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REGULAR PAPER

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Expression of oligodendrocyte lineage genes in oligodendroglial and astrocytic gliomas

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Abstract The oligodendrocyte lineage genes *OLIG1* and *OLIG2* have been reported as potential diagnostic markers for oligodendrogliomas [Lu et al. (2001) Proc Natl Acad Sci USA 98:10851–10856; Marie et al. (2001) Lancet 358:298–300]. We investigated the mRNA expression of *OLIG1* and *OLIG2*, as well as four other genes involved in oligodendrocyte development (*E2A, HEB*, *NKX2.2*, and *PDGFRA*) in a panel of 70 gliomas, including 9 oligodendrogliomas, 11 anaplastic oligodendrogliomas, 5 oligoastrocytomas, 10 anaplastic oligoastrocytomas, 10 diffuse astrocytomas, 10 anaplastic astrocytomas, and 15 glioblastomas. Most tumors demonstrated higher transcript levels of these genes as compared to non-neoplastic adult brain tissue. Four glioblastomas showed markedly increased *PDGFRA* mRNA expression due to *PDGFRA* gene amplification. Statistical analyses revealed no significant expression differences between oligodendroglial and astrocytic tumors. In oligodendroglial tumors, expression of the six genes was not significantly correlated to loss of heterozygosity on chromosome arms 1p and 19q. Thus, expression of the investigated oligodendrocyte lineage genes is up-regulated relative to non-neoplastic brain tissue in the majority of oligodendroglial and astrocytic tumors, suggesting that glioma cells are arrested in or recapitulate molecular phenotypes corresponding to early stages of glial development. However, the determination of mRNA expression of these genes by means of reverse transcription-PCR does not appear to be diagnostically useful as a marker for oligodendrogliomas.

Keywords Oligodendroglioma · Oligodendrocyte lineage genes · Olig1 · Olig2

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Introduction

The differential diagnosis between astrocytic and oligodendroglial tumors is of paramount importance because most anaplastic oligodendrogliomas respond favorably to chemotherapy, while anaplastic astrocytomas and glioblastomas are largely resistant to chemotherapy [16]. A considerable fraction of gliomas show ambiguous histological features that make their classification as either oligodendroglial or astrocytic difficult. Unfortunately, immunohistochemical or molecular markers for a reliable distinction of oligodendrogliomas and astrocytomas are not available. Recent studies reported that the oligodendrocyte lineage genes *OLIG1* and *OLIG2* are consistently expressed in oligodendrogliomas, while most astrocytic gliomas showed either low or absent expression [12, 13]. Both genes code for basic helix-loop-helix (HLH) transcription factors critically involved in oligodendrocyte development [11, 20]. Olig1 and Olig2 proteins can heterodimerize with other HLH proteins, such as E2a and Heb, which also regulate proliferation, survival, and fate decisions in the oligodendrocyte lineage [19]. Furthermore, Olig2 induces expression of platelet-derived growth factor receptor α (PDGFRα), a key regulator of proliferation, migration, and differentiation of oligodendrocyte precursor cells [6, 11, 21]. Oligodendrocyte precursors additionally express Nkx2.2, a homeobox-containing transcription factor that cooperates with Olig2 in the maturation to oligodendrocytes [5, 15, 21].

We report on the expression of *OLIG1*, *OLIG2*, *E2A* (*TCF3*), *HEB* (*TCF12*), *NKX2.2* (*NKX2B*), and *PDGFRA* transcripts in 70 gliomas using real-time reverse transcription (RT)-PCR analysis. We found that mRNA expression of these genes is frequently increased in gliomas as compared to non-neoplastic adult brain tissue. However, we detected no significant expression differences between oligodendroglial and astrocytic tumors, indicating that the mRNA levels of these genes, as determined by real-time RT-PCR, are of limited value for glioma classification.

Materials and methods

Tumors

Frozen tumor samples from 70 glioma patients (41 male, 29 female; median age: 52.5 years, range: 2–78 years) were selected from the tumor archive at the Department of Neuropathology, Heinrich-Heine-University, Düsseldorf, Germany. All tumors were classified according to the WHO classification of tumors of the nervous system [10]. The series consisted of 9 oligodendrogliomas (WHO grade II), 11 anaplastic oligodendrogliomas (WHO grade III), 5 oligoastrocytomas (WHO grade II), 10 anaplastic oligoastrocytomas (WHO grade III), 10 diffuse astrocytomas (WHO grade II), 10 anaplastic astrocytomas (WHO grade III), and 15 glioblastomas (WHO grade IV). Only tissue pieces with a histologically estimated tumor cell content of 80% or more were used for the molecular analyses. As reference tissues, we used 10 non-neoplastic brain tissue samples (cerebral cortex and/or white matter) from 8 different individuals (6 male, 2 female; median age: 44 years, range 8–72 years). These samples were from patients operated on for chronic temporal lobe epilepsy (n=6) or traumatic brain injury (n=1), or obtained at autopsy (n=1).

DNA and RNA extraction

Extraction of DNA and RNA from frozen tumor tissue was carried out by ultracentrifugation as described elsewhere [9]. DNA extraction from peripheral blood leukocytes was performed according to a standard protocol [17].

Real-time RT-PCR analysis

Three micrograms of total RNA from each tumor were reverse-transcribed into cDNA using random hexanucleotide primers and Superscript reverse transcriptase (Invitrogen, Karlsruhe, Germany). Expression of the six investigated genes was determined by real-time RT-PCR using the ABI PRISM 5700 sequence detection system (Applied Biosystems, Foster City, CA), which allows the continuous measurement of the PCR product amount by means of incorporation of SybrGreen fluorescent dye. The respective primer sequences are listed in Table 1. The transcript level of each gene was normalized to the transcript level of the house-keeping gene *ARF1* (ADP-ribosylation factor 1). The target gene/*ARF1* ratios determined in the individual gliomas were calculated relative to the mean target gene/*ARF1* ratio of the ten non-neoplastic brain samples (Fig. 2a).

Duplex-PCR analysis

The glioblastomas were analyzed for amplification of the *PDGFRA* gene using duplex-PCR for the simultaneous amplification of fragments from *PDGFRA* and the reference genes *APRT* (16q24) or *IFNG* (12q15). The respective primer sequences are listed in Table 1. PCR products were separated on agarose gels and the ethidium bromide-stained bands were recorded using the Gel-Doc 1000 system (Bio-Rad, Hercules, CA). Quantitative analysis of the signal intensities obtained for the target and reference genes was performed with the Molecular Analyst software, version 2.1 (Bio-Rad). Increases in the target gene/reference gene ratio of more than three times the ratio obtained for constitutional DNA were considered as gene amplification.

Microsatellite analysis

Twenty-nine of the oligodendroglial tumors included in this study had been investigated before for 1p and 19q losses using loss of heterozygosity (LOH) analyses at multiple microsatellite markers from 1p34-pter and 19q13 [3].

Statistical analyses

Potential correlations of expression values with tumor type, WHO grade or allelic status on 1p and 19q were evaluated using two-sided Student's t-test analyses. A P value of ≤ 0.05 was considered as significant.

Table 1 PCR primers used for the expression and amplification analyses

Gene	Primer sequences	GenBank accession no.	Fragment size (bp)	Application[a]
OLIG1	5'-AGGTCATCCTGCCCTACTCA-3' 5'-CTGCCCAGCAGTAGGATGTAG-3'	XM_170977	109	1
OLIG2	5'-CACCAAGAAGGACAAGAAGCA-3' 5'-CATCCATGGCGATGTTGA-3'	NM_005806	110	1
E2A	5'-GGACGAGGACGACCTTCTC-3' 5'-AAGGCCTCGTTGATGTCACG-3'	M31523	111	1
HEB	5'-TTTCATCGCGGCAGTACCAG-3' 5'-GTGAGCTTCCAGCAGCATTC-3'	M80627	112	1
NKX2.2	5'-TCTACGACAGCAGCGACAAC-3' 5'-CTTGGAGCTTGAGTCCTGAG-3'	AH007038	113	1
PDGFRA	5'-CTAATCCTCTGCCAGCTTTC-3' 5'-TCACTTCCAAGACCGTCAC-3'	NM_006206	205	1, 2
ARF1	5'-GACCACGATCCTCTACAAGC-3' 5'TCCCACACAGTGAAGCTGATG-3'	M36340	111	1
APRT	5'-CAGGGAACACATTCCTTTGC-3' 5'-GTGGGAAAGCTGTTTACTGCG-3'	Y00486	136	2
IFNG	5'-AGTGATGGCTGAACTGTCGC-3' 5'-CTGGGATGCTCTTCGACCT-3'	J00219	85	2

[a] *1* Expression analysis by real-time reverse transcription-PCR; *2* amplification analysis by duplex-PCR

Results

All six investigated genes (*OLIG1*, *OLIG2*, *E2A, HEB*, *NKX2.2*, and *PDGFRA*) were expressed in non-neoplastic brain tissue and in gliomas. With few exceptions, the mean expression levels determined for the different glioma types were higher than the corresponding mean expression values obtained for the non-neoplastic brain tissue samples (Figs. 1a, 2a). However, the mRNA levels determined for each gene varied considerably from tumor to tumor (Fig. 2a). Statistical comparison of oligodendrogliomas of WHO grades II and III with astrocytomas of WHO grade II and III revealed higher mean expression levels in the oligodendrogliomas for all genes except *E2A*. However, these differences were not significant. Only *HEB* appeared to be expressed at significantly higher levels in anaplastic oligodendrogliomas as compared to anaplastic astrocytomas (Fig. 2a).

We also investigated a possible association between the expression of these genes and the WHO malignancy grade. *OLIG1* and *E2A* showed the highest mean expression levels in the WHO grade III gliomas, whereas *HEB* demonstrated strongest expression in the WHO grade II gliomas (Fig. 2b). *OLIG2*, *NKX2.2* and *PDGFRA* were expressed at similar levels in WHO grade II and III tumors (Fig. 2b). Glioblastomas (WHO grade IV) had lower mean expression levels of *OLIG1, OLIG2, E2A, HEB*, and *NKX2.2* as compared to anaplastic and low-grade gliomas, with the differences being significant for *OLIG2* and *HEB* (Fig. 2b). In contrast, mean *PDGFRA* expression was strongest in glioblastomas. The latter finding was due to 4 glioblastomas with *PDGFRA* gene amplification that showed particularly high mRNA levels (Fig. 1b).

We additionally evaluated whether the expression of the six genes correlated with the allelic status on 1p and 19q in oligodendroglial tumors (Fig. 2c). Microsatellite analyses had been carried out for 29 of the 35 oligodendroglial tumors [3]. Combined 1p and 19q losses were detected in 19 tumors. The mean expression levels of *OLIG1, OLIG2, E2A, HEB,* and *NKX2.2* were higher in gliomas with combined 1p and 19q losses (Fig. 2c). However, statistical analyses revealed no significant correlation between combined 1p and 19q deletions and the mRNA expression levels of any of the investigated genes.

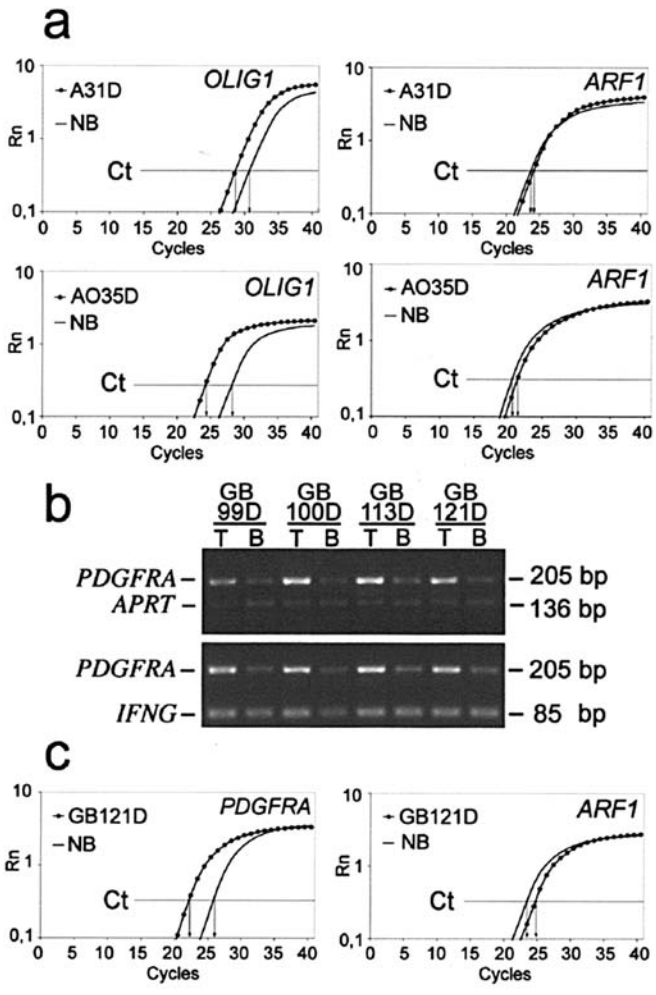

Fig. 1 **a** Examples of real-time reverse transcription-PCR analysis of *OLIG1* expression in gliomas. *Abscissa*, cycle number; *ordinate*, relative amount of PCR product. *OLIG1* expression is increased relative to non-neoplastic brain tissue (*NB*) in the astrocytoma A31D (*upper panel*) and the anaplastic oligodendroglioma AO35D (*lower panel*). The reference mRNA curves (*ARF1*) of NB and each tumor pass the threshold (*Ct*) at an approximately equal cycle number. In contrast, the *OLIG1* mRNA curves of both tumors are shifted to the left relative to the NB curves. The calculated *OLIG1* mRNA expression levels relative to NB are 14.6 (A31D) and 22.9 (AO35D). **b** Demonstration of *PDGFRA* amplification in four glioblastomas using duplex-PCR analyses with the reference genes *APRT* and *IFNG*. Case numbers are given on *top* of the panels (*T*, tumor DNA; *B*, blood DNA; *bp*, sizes of the individual PCR fragments in base pairs). All four glioblastomas demonstrate increased *PDGFRA* signals relative to the corresponding constitutional (blood) DNA. **c** Strong *PDGFRA* expression in glioblastoma GB121D. The *ARF1* mRNA curve of GB121D passes the threshold (*Ct*) at a slightly higher cycle number than the corresponding curve for non-neoplastic brain tissue (*NB*). In contrast, the *PDGFRA* curve of GB121D is shifted to the left relative to the NB curve. The calculated *PDGFRA* mRNA level in GB121D is 23.9 relative to NB

Discussion

Recent studies reported on a high expression of *OLIG1* and *OLIG2* transcripts in oligodendrogliomas and suggested these genes as potentially useful markers for the differential diagnosis of gliomas [12, 13]. Here, we report on mRNA expression analyses of six oligodendrocyte lineage genes (*OLIG1*, *OLIG2*, *E2A, HEB*, *NKX2.2*, and *PDGFRA*) in 70 gliomas using real-time RT-PCR analysis. In line with Lu et al. [12] and Marie et al. [13], we detected higher mean expression values for *OLIG1* and *OLIG2* in oligodendroglial tumors as compared to astrocytic tumors. However, these differences were not significant. Similarly, we found that the expression of four other oligodendrocyte lineage genes (*E2A, HEB, NKX2.2, PDGFRA*) did not significantly differ between astrocytic and oligodendroglial tumors, except for a higher *HEB* expression in anaplastic oligodendrogliomas as compared to

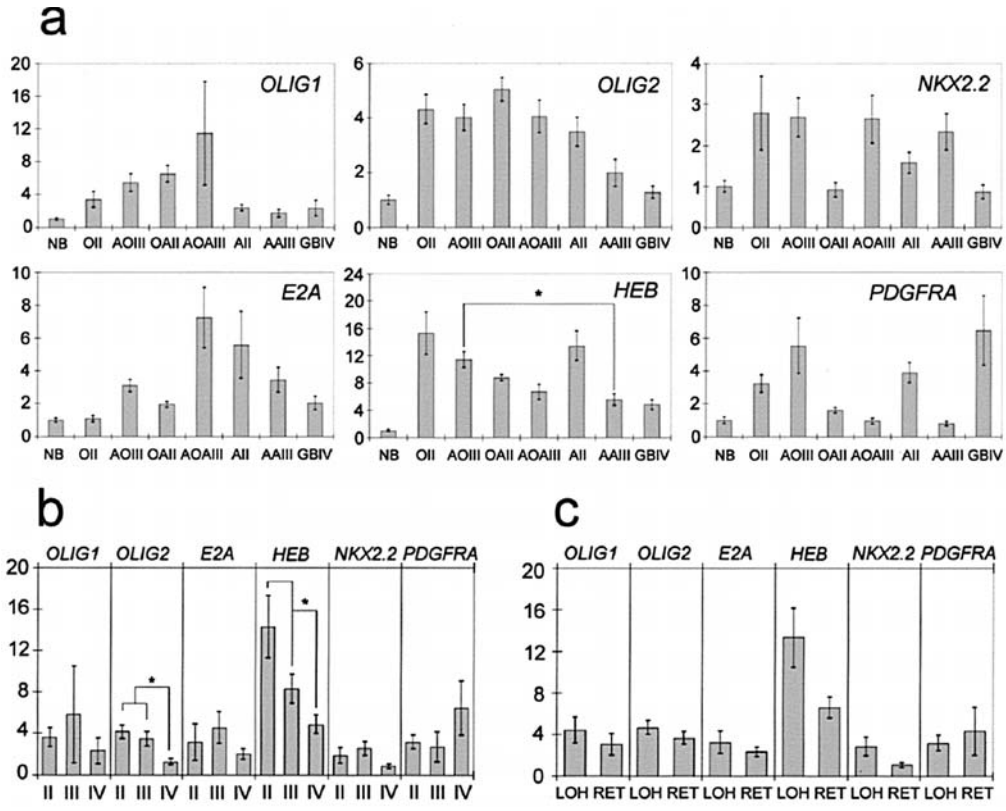

Fig. 2 **a** Mean transcript levels of six oligodendrocyte lineage genes in non-neoplastic brain tissue and different glioma types. *Abscissa*, glioma types: *NB*, non-neoplastic brain tissue; *OII*, oligodendroglioma (WHO grade II); *AOIII*, anaplastic oligodendroglioma (WHO grade III); *OAII*, oligoastrocytoma (WHO grade II); *AOAIII*, anaplastic oligoastrocytoma (WHO grade III); *AII*, diffuse astrocytoma (WHO grade II); *AAIII*, anaplastic astrocytoma (WHO grade III); *GBIV*, glioblastoma (WHO grade IV). *Ordinate*, expression level relative to non-neoplastic brain tissue (arbitrarily set to expression level = 1). In most instances, the mean expression levels of the six investigated genes are higher in the various glioma types as compared to the non-neoplastic brain samples (*error bars* represent mean value ±20% standard deviation). Statistical analysis showed no significant differences in the mean expression levels between astrocytic and oligodendroglial tumors. Only *HEB* expression was significantly higher in anaplastic oligodendrogliomas as compared to anaplastic astrocytomas (*). **b** Mean transcript levels of six oligodendrocyte lineage genes in gliomas of different WHO grades. *Abscissa*, WHO malignancy grades. *Ordinate*, expression level relative to non-neoplastic brain tissue. *Error bars* represent mean value plus/minus 25% standard error. WHO grade IV tumors demonstrated lower mean expression of *OLIG1*, *OLIG2*, *E2A*, *HEB* and *NKX2.2* as compared to gliomas of WHO grade II or III (*, significant differences). **c** Graphical representation of the mean expression levels of oligodendrocyte lineage gene transcripts in 29 oligodendroglial tumors grouped by LOH status on 1p and 19q. *Error bars* represent mean value ± 25% standard deviation. *LOH*, tumors with a combined loss of heterozygosity on 1p and 19q (n=19); *RET*, tumors with retention of heterozygosity on one or both chromosome arms (n=10). All genes except for *PDGFRA* showed higher mean expression levels in tumors with combined LOH on 1p and 19q, but these differences were not significant

anaplastic astrocytomas. The mean expression levels of *E2A* were even higher in astrocytic gliomas as compared to oligodendroglial tumors. It appears unlikely that the expression detected in astrocytic neoplasms is solely due to contaminating non-neoplastic oligodendrocytes because in most instances the mean expression levels detected in the astrocytic tumors were higher as compared to mean expression levels determined for non-neoplastic brain tissues (Fig. 2).

Taken together, our findings indicate that none of the investigated genes is expressed in a glioma type-specific manner that would suggest a diagnostic utility, at least when expression is determined at the mRNA level using real-time RT-PCR. Possible reasons for the differences between our data and the findings reported by Lu et al. [12] and Marie et al. [13] include the use of different detection methods, i.e., in situ hybridization [12, 13] versus real-time RT-PCR (present study), as well as differences in the number of investigated tumors, i.e., 23 gliomas including 6 oligodendrogliomas [12], 21 gliomas including 9 oligodendrogliomas [13], versus 70 gliomas including 20 oligodendroglial and 15 oligoastrocytic tumors (present study). Two recent studies published during the preparation of our manuscript reported on findings that support our data. Bouvier et al. [1] investigated a series of 89 brain tumors, including 71 oligodendroglial and astrocytic gliomas, for the expression of *OLIG1* and *OLIG2* transcripts using semiquantitative RT-PCR. Both genes were found to be expressed at similar levels in oligodendrogliomas and astrocytomas, with pilocytic astrocytomas showing the highest expression levels [1]. Similarly, Ohnishi et al. [14] reported on high levels of *OLIG1* and *OLIG2* transcripts in both anaplastic oligodendroglioma and anaplastic astrocytoma. However, on immunohistochemical analysis,

these authors found a higher fraction of Olig2-immunopositive tumor cells in anaplastic oligodendrogliomas as compared to anaplastic astrocytomas [14].

Correlation of oligodendrocyte lineage gene expression and tumor grade revealed that glioblastomas showed lower mean transcript levels of *OLIG1, OLIG2, E2A*, *HEB* and *NKX2.2* as compared to WHO grade II and III gliomas. Ohnishi et al. [14] also detected significantly lower *OLIG1* and *OLIG2* expression levels in glioblastomas than in lower grade gliomas. The reasons underlying this finding are unclear but may be related to the poor cellular differentiation in most glioblastomas.

In line with previous studies [2, 7], we detected increased *PDGFRA* expression relative to non-neoplastic brain tissue in many astrocytic and oligodendroglial tumors. The mean *PDGFRA* expression levels varied but did not differ significantly between the individual glioma entities. The higher mean expression determined for glioblastomas was due to four tumors with *PDGFRA* gene amplification. *PDGFRA* amplification has been reported in 8–16% of glioblastomas [4, 8]. More recently, Smith et al. [18] suggested that *PDGFRA* amplification is restricted to highly anaplastic oligodendrogliomas. However, histological review of the glioblastomas with *PDGFRA* amplification in our series did not demonstrate any features of oligodendroglial differentiation.

Oligodendroglial tumors frequently show combined LOH on 1p and 19q, which is associated with favorable response to chemotherapy and good prognosis [16]. To investigate the relationship between oligodendrocyte lineage gene expression and allelic status on 1p and 19q, we correlated our expression data with previously reported LOH data [3]. These analyses revealed that expression of the six genes was not significantly correlated with 1p and 19q losses. Thus, expression analysis of these genes by real-time RT-PCR cannot substitute for 1p/19q deletion testing in the diagnostic assessment of oligodendroglial tumors.

In summary, we show that expression of the oligodendrocyte lineage genes *OLIG1, OLIG2, E2A, HEB, NKX2.2*, and *PDGFRA* is frequently up-regulated relative to non-neoplastic brain tissue in both oligodendroglial and astrocytic gliomas. Our findings support the hypothesis that glial tumor cells are arrested in or recapitulate molecular phenotypes that correspond to early stages of glial development. However, expression analysis of these genes using real-time RT-PCR appears to be of limited significance for the differential diagnosis of different types of gliomas. Nevertheless, our data do not exclude that investigation of these genes by other methods, such as in situ hybridization or immunohistochemistry, may be diagnostically useful due to differences in detection sensitivity and/or the ability to demonstrate particular expression patterns.

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