < 0.001). INA is a class-IV neuronal intermediate filament.

---

**Table 2: Real-time RT-PCR study of 22 differentially expressed genes**

| Gene symbol | Description | Gene ontology | Fold difference of geom means 1p19q/EGFR (microarray)* | Fold difference of geom means 1p19q/EGFR in validation sample set (RT-PCR)** |
|-------------|-------------|---------------|--------------------------------------------------------|--------------------------------------------------------------------------------|
| AKR1C3      | Aldo-keto reductase family 1, member C3 | Prostaglandin metabolism | 17.5 | 7.1 |
| ATOH8       | Atonal homolog 8 (Drosophila) | Regulation of transcription | 26 | 23.5 |
| BMP2        | Bone morphogenetic protein 2 | Positive regulation of astrocyte differentiation | 10.1 | 10.2 |
| C20orf42    | Chromosome 20 open reading frame 42 | Cell adhesion | 16.2 | 15.7 |
| CTTNBP2     | Cortactin binding protein 2 | --- | 5.4 | 3.9 |
| DCX         | Doublecortex; lissencephaly, X-linked (doublecortin) | CNS development | 5.9 | 6.3 |
| GALNT13     | UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylneuraminyltransferase 13 (GalNAC-T13) | Protein amino acid O-linked glycosylation | 31.7 | 38.4 |
| L1CAM       | L1 cell adhesion molecule | Nervous system development | 14.6 | 24.5 |
| NCAM1       | Neural cell adhesion molecule 1 | Synaptic transmission | 4.8 | 1.7(NS) |
| Nog         | Noggin | Nervous system development | 11.2 | 18.4 |
| OLIG2       | Oligodendrocyte lineage transcription factor 2 | Nervous system development | 4.7 | 3.7 |
| CCNB1       | Cyclin B1 | Mitosis | 0.2 | 0.2 |
| CDK2        | Cyclin-dependent kinase 2 | Mitosis | 0.2 | 0.2 |
| CHI3L1      | Chitinase 3-like 1 (cartilage glycoprotein-39) | Chitin catabolism | 0.01 | 0.003 |
| EGFR        | Epidermal growth factor receptor (erythroblastemic leukemia viral (v-erb-b) oncogene homolog, avian) | Cell proliferation | 0.1 | 0.2 |
| GBP1        | Guanylate binding protein 1, interferon-inducible, 67kDa | Immune response | 0.05 | 0.05 |
| IGFBP2      | Insulin-like growth factor binding protein 2, 36 kDa | Regulation of cell growth | 0.02 | 0.01 |
| IQGAP1      | IQ motif containing GTPase activating protein 1 | Signal transduction | 0.11 | 0.1 |
| PDPN        | Podoplanin | Positive regulation of cell motility | 0.02 | 0.008 |
| PLAT        | Plasminogen activator, tissue | Proteolysis | 0.07 | 0.06 |
| POSTN       | Periostin, osteoblast specific factor | Cell adhesion | 0.01 | 0.01 |
| RNf135      | Ring finger protein 135 | --- | 0.2 | 0.2 |

Fold difference of geometrical means in microarray and in real-time RT-PCR of the 22 genes studied in the independent sample set. * All genes were differentially expressed with a p-value < 0.001 except DCX (p-value = 0.004). ** All genes were differentially expressed with a p-value < 0.05 except when NS (non significant) is specified.
Real-time RT PCR study of 22 genes differentially expressed between 1p19q codeleted gliomas and EGFR amplified gliomas. Real-time RT-PCR study of 11 genes overexpressed in gliomas with EGFR amplification (top) and 11 genes overexpressed in gliomas with 1p19q codeletion (bottom) was performed in an independent data set of 16 gliomas (8 gliomas with EGFR amplification (triangles), 8 gliomas with 1p19q codeletion (circles)). Each dot represents the relative expression (log2 transformed) of a given gene in one glioma compared with normal brain (median expression in the 3 normal brain samples). Dots above the upper dashed line are upregulated with a fold change larger than 2 in comparison to normal brain; dots below the lower dashed line are downregulated in comparison to normal brain with a fold change larger than 2. For example, NOG, BMP2 and ATOH8 were overexpressed in all 8 gliomas with 1p19q codeletion (circles) in comparison to all 8 gliomas with EGFR amplification (triangles) and in comparison to normal brain. CHI3L1, PLAT, IQGAP1, IGFBP2 and GBP1 were overexpressed in all gliomas with EGFR amplification (triangles) in comparison to gliomas with 1p19q codeletion (circles) and in comparison to normal brain. Except for NCAM1, all 22 genes were differentially expressed (p < 0.05).
involved in the morphogenesis of neurons [29]. Immunostaining for INA was positive in all five oligodendrogliomas with 1p19q codeletion examined. Immunopositivity was observed in some normal infiltrated neurons but was mostly seen in a specific cytoplasmic perinuclear staining pattern in tumor cells (Figure 7). Between 20 to 50% of tumor cells displayed this staining, which was different from the staining observed in the infiltrated normal neurons (Figure 7). Among the five glioblastomas with EGFR amplification, immunostaining was negative in four and positive in a scattered pattern in one, in a region displaying some features of oligodendroglial differentiation.

**Discussion**

EGFR amplification and whole 1p19q codeletion are mutually exclusive and predictive of completely different outcomes [3,4]. To date, no studies have compared the gene expression profile of these two types of gliomas. Indeed, among microarray studies of gliomas [5,28,30,31], only a few have compared genetically well-defined tumors [32-35]. In addition, these studies were based on LOH or FISH [32-34], and not on CGH-array. Yet, there is a need when interpreting a difference in gene expression to analyze it in relation to the genomic profile. Our data reveals clearly distinct gene expression profiles in these 2 groups of gliomas: those with EGFR amplification express the proliferative and mesenchymal gene set defined by Phillips et al., while 1p19q codeleted gliomas express the proneural group [5]. Moreover, gliomas with EGFR amplification clustered close to tumor stem cells. Indeed, the EGFR pathway is involved in the proliferation of normal neural stem cells and cancer stem cells [36]. This result is consistent with the fact that several studies

![Figure 7](image)

**Figure 7**

INA immunohistochemistry in 1p19q codeleted and EGFR amplified gliomas. Representative alpha-internexin (INA) immunohistochemistry in oligodendrogliomas with 1p19q codeletion (A, C, D) and in glioblastomas with EGFR amplification (B). C: the arrow shows immunopositivity in an entrapped neuron surrounded by immunopositive tumor cells.
isolated stem like tumor cells from glioblastoma but not from oligodendroglioma. Such studies did not include genetic profiles of the tumors, but data from our group suggest indeed that the capacity of cell renewal (as reflected by the formation of spheroids derived from the tumor) in vitro is tightly correlated with the presence of EGFR amplification (unpublished results). EGFR activation upregulates genes involved in neural stem cell proliferation: one of these genes is ASPM (abnormal spindle-like microcephaly associated) that promotes neuroblast proliferation and symmetric division and is strongly upregulated in glioblastomas. Inhibition of ASPM inhibits glioblastoma cell growth and neural stem cell proliferation [37].

A proneural/normal brain gene expression profile is a factor related to good prognosis and correlates with younger age and grade III histology, with most anaplastic oligodendrogliomas being classified as proneural [5,28]. As shown here, this gene expression profile can be determined by a simple, highly discriminating RT-PCR test, and this may be useful for clinical practice. Until now, a proneural gene expression profile has not been reported to be associated with 1p19q codeletion. In Freije’s study the number of gliomas with 1p19q codeletion was too small (4 out 74 patients) to address this question [28]. In Phillips' study the genomic/transcriptomic correlation was limited to patients with astrocytoma histology, and this may have limited the possibility of finding an association between 1p19q codeletion and the proneural gene expression profile [5]. However, the authors noticed a negative correlation between the proneural gene expression profile and EGFR amplification, similar to the negative correlation between 1p19q codeletion and EGFR amplification [3,5]. Our study demonstrates that there is a strong correlation between 1p19q codeletion and the expression of proneural genes, suggesting that gliomas with a 1p19q codeletion represent a subgroup of proneural gliomas. In addition, the expression of neuronal genes in 1p19q codeleted tumors is consistent with a previous study showing selective expression of neuronal genes in oligodendrogliomas with 1p loss [33]. Whether there is a link between the good prognosis of proneural gliomas and the fact that gliomas with 1p19q codeletion display a proneural gene expression profile remains to be elucidated. We make the hypothesis that gliomas without 1p19q codeletion but with a gene expression profile similar to the 1p19q codeleted gliomas might also harbor a better prognosis.

The expression of "neuronal genes" in 1p19q codeleted gliomas can be interpreted in different ways. As advocated by some authors, this expression is probably due in part to the presence of infiltrated neurons in the tumor [30]. Indeed, 1p19q codeletion has been suggested to be more frequent in tumors with indistinct, irregular borders, which therefore, are more likely to be contaminated with normal brain tissue [38]. However, as shown here, this normal brain infiltration cannot completely explain the expression of neuronal genes by 1p19q codeleted gliomas. Indeed, these tumors only express a specific subset of neuronal genes (Figure 3). In addition, if the expression of neuronal genes was only due to infiltration of normal brain tissue, the expression pattern of the neuronal genes in these tumors would be similar to their expression in the normal brain samples, which was not the case. Furthermore, we have demonstrated that alpha-internexin (INA), a neuronal protein, was specifically expressed by 1p19q codeleted glioma tumor cells. Thus INA expression might be used as a simple surrogate marker of 1p19q codeletion. This hypothesis is currently being tested in a larger series of gliomas. Interestingly, recent ultrastructural analysis of oligodendrogliomas has shown neuronal structures such as synapses and neurosecretory granules [39]. Thus, another hypothesis for the expression of neuronal genes in 1p19q codeleted glioma tumor cells is that the cell of origin of these tumors could be a progenitor cell giving rise to both neurons and oligodendrocytes [40,41]. This progenitor has less capacity of self renewal than the more multipotent neural stem cells. This is consistent with the fact that 1p19q codeleted oligodendroglioma fails in our hands to form spheroids in vitro (unpublished data). In this setting it is interesting to note that concomitant overexpression of both BMPs and BMP antagonists, such as the concomitant overexpression of BMP2 and NOG observed in 1p19q codeleted gliomas in our study, has been demonstrated in white matter progenitor cells, which can give rise to both oligodendrocytes and neurons [42]. Another non-exclusive explanation for the expression of "neuronal" genes in oligodendrogliomas could rely on the fact that some genes involved in neurogenesis and classified as "neuronal" may also play a role in oligodendroglial development, e.g. ASCL1/MASH1. This proneural gene specifies a population of telencephalic oligodendrocytes [43] and is also required for oligodendrocyte development in the spinal cord [44]. On the other hand, Olig2 -implicated in oligodendroglial specification-is also involved in neurogenesis: during development, Olig2+ progenitors give rise to both motoneurons and oligodendrocytes in the ventral spinal cord, [45]. Consistently with our results, these data, illustrating the tight connection that exists between neurons and oligodendrocytes fates, bring a new light on the pathogenesis of oligodendrogliaomas with 1p19q codeletion. Finally it is important to remember that current WHO classification is only based on morphological similarity between normal cells and tumor cells, and the link between oligodendrocytes and oligodendrogliomas has never been demonstrated.
Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
FD performed the major part of experiments and analysis. FD and MS drafted the manuscript. AI performed the CGH-array study and analysis. AR and SL provided the bioinformatic tools and participated to the analysis. IB and MV performed the real-time RT-PCR validation. YM, SP, JT and KM selected the samples, helped extracting the RNA and performed the immunohistochemistry validation. KHX, OD and JYD assisted with design of the study and with critical examination of the manuscript. MS conceived of and designed the study, participated in its experimental design and interpretation of results, and helped edit the manuscript. All authors read and approved the final manuscript.

Additional material

Additional file 1
Complete list of genes differentially expressed (t-test, p < 0.001) between 1p19q codeluted oligodendrogliomas (n = 4) and 1 gliomas with EGFR amplification (n = 9), 2) cerebral cortex samples (n = 5), 3) corpus callosum samples (n = 5) 4) Beier’s et al. gliomas cancer stem cells (n = 6) [7].
Click here for file [http://www.biomedcentral.com/content/full/1476-4598-7-41-S1.xls]

Additional file 2
Detailed results of the 22 genes studied by real-time RT-PCR in the independent sample set of 7 oligodendrogliomas with 1p19q codelution and 8 gliomas with EGFR amplification.
Click here for file [http://www.biomedcentral.com/content/full/1476-4598-7-41-S2.xls]

Acknowledgements
This study was supported by a grant of the Institut National du Cancer (INCA, PL032) and by the Ligue Nationale contre le Cancer.

References
1. Idbaa A, Marie Y, Pierron G, Brennetot C, Hoang-Xuan K, Kujas M, Mokhtari K, Sanson M, Lejeune J, Aurias A, Delattre O, Delattre JY: Two types of chromosome 1p losses with opposite significance in gliomas. Ann Neurol 2005, 58(3):483-487.
2. Jenkins RB, Blair H, Ballman KV, Giannini C, Arusell RM, Law M, Flynn H, Passe S, Felten S, Brown PD, Shaw EG, Buckner JC: A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. Cancer Res 2006, 66(20):9852-9861.
3. Idbaa A, Marie Y, Lucchesi C, Pierron G, Manie E, Raynal V, Mosseri V, Hoang-Xuan K, Kujas M, Brito I, Mokhtari K, Sanson M, Barillot E, Aurias A, Delattre JA, Delattre O: BAC array CGH distinguishes mutually exclusive alterations that define clinicogenetic subtypes of gliomas. Int J Cancer 2008, 122(9):1778-1786.
4. Dehais C, Lagie-Donsday F, Marie Y, Kujas M, Lejeune J, Benouaich-Amiel A, Pedretti M, Polivka M, Xuan KH, Thillet J, Delattre JY, Sanson M: Prognostic stratification of patients with anaplastic gliomas according to genetic profile. Cancer 2006, 107(8):1891-1897.
5. Phillips HS, Kharbanda S, Chen R, Forrest WF, Soriano RH, Wu TD, Misra A, Nigro JM, Colman H, Sorocoenu L, Williams PM, Modrusan Z, Feuerstein BG, Aldape K: Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. Cancer Cell 2006, 9(3):157-173.
6. Gene Expression Omnibus repository (GSE7307) [http://www.ncbi.nlm.nih.gov/geo/].
7. Beier D, Hau P, Poescholdt M, Lohmeier A, Wirschhusen J, Oefner PJ, Agner L, Brawanski A, Bogdahn U, Beier CP: CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Res 2007, 67(9):4010-4015.
8. ArrayExpress: [http://www.ebi.ac.uk/arrayexpress].
9. Bioconductor: version 2.0, [http://www.bioconductor.org].
10. NCBI BLAST, OLGA: BAC array CGH distinguishes mutually exclusive alterations that define clinicogenetic subtypes of gliomas. [http://www.ncbi.nlm.nih.gov/geo].
11. Simon R, Peng-Lam A: BRB Array Tools (version 3.5.0), [http://linus.nci.nih.gov/BRB-ArrayTools.html].
12. DAVID: v2.0 software, [http://www.broad.mit.edu/gsea/].
13. GOA: Gene Ontology Database. ftp://ftp.ebi.ac.uk/pub/databases/GO/goa/.
14. David Center for Genes and Disease at Columbia University: [http://david.abcc.ncifcrf.gov/].
15. Geneset enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005, 102(43):15459-15530.
16. Affymetrix: annotation file HG-U133_Plus_2.annot. [http://www.affymetrix.com]. 2007.
17. The GeneOntology: [http://www.geneontology.org]. 2007.
18. GOA: Gene Ontology Database. ftp://ftp.ebi.ac.uk/pub/databases/GO/goa/.
19. David Center for Genes and Disease at Columbia University: [http://david.abcc.ncifcrf.gov/].
20. Beeche I, Parfait B, Le Doussal V, Olimi M, Rieu MC, Lidereau R, Mesirov JP: Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005, 102(43):15459-15530.
21. Affymetrix: annotation file HG-U133_Plus_2.annot. [http://www.affymetrix.com]. 2007.
22. NOVACORE: [http://www.leica-microsystems.com/ Histology_Systems, product code : NCL-A-INTER, clone 2E3].
23. Couillard-Despres S, Winner B, Schaubek S, Aigner R, Vroemen M, Weidner N, Bogdahn U, Winkler J, Kuhn HG, Aigner L: Doublecortin expression levels in adult brain reflect neurogenesis. Eur J Neurosci 2005, 21(1):1-14.
24. Zhang Y, Iwaski H, Wang H, Kudo T, Kalka TB, Hennet T, Kubota T, Cheng L, Inaba N, Gotoh M, Togayachi A, Guo J, Hisatomi H, Nakanima K, Nishihara S, Nakamura M, Martz JD, Narimatsu H: Cloning and characterization of a new human UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase, designated pp-GalNAc-T13, that is specifically expressed in neurons and synthesizes GalNAc alpha-serine/threonine antigen. J Biol Chem 2003, 278(1):573-584.
25. Linou C, Bae SK, Takatsuka K, Inoue T, Beshto Y, Kageyama R: Math3, a BHLH gene expressed in the developing nervous system, regulates neuronal versus glial differentiation. Genes Cells 2001, 6(11):977-986.
26. Steele-Pinkers G, Plachez C, Butz KG, Yang G, Bachurski CJ, Kinsman SL, Litwack ED, Richards LJ, Gronostajski RM: Noggin antagonizes BMP signaling to control the sequential onset of neurogenesis and gliogenesis via induction of BMPs. Genes Cells 2005, 10(8):777-783.
27. Kasai M, Satoh K, Akiyama T: Wnt signaling regulates the sequential onset of neurogenesis and gliogenesis via induction of BMPs. Mol Chem Neurobiol 2005, 22(2):685-698.
28. Lin DA, Tramontin AD, Trevejo JM, Herrera DG, Garcia-Verdugo JM, Alvarez-Buylla A: Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. Neuron 2000, 28(3):713-726.
29. Mabie PC, Mehler MF, Ruffin M, Papavassiliou A, Song Q, Kessler JA: Bone morphogenetic proteins induce astroglial differentia-
tion of oligodendrogial-astroglial progenitor cells. J Neurosci 1997, 17(11):4112-4120.

18. Freije WA, Castro-Vargas FE, Fang Z, Horvath S, Cloughesy T, Liu LM, Mischel PS, Nelson SF. Gene expression profiling of gliomas strongly predicts survival. Cancer Res 2004, 64(18):6503-6510.

19. Chan SO, Chiu FC: Cloning and developmental expression of human 66 kd neurofilament protein. Brain Res Mol Brain Res 1995, 29(1):177-184.

20. Liang Y, Diehn M, Watson N, Bollen AW, Aldape KD, Nicholas MK, Lamborn KR, Berger MS, Botstein D, Brown PO, Israel MA: Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. Proc Natl Acad Sci USA 2005, 102(16):5814-5819.

21. Nut CL, Mani DR, Betensky RA, Tamayo P, Cairncross JG, Ladd C, Pohl U, Hartmann C, McLaughlin ME, Batchelor TT, Black PM, von Deimling A, Pomery SL, Golub TR, Louis DN: Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. Cancer Res 2003, 63(7):1602-1607.

22. French PJ, Swagemakers SM, Nagel JH, Kouwenhoven MC, Brouwer E, van der Spek P, Luider TM, Kros JM, van den Bent MJ, Sillevis Smitt PA: Gene expression profiles associated with treatment response in oligodendrogliomas. Cancer Res 2005, 65(24):11335-11344.

23. Mukasa A, Ueki K, Ge X, Ishikawa S, Ide T, Fujimaki T, Nishikawa R, Asai A, Kirino T, Aburatani H: Selective expression of a subset of neuronal genes in oligodendroglioma with chromosome 1p loss. Brain Pathol 2004, 14(1):34-42.

24. Mukasa A, Ueki K, Matsumoto S, Tsutsumi S, Nishikawa R, Fujimaki T, Asai A, Kirino T, Aburatani H: Distinction in gene expression profiles of oligodendrogliomas with and without allelic loss of 1p. Oncogene 2002, 21(25):3961-3968.

25. Tews B, Felsberg J, Hartmann C, Kunikh A, Hahn M, Toedt G, Neben K, Hummerich L, von Deimling A, Reifenberger G, Lichter P: Identification of novel oligodendroglialoma-associated candidate tumor suppressor genes in 1p36 and 19q13 using microarray-based expression profiling. Int J Cancer 2006, 119(4):792-800.

26. Lee J, Kodiarova S, Kodiarov Y, Li A, Su Q, Donin NM, Pastorino S, Purow BW, Christopher N, Zhang W, Park JK, Fine HA: Tumor stem cells derived from glioblastomas cultured in BFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 2006, 9(5):391-403.

27. Horvath S, Zhang B, Carlson M, Lu KV, Zhu S, Felciano RM, Laurance J, Kotliarova S, Kotliarov Y, Li A, Su Q, Donin NM, Pastorino S, Dubrow KJ, Natesan S, Merrill JE, Goldman SA: Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target. Proc Natl Acad Sci USA 2006, 103(46):17402-17407.

28. Jenkinson MD, du Plessis DG, Smith TS, Joyce KA, Warnke PC, Walker C: Distinction in gene expression profiles of oligodendrogliomas with and without allelic loss of 1p. Brain Res Mol Brain Res 2004, 129:1884-1891.

29. Vyberg M, Ulhøi BP, Teglbjaerg PS: Neuronal features of oligodendroglialomas-an ultrastructural and immunohistochemical study. Histopathology 2007, 50:887-896.

30. Penn B, Garcia-Verdugo JM, Yashine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A: Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci 2006, 26(30):7907-7918.

31. Nutt CL, Mani DR, Betensky RA, Tamayo P, Cairncross JG, Ladd C, Pohl U, Hartmann C, McLaughlin ME, Batchelor TT, Black PM, von Deimling A, Pomery SL, Golub TR, Louis DN: Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. Cancer Res 2003, 63(7):1602-1607.

32. French PJ, Swagemakers SM, Nagel JH, Kouwenhoven MC, Brouwer E, van der Spek P, Luider TM, Kros JM, van den Bent MJ, Sillevis Smitt PA: Gene expression profiles associated with treatment response in oligodendrogliomas. Cancer Res 2005, 65(24):11335-11344.

33. Mukasa A, Ueki K, Ge X, Ishikawa S, Ide T, Fujimaki T, Nishikawa R, Asai A, Kirino T, Aburatani H: Selective expression of a subset of neuronal genes in oligodendroglioma with chromosome 1p loss. Brain Pathol 2004, 14(1):34-42.

34. Mukasa A, Ueki K, Matsumoto S, Tsutsumi S, Nishikawa R, Fujimaki T, Asai A, Kirino T, Aburatani H: Distinction in gene expression profiles of oligodendrogliomas with and without allelic loss of 1p. Oncogene 2002, 21(25):3961-3968.

35. Tews B, Felsberg J, Hartmann C, Kunikh A, Hahn M, Toedt G, Neben K, Hummerich L, von Deimling A, Reifenberger G, Lichter P: Identification of novel oligodendroglialoma-associated candidate tumor suppressor genes in 1p36 and 19q13 using microarray-based expression profiling. Int J Cancer 2006, 119(4):792-800.

36. Lee J, Kodiarova S, Kodiarov Y, Li A, Su Q, Donin NM, Pastorino S, Purow BW, Christopher N, Zhang W, Park JK, Fine HA: Tumor stem cells derived from glioblastomas cultured in BFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. Cancer Cell 2006, 9(5):391-403.

37. Horvath S, Zhang B, Carlson M, Lu KV, Zhu S, Felciano RM, Laurance MF, Zhao W, Qi S, Chen Z, Lee Y, Scheck AC, Liu LM, Wu H, Geschwind DH, Fobbio PG, Kornblum H, Cloughesy TF, Nelson SF, Mischel PS: Analysis of oncogenic signaling networks in glioblastoma identifies ASPM as a molecular target. Proc Natl Acad Sci USA 2006, 103(46):17402-17407.

38. Jenkinson MD, du Plessis DG, Smith TS, Joyce KA, Warnke PC, Walker C: Distinction in gene expression profiles of oligodendrogliomas with and without allelic loss of 1p. Brain Res Mol Brain Res 2004, 129:1884-1891.

39. Vyberg M, Ulhøi BP, Teglbjaerg PS: Neuronal features of oligodendroglialomas-an ultrastructural and immunohistochemical study. Histopathology 2007, 50:887-896.

40. Penn B, Garcia-Verdugo JM, Yashine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A: Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci 2006, 26(30):7907-7918.

41. Nutt CL, Mani DR, Betensky RA, Tamayo P, Cairncross JG, Ladd C, Pohl U, Hartmann C, McLaughlin ME, Batchelor TT, Black PM, von Deimling A, Pomery SL, Golub TR, Louis DN: Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. Cancer Res 2003, 63(7):1602-1607.

42. French PJ, Swagemakers SM, Nagel JH, Kouwenhoven MC, Brouwer E, van der Spek P, Luider TM, Kros JM, van den Bent MJ, Sillevis Smitt PA: Gene expression profiles associated with treatment response in oligodendrogliomas. Cancer Res 2005, 65(24):11335-11344.

43. Mukasa A, Ueki K, Ge X, Ishikawa S, Ide T, Fujimaki T, Nishikawa R, Asai A, Kirino T, Aburatani H: Selective expression of a subset of neuronal genes in oligodendroglioma with chromosome 1p loss. Brain Pathol 2004, 14(1):34-42.

44. Sugimori M, Nagao M, Parras CM, Nakafuku M, Lebel M, Guillemot F, Nakafuku M: Ascl1 is required for oligodendrocyte development in the spinal cord. Development 2008, 135(7):1271-1281.

45. Lu QR, Sun T, Zhu Z, Ma N, Garcia M, Stiles CD, Rowitch DH: Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. Cell 2002, 109(1):75-86.