Expression of CD40 Correlates Negatively with Overall and Progression-Free Survival of Low- and High-Grade Gliomas

Jan-Michael Werner[1,2], Saskia Kuhl[1], Katharina Ulrich[3], Boris Krischek[1], Pantelis Stavrinou[1], Roland Goldbrunner[1], Marco Timmer[1,4]

■ BACKGROUND: **Low-grade gliomas (LGGs) are known to progress to glioblastoma (GBM), decreasing the chances of survival. The tumor necrosis factor receptor CD40 and its ligand CD40L have shown value as biomarkers for GBM. The present study evaluated the role of CD40/CD40L in LGG and GBM in differentiating isocitrate dehydrogenase (IDH) wild-type and IDH-mutant GBM.**

■ METHODS: **The present study was based on patient-derived samples (74 grade II gliomas, 36 grade III gliomas, and 40 cases of GBM) and expression analysis using real-time polymerase chain reaction. Open-access data from The Cancer Genome Atlas (TCGA) and the strong cohorts of TCGA data sets "brain lower grade glioma" and "glioblastoma" were used to run the analysis on mRNA expression as a validation data set.**

■ RESULTS: **We found that patients with LGG and CD40 overexpression experienced shorter progression-free survival (43 vs. 29 months; hazard ratio, 0.5715; $P = 0.0262$) and overall survival (116 vs. 54 months; hazard ratio, 0.3431; $P < 0.0001$). Consistently, relapsed grade II glioma showed greater CD40 expression compared with primary grade II glioma ($P = 0.0028$). Just as with LGG, CD40 was a negative marker for overall survival in GBM (12 vs. 10 months; hazard ratio, 0.5178; $P = 0.0491$). In this context, we found greater CD40 expression in IDH wild-type GBM than in IDH-mutant GBM. The data obtained from TCGA supported our findings, with similar results for PFS and OS in LGG and GBM. CD40L expression showed no correlation with the survival data.**

■ CONCLUSION: **High CD40 expression showed a significant correlation with poor outcomes for both LGG and GBM and was overexpressed in IDH wild-type GBM.**

INTRODUCTION

High-grade gliomas and brain metastases are the most common tumors of the central nervous system, with astrocytic tumors representing the largest proportion of all gliomas. Despite all the research efforts, the prognosis for patients with glioblastoma (GBM) has remained poor.[1,2] Diffuse astrocytomas can have an aggressive course and transform into GBM, especially in the case of a somatic missense mutation in codon 132 of isocitrate dehydrogenase (IDH) 1 (IDH1). The latter is found in 12% of all cases of GBM and the vast majority of diffuse and anaplastic astrocytomas.[3-5] The presence of an IDH mutation

Key words
- CD40
- CD40L
- Glioblastoma
- Glioma
- Low-grade glioma
- Prognosis
- TNF receptor

Abbreviations and Acronyms
CD40L: CD40 ligand
GBM: Glioblastoma
GII: World Health Organization grade II
IDH: Isocitrate dehydrogenase
IDHmut: Isocitrate dehydrogenase mutated
IDHwt: Isocitrate dehydrogenase wild-type
LGG: Low-grade glioma
OS: Overall survival
PFS: Progression-free survival
rt-PCR: Real-time polymerase chain reaction
SDHA: Succinate dehydrogenase complex
subunit A: Flavoprotein variant
TCGA: The Cancer Genome Atlas
TNF-R: Tumor necrosis factor receptor
WHO: World Health Organization

From the [1]Laboratory for Neurooncology and Experimental Neurosurgery, Department of General Neurosurgery, Center for Neurosurgery, and [2]Department of Neurology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne; [3]German Center for Neurodegenerative Diseases, Bonn; and [4]Max Planck Institute for Metabolism Research, Cologne, Germany

To whom correspondence should be addressed: Marco Timmer, M.Sc., M.D. [E-mail: marco.timmer@uk-koeln.de]

Citation: World Neurosurg. (2019) 130:e17-e25.
https://doi.org/10.1016/j.wneu.2019.05.112

*Journal homepage: **www.journals.elsevier.com/world-neurosurgery***

*Available online: **www.sciencedirect.com***

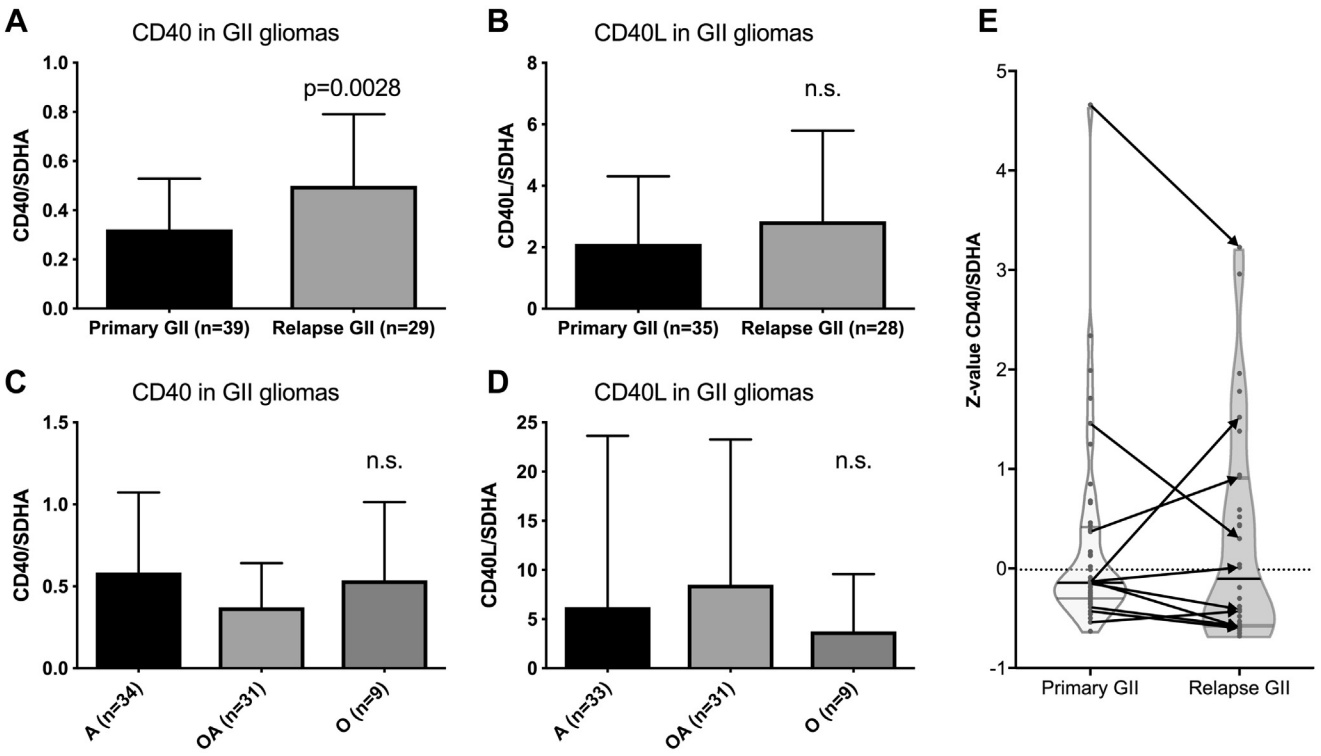

Figure 1. CD40 expression was retained and was significantly greater in relapsed grade II gliomas compared with primary grade II gliomas. In contrast, differentiation into astrocytoma (A), oligoastrocytoma (OA), and oligodendroglioma (O) showed no differences. Comparisons of CD40 and CD40L expression levels in (**A,B**) primary and relapsed grade II (GII) gliomas and (**C**,**D**) astrocytoma, oligoastrocytoma, and oligodendrogliomas. (**E**) Comparison of 10 patients with primary and relapsed GII samples showing individual retention of relative CD40 expression (displayed as Z values; patient samples connected by *arrows*). *Columns* display the mean values and *error bar*, the standard deviation. n.s., no statistical significance; SDHA, succinate dehydrogenase complex, subunit A, flavoprotein variant.

has been an independent favorable prognostic factor and was included in the novel classification of primary brain tumors by the World Health Organization (WHO).[6,7] The presence of an IDH mutation can be used to differentiate GBM into IDH wild-type (IDHwt; de novo) GBM and secondary IDH-mutant (IDHmut) GBM, which arise from previous astrocytic gliomas via malignant progression.[6,8]

The 48-kDa type I glycoprotein receptor protein CD40 is a member of the tumor necrosis factor receptor (TNF-R) family. Its principal function is B-cell activation and cellular viability. CD40 is a costimulatory molecule, which can act in both a proapoptotic and an antiapoptotic manner, and is known to mediate the proliferation and differentiation of B cells.[9] CD40 plays a role in the efficient activation of T cells by antigen-presenting cells and can be expressed by nonlymphoid cells, including epithelial cells, fibroblasts, and neuronal cells, as well as malignant tumors.[10,11] CD40 is expressed by 70% of solid tumors, and CD40 activation leads to apoptosis and activation of TNF-R–associated factors in many tumor models.[12] The physiological ligand CD154 (CD40L), a type II membrane protein, is expressed on T cells, platelets, monocytes, macrophages, and endothelial cells.[13,14] CD40 signaling is context specific and can either promote survival or induce apoptosis.[11]

In contrast to other TNF-Rs, CD40 does not possess its own death domain. Soluble CD40L inhibits growth in both vital and malignant cells but only induces apoptosis in tumor cells.[11] The stimulation of CD40 with recombinant soluble CD40L was shown to increase survival in a breast cancer mouse model and had similar effects in breast cancer cell lines.[15] The expression of CD40 has been correlated with the histological grade in colon cancer.[16] CD40-mediated therapy using an adenoviral vector expressing CD40L showed positive treatment effects in metastatic colon cancer.[17] Similar results were shown for ovarian cancer using anti-CD40 agonists, which significantly inhibited cell growth.[18] Additionally, recent studies have shown an enhanced effect of cisplatin in ovarian cancer by sensitizing the tumors with soluble CD40L.[19]

CD40 is expressed in human glioma cells in vitro and in vivo. Bispecific antibodies against CD40 and CD95, a member of the TNF-α receptor family with a death domain mostly known as FAS or APO-1, specifically kill glioma cells.[20] A recent study showed greater levels of CD40/CD40L in grade III gliomas compared with GBM and identified high CD40/CD40L expression as a favorable prognostic marker.[21] Direct antitumor effects of an anti-CD40 agonistic monoclonal antibody (FGK45) against glioma cell lines were shown in vitro.[21] Local convection-enhanced

delivery of an anti-CD40 agonistic monoclonal antibody induced antitumor effects in a mouse model of glioma.[22]

Our study aimed to further deepen our understanding of the expression patterns of CD40 in glioma and to gain new insights into the prognostic value of CD40/CD40L expression in WHO grade II (GII) gliomas.

METHODS

Samples and Patient Data

The tumor samples were obtained directly during surgery, cut, and immediately frozen in liquid nitrogen and maintained at −80°C until RNA extraction. Surgery was performed from 1991 to 2014. Patient data were last updated on May 3, 2018. Nontumorous tissue was obtained from operative fields distant from the tumor. Two independent neuropathologists performed the histopathological diagnosis and grading of the extracted tissue. The local ethics committee of University Hospital Cologne approved the present study (application no. 03-170), which was performed in accordance with the Declaration of Helsinki.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction

Each sample was confirmed to only contain tumor before proceeding with real-time polymerase chain reaction (rt-PCR). RNA was isolated from frozen human tumor samples (RNeasy Kit [Qiagen, Hilden, Germany]), and cDNA was synthesized (QuantiTect Reverse Transcription Kit [Qiagen]). The Cycler Rotor-Gene Q and the Rotor-Gene SYBR Green PCR Kit (Qiagen) were used for rt-PCR. Every rt-PCR was performed 3 times in a 2-step protocol (95°F for 5 minutes, 95°F for 5 seconds, 60°F for 10 seconds).

The succinate dehydrogenase complex, subunit A, flavoprotein variant (SDHA), was used as a housekeeping gene, and the expression level was calculated by dividing the value of CD40 or CD40L by the value of SDHA. The primers used were as follows: SDHA (QT00059486 [Qiagen]), CD40 (QT00998326 [Qiagen]), and CD40L (sense: CACCCCCTGTTAACTGCCTA; antisense: CTGGATGTCTGCATCAGTGG [Eurofins, Luxemburg]).

IDH1 Mutation Analysis

IDH1 mutations were detected by direct sequencing (ABI PRISM BigDye Terminator, version 1.1, Cycle Sequencing Kit and the ABI 3730 sequencing instrument [Applied Biosystems, Foster City, California, USA]) of amplified cDNA (primers: sense—GTGCCACTATCACTCCTGATG; antisense—AAGGCCAACCCTTAGACAGAG).

The Cancer Genome Atlas Data Analysis

The data were generated from The Cancer Genome Atlas (TCGA) Research Network (available at: http://www.cancergenome.nih.gov/). Data access and analysis were performed using the cBioPortal for Cancer Genomics (available at: http://www.cbioportal.org).[23,24] The cancer studies "brain lower grade glioma (TCGA, provisional)" and "glioblastoma (TCGA, provisional)" were selected, and the genomic profile mRNA expression z scores (RNA sequence, version 2, RSEM) were chosen. The patient/case set was limited to tumor samples with mRNA data (RNA sequence, version 2). The Onco Query Language command "CD40:EXP>1" was used to select cases with mRNA expression >1 standard deviation greater than the mean. Cases with negative survival data were excluded before statistical analysis.

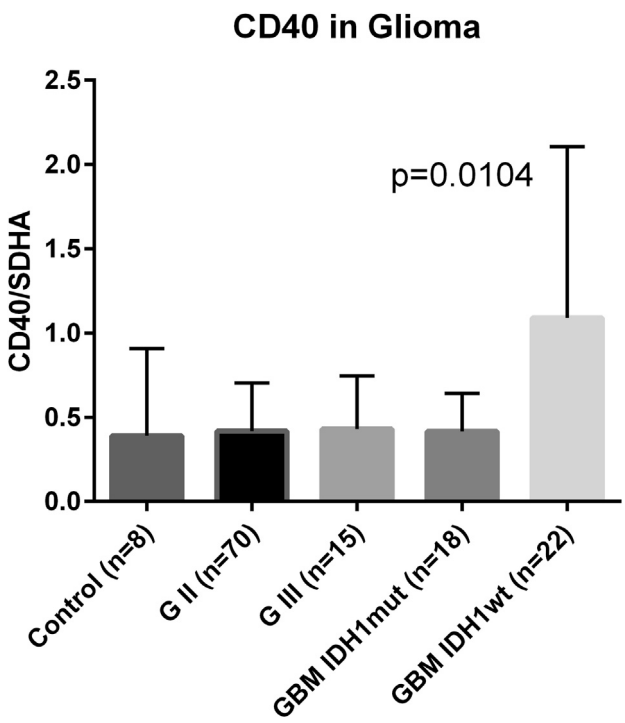

Figure 2. Isocitrate dehydrogenase 1 (IDH1) wild-type (wt) glioblastoma (GBM) showed significantly greater expression of CD40 compared with IDH1-mutated (mut) GBM, grade II (GII) and grade III (GIII) gliomas, and peritumoral control tissue (control). *Columns* display mean values and *error bar*, the standard deviation. SDHA, succinate dehydrogenase complex, subunit A, flavoprotein variant.

Statistical Analysis

Statistical analyses were executed using Prism 7 software (GraphPad Software, San Diego, California, USA). The ROUT test ($Q = 0.5\%$) was used to exclude outliers in the comparison of CD40 and CD40L expression. The 2-tailed Mann-Whitney U test was used for the comparison of primary and relapsed GII glioma (**Figure 1A,B**). The Kruskal-Wallis test was used for the comparison of histological subgroups and different WHO grades (**Figures 1** and **2**). For the survival analysis, the log-rank test (Mantel-Cox test) and the Gehan-Breslow-Wilcoxon test were used to the compare the survival curves (**Figures 3** and **4**). The 2-tailed nonparametric Spearman correlation was used for the correlation analysis (**Figure 3C**).

RESULTS

A total of 75 GII samples were included in the present study. Detailed data on the patients' sex, age, Karnofsky performance scale score, therapy, operative results, progression, survival, tumor histological data, and IDH1 status are presented in **Table 1**. To place the results into context with the reported data of CD40

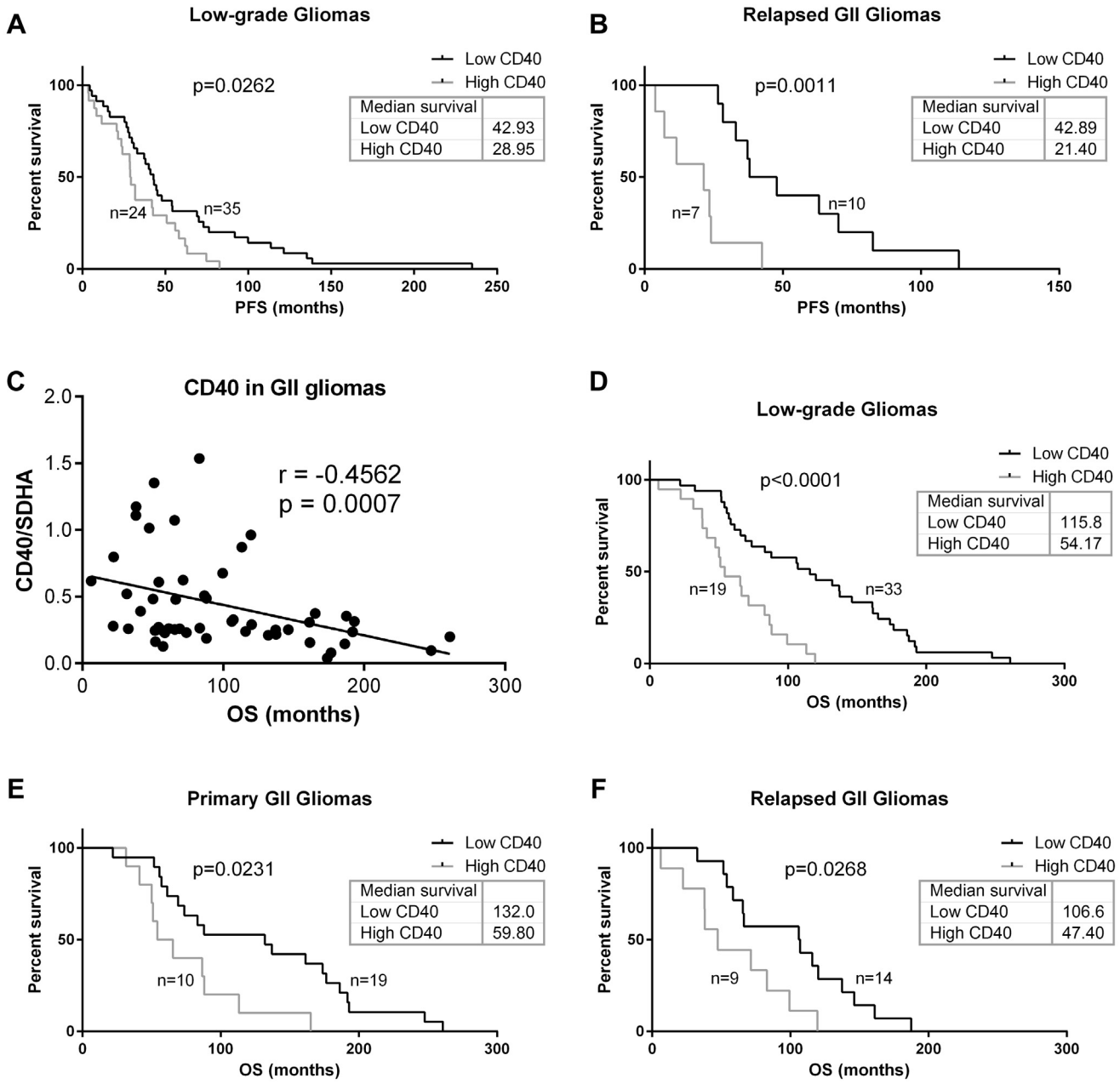

Figure 3. Patients with high expression of CD40 in grade II (GII) gliomas had significantly shorter progression-free survival (PFS) and overall survival (OS). Kaplan-Meier plots of PFS for (**A**) all low-grade gliomas and (**B**) relapsed GII gliomas according to CD40 expression. (**C**) Negative correlation between CD40 expression and survival. Kaplan-Meier plots of OS of (**D**) all low-grade gliomas, (**E**) primary GII gliomas, and (**F**) relapsed GII gliomas according to CD40 expression. *r*, Spearman's rho; SDHA, succinate dehydrogenase complex, subunit A, flavoprotein variant.

expression in high-grade gliomas, samples from 40 patients with GBM were collected. rt-PCR of CD40 expression was performed on 22 IDHwt and 18 IDHmut GBM samples. The IDH1mut cases included 14 IDH (R132H) mutations, 3 IDH (R132S) mutations, and 1 IDH (R132S) mutation. The mean survival was 17.40 months for the patients with IDHwt GBM and 19.04 months for the patients with IDHmut GBM. The mean Karnofsky performance scale score before surgery was 80 for those with IDHwt GBM and 75 for those with IDHmut GBM.

Differences in CD40 and CD40L Expression

Relapsed GII gliomas had significantly greater levels of CD40 compared with primary GII gliomas ($P = 0.0028$, Mann-Whitney U test; Figure 1A). CD40L expression showed no statistically

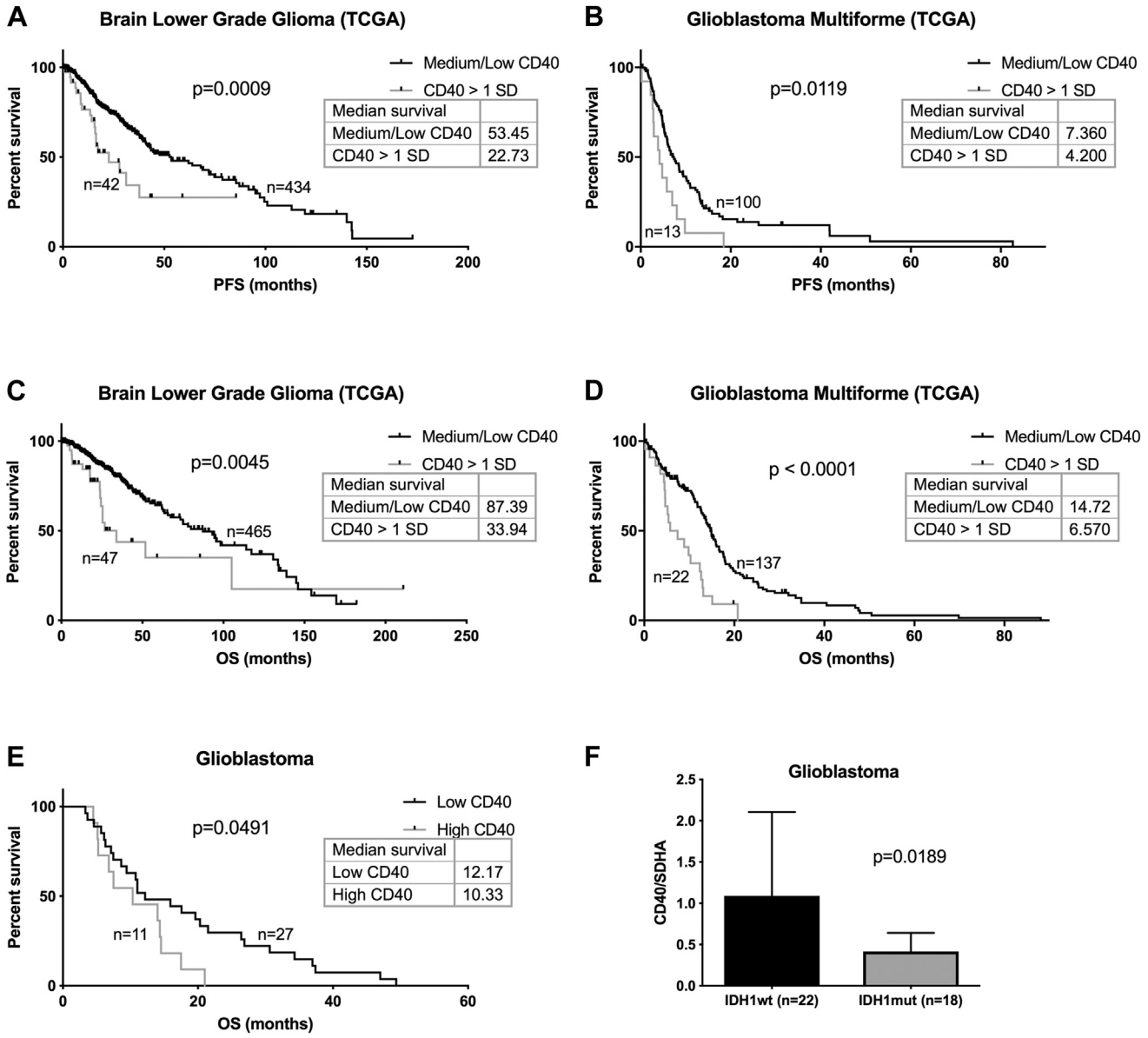

Figure 4. Patients with high expression of CD40 showed significantly shorter progression-free survival (PFS) and overall survival (OS) in glioblastoma (GBM). Additionally, CD40 was overexpressed in isocitrate dehydrogenase 1 (IDH1) wild-type (wt) compared with IDH1 mutated (mut) GBM. Kaplan-Meier plots of The Cancer Genome Atlas (TCGA) data, showing (**A,B**) PFS and (**C,D**) OS of low-grade glioma and glioblastomas according to CD40 expression. (**E**) Kaplan-Meier plot of our findings showing OS of GBM according to CD40 expression. (**F**) Comparison of CD40 expression in IDHwt and IDHmut GBM. SD, standard deviation; SDHA, succinate dehydrogenase complex, subunit A, flavoprotein variant.

significant differences between primary and relapsed GII glioma ($P = 0.4437$, Mann-Whitney U test; Figure 1B). CD40 and CD40L expression both showed significant differences with respect to the histological features of GII glioma (CD40, $P = 0.0728$; CD40L, $P = 0.528$, Kruskal-Wallis test; Figure 1C,D). Tissue samples from the initial diagnosis and recurrent tumor were available for 10 patients. CD40 expression of primary and recurrent GII glioma was normalized into Z values for comparison (Figure 1E). CD40 expression was preserved from the primary to recurrent tumor. The comparison of CD40 expression of the corresponding primary and recurrent tumor samples from the 10 patients revealed no statistically significant differences ($P = 0.67$). Further development to either recurrence of a GII glioma or malignant progression to high-grade glioma showed no statistically significant differences in CD40 ($P = 0.534$) or CD40L ($P = 0.344$) expression.

Table 1. Detailed Data on Patients with Grade II Glioma

Characteristic	Patients (n; %)
Primary or relapsed GII*	
Primary GII	44 (59.5)
Relapse GII	30 (40.5)
Age at surgery (years)	
<40	39 (52.0)
≥40	36 (48.0)
Age (years)	
Mean	40.95
95% CI	38.01–43.89
Sex	
Female	35 (46.7)
Male	40 (53.3)
KPS score before surgery	
<70	2 (3.5)
>70	55 (96.5)
KPS score	
Mean	87.11
95% CI	84.46–89.75
Histological type	
Astrocytoma	35 (46.7)
Oligoastrocytoma	31 (41.3)
Oligodendroglioma	9 (12.0)
IDH1 status	
IDH1 mutation	65 (86.7)
IDH1 wild type	10 (13.3)
Radiotherapy	
Radiotherapy or brachytherapy	21 (32.8)
No radiotherapy	34 (53.1)
No data	9 (14.1)
Operative result	
Partial resection	21 (28.0)
Gross total resection	24 (32.0)
No data	30 (40.0)
Progression	
Stable (relapse GII)	26 (34.7)
Malignant progression (relapse GIII/GBM)	33 (44.0)
No data or no relapse	16 (21.3)
PFS (months)	
Mean	46.64
95% CI	33.75–59.54
OS (months)	
Mean	101.24
95% CI	83.47–119.02

GII, grade II glioma; CI, confidence interval; KPS, Karnofsky performance scale; IDH1, isocitrate dehydrogenase 1; GIII, grade III glioma; GBM, glioblastoma; PFS, progression-free survival; OS, overall survival.
*One sample could not be defined as primary or relapsed GII.

Prognostic Value of CD40 and CD40L Expression in Low-Grade Gliomas

The evaluation of the prognostic value of CD40 and CD40L expression with respect to progression-free survival (PFS) and overall survival (OS) showed a statistically significant negative rank correlation between CD40 expression and OS (Spearman $r = -0.456$; $P = 0.0007$; Figure 3C). However, no statistically significant correlation was found with PFS (Spearman $r = -0.252$; $P = 0.0569$). CD40L expression showed no clear correlation with OS or PFS (data not shown).

The data set was divided according to high and low CD40 expression. The division was performed after Z transformation of the CD40/SDHA values and designated as low expression (Z value <0; $n = 44$) and high expression (Z value >0; $n = 31$). The group comparison of high- and low-expression samples with respect to several prognostic factors such as the Karnofsky performance scale score, patient age, or operative result showed no differences between the 2 groups (Table 2). The median OS differed significantly ($P < 0.0001$) between the groups with low and high expression of CD40 (116 vs. 54 months, respectively). Although the regression analysis did not show a significant correlation between CD40 expression and PFS, the Kaplan-Meier analysis showed a statistically significant difference between the median PFS and low and high expression of CD40 (42.93 vs. 28.95 months; $P = 0.0262$; Figure 3A).

Data on OS were available for 52 patients (33 and 19 patients with low and high expression of CD40, respectively). Data on PFS were available for 59 patients (35 and 24 patients with low and high expression of CD40, respectively). Because CD40 expression showed differences between primary and relapsed GII gliomas (Figure 1A), individual analyses of both groups were performed with respect to OS and PFS (Figure 3). The positive predictive effect of low expression of CD40 with respect to OS was strongest in the combined group of primary and recurrent GII gliomas (median survival, 116 vs. 54 months; hazard ratio, 0.343 vs. 2.915; $P < 0.0001$, log-rank test; Figure 3D). Both primary (median survival, 132 vs. 60 months; hazard ratio, 0.408 vs. 2.452; $P = 0.0231$, log-rank test; Figure 3E) and relapsed (median survival, 107 vs. 47 months; hazard ratio, 0.403 vs. 2.484; $P = 0.0268$, log-rank test; Figure 3F) GII gliomas showed longer OS when CD40 expression was low. In addition, a positive predictive effect of low CD40 expression in relation to PFS was shown in the combined group of primary and recurrent GII gliomas (median PFS, 43 vs. 29 months; hazard ratio, 0.572 vs. 1.75; $P = 0.0262$, log-rank test; Figure 3A). The same effect

Table 2. Group Comparison of Low and High Expression of CD40 in Grade II Glioma

Variable	Low CD40 *n* (%, Row; %, Column)	High CD40 *n* (%, Row; %, Column)	*P* Value*
Patients	44 (59.5; NA)	30 (40.5; NA)	NA
Age group (years)			0.348 (NS)†
<40	21 (53.9; 47.7)	18 (46.1; 60.0)	
>40	23 (65.7; 52.3)	12 (34.3; 40.0)	
Age			0.196 (NS)‡
Mean	41.61	37.59	
95% CI	38.11–45.11	34.65–40.53	
Sex			0.479 (NS)†
Female	19 (54.3; 43.2)	16 (45.7; 53.3)	
Male	25 (64.1; 56.8)	14 (35.9; 46.7)	
KPS score			0.217 (NS)‡
Mean	88.97	85.45	
95% CI	85.94–92.00	80.97–89.94	
Histological type			0.221 (NS)§
Astrocytoma	17 (50.0; 38.6)	17 (50.0; 56.7)	
Oligoastrocytoma	22 (71.0; 50.0)	9 (29.0; 30.0)	
Oligodendroglioma	5 (55.6; 11.4)	4 (44.4; 13.3)	
IDH1			0.146 (NS)†
IDH1 wild type	3 (33.3; 6.8)	6 (66.7; 20.0)	
IDH1 mutated	41 (63.1; 93.2)	24 (36.9; 80.0)	
Operative results			0.241 (NS)§
Partial resection	15 (71.4; 34.1)	6 (28.6; 20.0)	
GTR	15 (62.5; 34.1)	9 (37.5; 30.0)	
Missing data	14 (48.3; 31.8)	15 (51.7; 50.0)	
Radiotherapy			0.451 (NS)§
None	22 (64.7; 50.0)	12 (35.3; 40.0)	
Radiotherapy or brachytherapy	13 (61.9; 30.0)	8 (38.1; 26.7)	
No data	9 (47.4; 20.0)	10 (52.6; 33.3)	
OS (months)			<0.0001‖¶
Median	115.80	54.17	
HR	0.343	2.915	
95% CI	0.165–0.714	1.401–6.063	
PFS (months)			0.0262‖¶
Median	42.93	28.95	
HR	0.572	1.75	
95% CI	0.324–1.008	0.992–3.086	

Continues

NA, not applicable; NS, not statistically significant; CI, confidence interval; KPS, Karnofsky performance scale; IDH1, isocitrate dehydrogenase 1; GTR, gross total resection; OS, overall survival; HR, hazard ratio; PFS, progression-free survival.
*Comparison of low and high CD40 expression.
†Fisher's exact test.
‡Mann-Whitney *U* test.
§χ^2 test.
‖Statistically significant.
¶Log-rank (Mantel-Cox) test.

appeared in the analysis of the group of recurrent GII gliomas alone (median PFS, 43 vs. 21 months; hazard ratio, 0.251 vs. 3.985; P = 0.0011, log-rank test; Figure 3B).

CD40 Expression in GBM

Our CD40 data from GII gliomas—in contrast to a previous report[21] of high-grade gliomas (see the Introduction section)—showed a negative correlation between CD40 expression and patient survival. Therefore, we also analyzed our higher grade gliomas. IDHwt GBM showed significantly greater expression of CD40 compared with the other gliomas or control tissue (P = 0.0104; Figure 2). Correspondingly, a direct comparison showed significantly greater expression of CD40 in IDHwt compared with IDHmut GBM (95% confidence interval, 0.6389–1.54 vs. 95% confidence interval, 0.3037–0.5278, P = 0.0189, Mann-Whitney U test; Figure 4F). Furthermore, a negative correlation was found between the expression of CD40 and OS in the combined GBM data set (Spearman r = −0.3491; P = 0.0317) and the IDHwt GBM data set (Spearman r = −0.4229; P = 0.0499). However, IDHmut GBM showed no significant correlation between the survival data and CD40 expression. The GBM data set was divided into high (n = 11; 9 with IDHwt and 2 with IDHmut) and low (n = 29; 13 with IDHwt and 16 with IDHmut) expression of CD40 according to positive (high) or negative (low) z values of the CD40/SDHA. Kaplan-Meier analysis showed longer survival for GBM with low CD40 expression (median survival, 12 vs. 10 months; hazard ratio, 0.5178; P = 0.0491, log-rank test; Figure 4E). The GBM data set was too small to repeat the Kaplan-Meier analysis for the subset of IDHwt GBM cases. Therefore, a subsequent TCGA data analysis was performed.

TCGA Data Analysis

CD40 expression analysis was repeated with data from TCGA. High expression of CD40 mRNA was defined as >1 standard deviation greater than the mean value. Analysis of TCGA low-

grade glioma (LGG) data revealed a positive prognostic value of low CD40 expression with respect to OS (median survival, 87 vs. 34 months; hazard ratio, 0.3696; P = 0.0045, log-rank test; **Figure 4C**) and PFS (median survival, 54 vs. 23 months; hazard ratio, 0.4574; P = 0.0009, log-rank test; **Figure 4A**).

In accordance with the previous results from LGGs and our results with GBM, the positive prognostic value of low CD40 expression was also shown in TCGA data for IDHwt GBM with respect to both OS (median survival, 15 vs. 7 months; hazard ratio, 0.4090; $P < 0.0001$, log-rank test; **Figure 4D**) and PFS (median survival, 7 vs. 4 months; hazard ratio, 0.4821; $P < 0.0001$, log-rank test; **Figure 4**).

DISCUSSION

Our study resulted in 3 major findings. First, patients with LGG or high-grade glioma and low CD40 expression had longer OS and PFS. Second, relapsed GII gliomas had greater CD40 expression compared with primary GII gliomas, although CD40 expression was preserved in the individual patient from the primary tumor to recurrent GII glioma. Finally, CD40 expression was greater in IDHwt GBM than in IDHmut GBM.

Our Findings Compared with Reported Data

Our findings have confirmed previous studies that suggested CD40 as a possible biomarker for glioma.[21] To the best of our knowledge, we have shown, for the first time, that low CD40 values imply a significantly better prognosis for patients with LGG. From our results for LGGs and the reported data for GBM, it might be reasonable to assume that GII gliomas with high CD40 expression will be more likely to progress to high-grade glioma. Thus, an inverse correlation could exist for LGGs and high-grade gliomas between CD40 expression and survival. However, as our data have shown, no correlation between CD40 expression and further progression of LGGs could be derived. Additionally, and in contrast to the GBM study by Chonan et al.,[21] we found a negative correlation between CD40 expression and OS for patients with IDHwt GBM. Our findings were confirmed by analysis of the open accessible data from TCGA. One possible explanation for the discrepancy between the previous data and our findings could be the difference in CD40 expression with respect to IDH mutation status. Chonan et al.[21] did not report on the IDH mutation status of the included GBM samples. Thus, the composition of the respective cohorts could have affected the results, because we, in particular, did not find a correlation between CD40 expression and survival in those with IDHmut GBM.

Our findings were confirmed by an analysis of data from TCGA, which additionally revealed a positive predictive value for low CD40 expression with respect to PFS for both LGG and high-grade glioma. The median survival of the "brain lower grade glioma (TCGA, provisional)" data set was considerably shorter compared with our LGG data (most likely because TCGA data also included grade III gliomas).

IDH Mutation Status

The expression of CD40 differed depending on the IDH mutation status. Therefore, IDH mutation status has been as a confirmed positive prognostic marker and should be considered in the analysis of survival data.[7] However, we were able to show an IDH-independent prognostic value of CD40 expression in IDHwt GBM.

CD40 Expression in Cancer

Studies of other cancer entities have supported the negative correlation between CD40 expression and survival. Positive CD40 expression in esophageal squamous cell carcinoma and gastric cancer has been associated with a worse prognosis, including shorter OS.[25,26] Immunotherapy for glioma has remained challenging because the tumors are known for their immunosuppressive milieu. Nevertheless, agents against CD40 are available and have been tested in other types of cancer. Treatment of multiple myeloma using the monoclonal CD40 antibodies lucatumumab and dacetuzumab was safely tolerated and achieved stable disease in 43% and 20% of patients, respectively.[27,28] Glioma vaccination could be one method of using CD40 as a therapeutic target for future treatment options.[21,29] Moreover, immunostimulatory AdCD40L therapy for malignant melanoma was well tolerated in a clinical study, and the local and distant responses and the better survival in the low-dose cyclophosphamide group were encouraging.[30]

CONCLUSION

In our study, increased expression of CD40 was a negative prognostic marker in GII gliomas and IDHwt GBM.

ACKNOWLEDGMENTS

We thank B. Disler for experimental help, F. and W. von Heymann for assistance with the revision of the manuscript. Furthermore, we are grateful to Dr. G. Röhn for support with the polymerase chain reaction analysis.

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Conflict of interest statement: The present study was supported by the German Foundation for Young Adults with Cancer.

Received 13 March 2019; accepted 13 May 2019

Citation: World Neurosurg. (2019) 130:e17-e25. https://doi.org/10.1016/j.wneu.2019.05.112

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