Original Article

Discoidin domain receptor 1 as a promising biomarker for high-grade gliomas

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ABSTRACT

Background: Two fundamental challenges in the current therapeutic approach for central nervous system tumors are the tumor heterogeneity and the absence of specific treatments and biomarkers that selectively target the tumor tissue. Therefore, we aimed to investigate the potential relationship between discoidin domain receptor 1 (DDR1) expression and the prognosis and characteristics of glioma patients.

Materials and Methods: Tissue and serum samples from 34 brain tumor patients were evaluated for DDR1 messenger ribonucleic acid levels in comparison to 10 samples from the control group, and Kaplan–Meier survival analysis has performed.

Results: DDR1 expression was observed in both tissue and serum samples of the patient and control groups. DDR1 expression levels in tissue and serum samples from patients were higher in comparison to the control group, although not statistically significant ($P > 0.05$). A significant correlation between tumor size and DDR1 serum measurements at the level of 0.370 was reported ($r = 0.370$; $P = 0.034$). The levels of DDR1 in serum showed a positive correlation with the increasing size of tumor. The results of the 5-year survival analysis depending on the DDR1 tissue levels showed a significantly higher survival rate ($P = 0.041$) for patients who have DDR1 tissue levels above cutoff value.

Conclusions: DDR1 expression was significantly higher among brain tumor tissues and serum samples and its levels showed a positive correlation with the increased size of tumor. This study can be a starting point, since it investigated and indicated, for the first time, that DDR1 can be a novel therapeutic and prognostic target for aggressive high-grade gliomas.

KEY WORDS: Biomarker, brain tumors, discoidin domain receptor, high-grade gliomas

INTRODUCTION

The biggest challenge in the current therapeutic approach for central nervous system tumors is the tumor heterogeneity. Another challenge is the absence of specific treatments and biomarkers that selectively target the tumor tissue.[1] To overcome this challenge, specific markers that are overexpressed in the tumor tissues must be recognized. Thus, the search for a promising therapeutic target and a novel prognostic biomarker for brain tumors is of great interest to the scientific body. Members of the tyrosine kinase receptor (TKR) family, which are a type of growth factor receptors, are thought to be suitable targets as they are reported to have an abnormal expression pattern in tumor cells.[2] TKRs play important roles in the control of cell proliferation, migration, differentiation, and cell death mechanisms. Mutated or abnormally expressed TKRs have been reported to play a role in the pathophysiology of brain tumors. However, there is not enough evidence to prove that there is a constant abnormal level of TKRs in brain tumors; therefore, their application in the investigation of potential targets is limited.[3-6]

Discoidin domain receptor 1 (DDR1) is a type of transmembrane TKR that binds to collagen specifically along with the integrin family. The integrin and DDR families are the most widely

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expressed collagen receptors in vertebrates.[7] In contrast to other TKRs, DDR1 can be activated by different types of collagen, including Types I, II, III, IV, V, VIII, and XI, which are major components of the extracellular matrix (ECM) in solid tumors.[8] Thus, DDR1 plays a role as a sensor in the ECM microenvironment by regulating cell adhesion and supporting tumor invasion at its abnormal levels.[9] In addition, it has a role in the survival of cancer stem cells in a collagen-rich environment through E-cadherin-mediated adhesion.[10] In the human body, five isoforms of DDR1 have been described, with all the isoforms present in the human brain. Although little is known about the role of DDRs in brain tumors, isoform-dependent functions have been described, such as DDR1a, which plays a role in cell invasion and adhesion in gliomas by activating matrix metalloproteinase-2 (MMP2).[11] Therefore, multiple studies have reported an association of increased DDR1 expression with poor prognosis, higher metastasis, and higher recurrence rate and therapy resistance mechanisms in solid tumors.[12,13] Given that DDR is the only member of the TKR family that activates unique signaling pathways and elicits distinct cellular responses in response to collagen, its role in the etiology of cancer progression must be investigated and elicited.[14]

Increased activation of DDR1 stimulates the pro-survival Ras/Raf/ERK, PI3K/Akt, and Notch1 pathways, increased production of anti-apoptotic Bcl-xL protein, and increased NF-κB and cyclooxygenase-2 expression in various cancer cell lines.[15] Yoshimura *et al*. also identified that MAPK and NF-κB are signaling molecules activated by the interaction of DDR1b with collagen in macrophages, dendritic cells, neutrophils, and lymphocytes, wherein the activation of this receptor likely influences their functions.[16] DDR1 activation also stimulates epithelial-to-mesenchymal-like cell scattering in response to the upregulation of N-cadherin.[14] Although its role in certain types of cancer, such as that of the stomach, lungs, liver, ovary, pancreas, and breast, has been well-studied,[4,17,18] its role in brain tumors is still not considered remarkable and must be investigated further.

DDR1 messenger ribonucleic acid (mRNA) was found to be highly expressed in tissue samples from high-grade brain tumors in pediatric and adult populations regardless of cell type (glioblastoma multiforme [GBM], anaplastic astrocytoma, anaplastic mixed glioma, primitive neuroectodermal tumors, ependymoma, and meningeal sarcoma).[3] A higher level of DDR1 was also reported in high-grade primary neuroepithelial and metastatic tumors, GH-or PRL-producing pituitary adenomas and macroadenomas, and malignant GBM.[14] In another study, it was observed that DDR1-enriched cells were more aggressive, and were held responsible for the progression, invasion, and poor clinical outcomes of astroglial tumors.[19] Since the collagen matrix within the brain parenchyma is limited to the vascular and perivascular areas, the high expression of DDR1 in brain tumors is thought to play a role in the invasiveness of glioma cells along with the perivascular matrix.[14] Currently, therapies targeting the inhibition of DDR1 are becoming more popular in multimodal treatment approaches since its inhibition will also cause the inhibition of MMP2 expression and result in the reduction of aggressiveness of tumor cells.[20]

In this study, we examined the differential gene expression patterns of DDR1 mRNA in both tissue and serum samples from different groups of patients with malignant brain tumors. We also examined the significance and prognostic value of DDR1 by investigating the possible relationship between DDR1 expression levels and clinical and pathological parameters of patients, including their overall survival rate.

MATERIALS AND METHODS

Patients and tissue specimens

We analyzed the clinical and pathological characteristics of 34 patients with resectable brain tumors of low-grade astrocytoma, oligodendroglioma, and GBM. Patients who had a history of other solid tumors or who received preoperative chemotherapy, radiotherapy (RT), or other anticancer therapies were excluded from this study. The diagnosis was confirmed by two different clinical pathologists. Data regarding the clinical characteristics of the patients and pathological characteristics of brain tumors have been obtained from the patients' reports. For the control group, tissue and blood samples from patients who had undergone epileptic surgery for similar brain regions and had similar characteristics to the patient group were used. This research project was approved by the ethics committee of a tertiary institution, and written informed consent was obtained from each patient involved in this study.

Sample collection, RNA extraction, and real-time quantitative polymerase chain reaction

Immediately after resection, tissue samples and blood samples were collected in tubes. The tubes containing blood samples were centrifuged at 2,000×g for 10 min to separate the plasma

and serum. The tissue and serum samples were kept at − 80°C until analysis of DDR1 levels was done. RNA was extracted from tissue and serum samples using Trizol reagent (Invitrogen, Carlsbad, CA, USA) to obtain the total RNA according to the manufacturer's recommendations.[21] Complementary DNA (cDNA) was transcribed from 100 ng of total RNA using a High-Capacity cDNA Reverse Transcription (RT) Kit (Applied Biosystems, Foster City, CA, USA) in a total volume of 20 μL. The RT master mix contained the following: 10× RT buffer, 25× dNTP (deoxynucleotide) mix (100 mM), 10× RT random primers, MultiScribe™ reverse transcriptase, RNase inhibitor, and nuclease-free water. The RT reaction was performed in a Thermocycler (Eppendorf, Hamburg, Germany) under the following conditions: 5 min at 25°C, followed by 60 min at 42°C, and then the samples were heated to 70°C for 5 min.[22]

The relative expression of DDR1 was assessed using TaqMan® probes (Applied Biosystems) for the studied gene (Hs01058430_m1, respectively), with *ACTB* (Hs99999903_m1) as the reference gene.[23] The procedure was performed in an Applied Biosystems 7500 Fast Real-Time Polymerase Chain Reaction (PCR) System for 40 cycles. The PCR mixture was as follows: CDNA (1–100 ng), 20× TaqMan® Gene Expression Assay, 2× TaqMan® Gene Expression Master Mix, and RNase-free water, in a total volume of 20 μL. Gene expression levels were quantified using the 7500 Fast Real-Time Sequence Detection System Software (Applied Biosystems, Foster City, CA, USA). Gene expression was defined based on the threshold cycle (Ct), and ACTB was used as a reference gene that acts as an internal reference to normalize the RNA expression, which was calculated as $2^{-\Delta\Delta CT}$.[24] The probe used to measure DDR1 detected all DDR1s' isoforms. Relative DDR1 mRNA levels in the control group were taken as the reference value when comparing the results between the two groups.

Statistical analysis

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for the overall statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, and maximum) were used when evaluating the study data. The suitability of quantitative data for normal distribution was tested using the Shapiro–Wilk test and graphical examinations. The Mann–Whitney U-test was used to compare the quantitative variables that did not show a normal distribution between the two groups. The Kruskal–Wallis test was used for comparisons of three or more groups that did not show a normal distribution, and the Bonferroni–Dunn test was used for binary comparisons. Fisher's exact test was used to compare qualitative data. Spearman correlation analysis was used to evaluate the relationships between the quantitative variables. The statistical significance level of the data was set at $P < 0.05$. The Spearman's correlation coefficient was evaluated as follows: $R = 0$, indicating no relation, and $r = 1$ indicating a significant relationship. For the analysis of the effect of DDR1 levels on overall survival, a log-rank test, which is a test statistic in the Kaplan–Meier method, was used.[25,26]

RESULTS

In this study, 34 patients, with brain tumors of different types and grades, and 10 control patients were examined for their tissue and serum levels of DDR1. The characteristics of the patient and control groups are shown in Table 1. Overall, 45.5% ($n = 20$) of the patients were female and 54.5% ($n = 24$) of the patients were male. Their ages ranged between 19 and 82 years, with an average of 51.30 ± 10.88 years. Relative DDR1 tissue mRNA levels ranged from 0.1 to 9.9, with an average of 1.76 ± 2.40, and DDR1 serum levels ranged from 13.7 to 252, with an average of 52.55 ± 48.16.

In the patient group, 32.4% ($n = 11$) of the patients were smokers. When the localization of the tumors was examined, the distribution of the tumors was found to be: 26.5% ($n = 9$) in the frontal lobe, 5.9% ($n = 2$) in the occipital lobe, 20.5% ($n = 7$) in the parietal lobe, and 47.1% ($n = 16$) in the temporal lobe. The size of the tumor ranged from 12 to 70 mm, with an average of 39.56 ± 12.94 mm. The grading distribution of the patients was as follows: 35.3% ($n = 12$) Grade 2, 2.9% ($n = 1$) Grade 3, and 61.8% ($n = 21$) Grade 4. While 17.6% ($n = 6$) of the patients had low-grade astrocytoma, 20.6% ($n = 7$) had oligodendroglioma, and 61.8% ($n = 21$) had GBM. Chemoradiotherapy was carried out for 76.5% ($n = 26$) of the cases, and RT for 5.9% ($n = 2$) of the cases. In addition, no treatment was applied to 17.6% ($n = 6$) of the cases. Total resection was seen in 20.6% ($n = 7$) of the cases and resection was subtotal in 79.4% ($n = 27$). The degree of edema was locally minimal in 29.4% ($n = 10$) of the cases, 35.3% ($n = 12$) were smaller than the mass, 29.4% ($n = 10$) were similar to the mass, and 5.9% ($n = 2$) were larger than the mass. IDH1 mutation status was found to be negative in 55.6% ($n = 15$) of the cases and positive in 44.4% ($n = 12$). The relative Ki-67 measurements ranged from 0.8 to 90, with an average of 12.49 ± 20.60. It was observed that 55.9% ($n = 19$) of the patients were alive at the end of the follow-up period and 44.1% ($n = 15$) expired by that time. The duration of follow-up ranged from 15 to 49 months, with an average of 26.75 ± 17.16 months.

The DDR1 levels in the serum and tissue samples of the patients were comparatively higher than in the control group, although the difference was not statistically significant ($P > 0.05$) [Table 2]. There was no significant difference in DDR1 tissue and serum levels in terms of the age and gender distribution between the groups ($P > 0.05$) [Table 2]. When the patient and control groups were evaluated separately, no statistically significant association was shown between the DDR1 tissue expression and DDR1 serum levels within either group ($P > 0.05$)[Table 3].

The DDR1 serum levels and tissue expression were evaluated according to the specific characteristics of the patient group. No significant relationship was found between DDR1 serum levels or tissue expression and sex, age, smoking status, localization,

Table 1: The distribution of the study populations' characteristics (a) and the clinical characteristics of the patient group (b)

	n (%)
a. All cases (*n*=44)	
Age (years)	
Minimum–maximum (median)	19–82 (49)
Average±SD	51.30±10.88
Gender	
Woman	20 (45.5)
Man	24 (54.5)
DDR1 levels (tissue)	
Minimum–maximum (median)	0.1–9.9 (1)
Average±SD	1.76±2.40
DDR1 levels (serum)	
Minimum–maximum (median)	13.7–252 (36.2)
Average±SD	52.55±48.16
b. Patient group (*n*=34)	
Smoking	
No	23 (67.6)
Yes	11 (32.4)
Localization	
Frontal	9 (26.5)
Occipital	2 (5.9)
Parietal	7 (20.5)
Temporal	16 (47.1)
Size (mm)	
Minimum–maximum (median)	12–70 (39.5)
Average±SD	39.56±12.94
Grade	
Grade 2	12 (35.3)
Grade 3	1 (2.9)
Grade 4	21 (61.8)
Pathological subtype	
Low-grade astrocytoma	6 (17.6)
Oligodendroglioma	7 (20.6)
GBM	21 (61.8)
Therapy	
KRT	26 (76.5)
Radiotherapy	2 (5.9)
None	6 (17.6)
Resection	
Total	7 (20.6)
ST	27 (79.4)
Degree of edema	
Locally minimal	10 (29.4)
Smaller than mass	12 (35.3)
Similar to mass	10 (29.4)
Bigger than mass	2 (5.9)
IDH1 (*n*=27)	
Negative	15 (55.6)
Positive	12 (44.4)
Ki-67	
Minimum–maximum (median)	0.8–90 (6)
Average±SD	12.49±20.60
Status	
Alive	19 (55.9)
Exitus	15 (44.1)
Follow-up time (months)	
Minimum–maximum (median)	0.5–49 (29.7)
Average±SD	26.75±17.16

SD=Standard deviation, DDR1=Discoidin domain receptor 1, GBM=Glioblastoma multiforme, KRT=Kidney replacement therapy, IDH1=Isocitrate dehydrogenase 1

grade, pathological subtype, therapy modality, resection type, degree of edema, IDH1 positivity, Ki-67 levels, or follow-up time. There was also no significant difference in DDR1 expression levels between alive and expired patients from both samples.

While no statistically significant relationship was found between size and DDR1 tissue expression ($P > 0.05$), a statistically significant correlation between size and DDR1 serum level was reported ($r = 0.370$; $P = 0.034$; $P < 0.05$) (effect size: 0.700; power: 0.734). The serum levels of DDR1 showed a positive correlation with the size of the tumor (effect size: 0.700; power: 0.734) [Table 4].

Higher DDR1 levels were observed in the serum of patients with frontal and IDH1-positive tumors, patients who were receiving RT, and patients who had edema that was larger than the tumor ($P > 0.05$). Higher DDR1 expression was also observed in the tumor tissue of the patients with parietal tumors, Grade III tumors, patients who were receiving RT, and patients who had edema smaller than the tumor ($P > 0.05$). Patients with Grade IV tumor showed higher DDR1 tissue expression but lower DDR1 serum levels compared to patients with Grade I, II, and III tumors, albeit not statistically significant ($P > 0.05$) [Figure 1].

For survival analysis, depending on the relative DDR1 tissue expression levels at the end of the 48th month, the cutoff value of 1 was determined as the median value for DDR1 tissue levels. It was seen that among the cases, wherein DDR1 tissue mRNA levels were below 1, 10 patients (50%) were alive and 10 had died at the end of the follow-up time. It was observed that, for this group, the average survival time was 40.66 ± 1.92 months below the cutoff value [Table 5 and Figure 2]. Among the cases in which DDR1 tissue value is above 1, 9 patients (64.3%) were alive and 5 had died. The mean survival time for this group was calculated at 47.36 ± 1.04 months. When the survival rates relative to the DDR1 tissue mRNA levels were evaluated with the log-rank test, a statistically significant difference between the 5-year survival rates ($P = 0.041$; $P < 0.05$) was found between the groups separated by cutoff values. The survival time was shorter for those with DDR1 tissue levels below 1.

For survival analysis based on the relative DDR1 serum levels, the cutoff value of 36 was determined as the median value and the survival rate was investigated below and above the cutoff value. It was observed that in the cases with a DDR1 serum value below 36, 8 patients (50%) were alive and 8 had died by the end of the follow-up time. The average survival time for this group was calculated at 42.88 ± 2.57 months [Table 6 and Figure 2]. For the cases with a DDR1 serum value above 36, 10 patients (58.8%) were alive and 7 deaths were observed. The average survival time for this group was calculated as 43.41 ± 1.92 months. When the survival rates according to the DDR1 serum levels were analyzed with the log-rank test, there was no statistically significant difference between the 5-year survival rates of the two groups.($P = 0.643$; $P > 0.05$).

DISCUSSION

In the literature, it was reported that upregulated DDR1 levels were associated with liver, pancreas, breast, lung, ovarian,

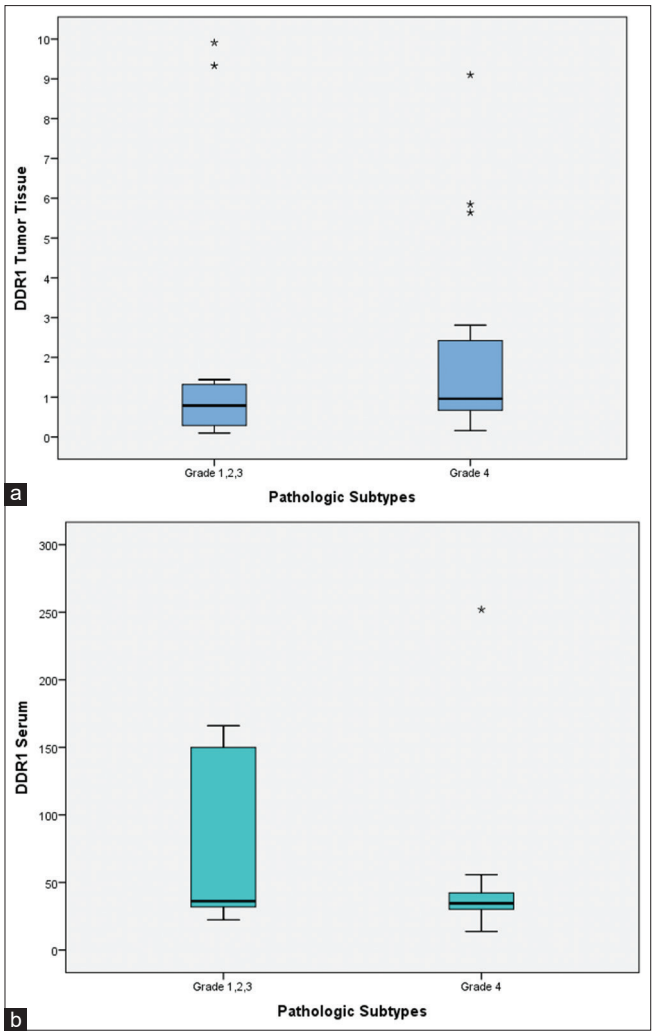

Figure 1: Discoidin domain receptor 1 tissue (a) and serum (b) mRNA levels according to pathological subtype of patients. Patients with Grade IV tumor showed higher discoidin domain receptor 1 tissue expression but lower discoidin domain receptor 1 serum levels compared to patients with Grade I, II, and III tumors, albeit not statistically significant

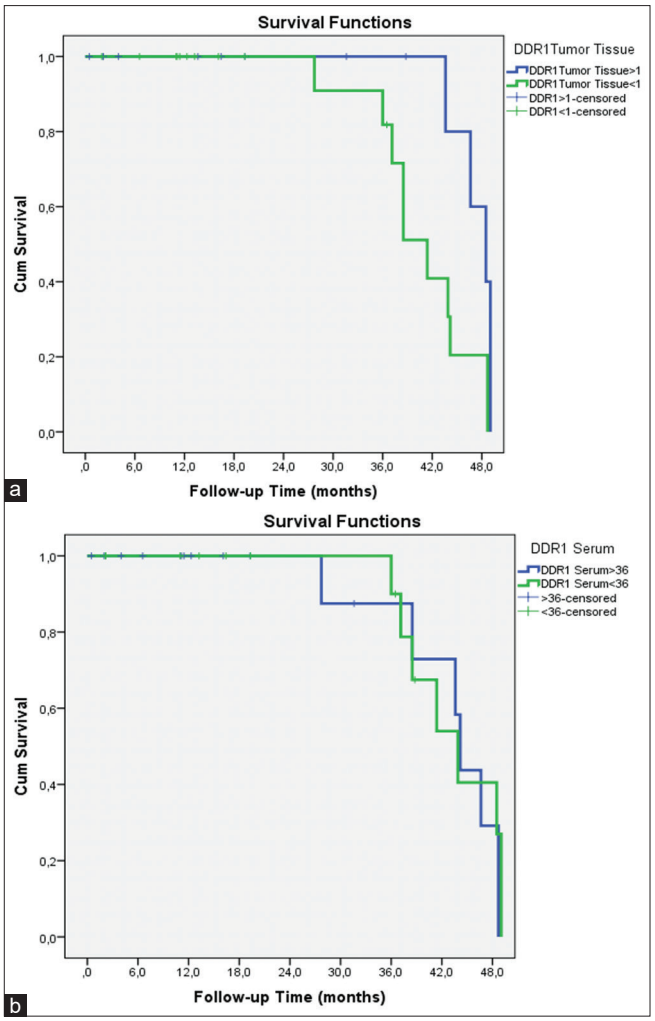

Figure 2: Kaplan–Meier survival analysis considering serum and tissue expressions of discoidin domain receptor 1 mRNA levels below and above cutoff value analyzed with log-rank test. (a) Tissue levels. A significantly higher survival rate was observed for patients who have discoidin domain receptor 1 tissue mRNA levels higher than cutoff value ($P = 0.041$). (b) Serum levels. No statistically significant different was found associated with serum levels below and above cutoff value ($P > 0.05$)

esophageal, head and neck, and brain cancers.[14] Despite accumulating evidence, a comparison of the role of DDRs and differential expression in the diverse types of brain tumors has not been conducted. Therefore, we aimed to investigate the significance of the relative and differential expression of DDR1 mRNA in different brain tumors, depending on the patient characteristics. We also aimed to investigate the association between DDR1 tissue expression and serum levels. Although we detected an overall increase in DDR1 expression in both serum and tissue samples from the patient group compared with the control group, it was not statistically significant.

Several cancer types exhibit a mutation or change in expression levels of DDR1, which promotes tumor cell behavior in a complex and context-dependent manner.[14] Therefore, loss of DDR1 activity increases apoptosis via decreased p53 and Notch levels, indicating its role in tumor growth and decreasing chemoresistance in tumors via decreased COX2 and NF-κB pathways.[27,28] DDR1 amplification is commonly observed in various cancers, including ~14% of metastatic breast cancer and aggressive neuroendocrine prostate cancer cases, indicating that the DDR1 signaling pathway is significantly deregulated in aggressive cancers.[27] Since increased expression of DDR1 confers resistance to chemotherapy and mediates pro-survival signals, DDR1 can be utilized as an important biomarker that reflects the prognosis through chemoresistance mechanisms. Patients who have high expression of DDR1 might instead benefit from small molecule inhibitors (imatinib, nilotinib, and dasatinib) that target breakpoint cluster region-Abelson kinase (BCR-ABL), since they were known to potently inhibit DDR1 activity in pancreatic tumor cells. Furthermore,

Table 2: The evaluation of age, gender, and discoidin domain receptor 1 serum and tissue levels in patient and control groups

	Patient group (*n*=34)	Control group (*n*=10)	*P*
Age (years)			
Minimum–maximum (median)	19–82 (48.5)	46–53 (49.5)	0.747[a]
Average±SD	51.82±12.30	49.50±2.46	
Gender, *n* (%)			
Woman	15 (44.1)	5 (50.0)	1.000[b]
Man	19 (55.9)	5 (50.0)	
DDR1 tissue			
Minimum–maximum (median)	0.1–9.9 (0.9)	1–1 (1)	0.398[a]
Average±SD	1.99±2.70	1.00±0	
DDR1 serum			
Minimum–maximum (median)	13.7–252 (36)	33.3–53.8 (41.1)	0.181[a]
Average±SD	55.91±54.61	41.47±6.69	

[a]Mann–Whitney U test, [b]Fisher's exact test. DDR1=Discoidin domain receptor 1, SD=Standard deviation

Table 3: The relationship between serum and tissue levels of discoidin domain receptor 1

DDR1 serum	DDR1 tissue	
	Total (*n*=44)	Patient group (*n*=34)
r	−0.029	−0.049
P	0.855	0.788

r=Spearman's correlation coefficient, DDR1=Discoidin domain receptor 1

Aguilera *et al*. demonstrated that an ATP-competitive orally available novel small-molecule kinase inhibitor abrogated collagen-induced DDR1 signaling, reduced colony formation, and improved chemoresponse of tumors.[17]

Since overexpressed DDR1a isotype levels are also demonstrated in high-grade gliomas, it is thought to contribute to enhanced invasion and migration of glioma cells concomitant with increased levels of MMPs that are responsible for the invasive phenotype.[20] Although collagen VIII is absent in the normal brain, it might be selectively expressed in glioma tissues, supporting DDR1 signaling. In GBM, collagen IV is present in almost all tumor vessels and in giant glioma cells along with collagen VIII; these are responsible for the tumor's invasiveness and poor prognosis.[29] Supporting data by Yamanaka *et al*.[2006] revealed that DDR1 expression is more closely related to patient survival than the histological grade of gliomas. This indicates that DDR1 mRNA and protein expression levels might even be better prognostic factors in patient survival than WHO grading.[30] The selective and constant mRNA expression pattern of DDRs in high-grade gliomas gave us our idea of investigating whether DDR1 can be a useful biomarker for fast-growing tumors. In our study, tissue samples from Grade IV tumors demonstrated significantly increased DDR1 expression compared to patients with Grade I, II, or III tumors. On the other hand, patients with Grade IV tumors did not show a specific difference in serum levels compared to patients with low-grade tumors. DDR1 tissue expression levels can be utilized as a biomarker in the comparison of low- and high-grade brain tumors, as it exhibits a specific differential expression pattern in high-grade gliomas.

Yoshida and Teramoto reported an increased expression of DDR1 levels in macroadenomas compared to microadenomas, which may indicate the significance of tumor size in the expression levels.[9] The DDR1-ERK signaling cascade is reported to be crucial for the functions of both cancer cells and endothelial cells to constitute a proper microenvironment via induction of angiogenesis.[31,32] Roig *et al*. identified the importance of the expression of DDR1 in capillary endothelial cells and perivascular cells.[31] These findings validate the increased need for angiogenesis due to increasing tumor size. In our findings, a statistically significant correlation between tumor size and DDR1 serum levels ($P < 0.05$), but not tissue levels ($P > 0.05$), was found. This may indicate the significant impact of DDR1 in the overall clinical picture of patients as tumor size increases through its role in angiogenesis.

It is known that DDR1 expression in astrocytes is upregulated under activation circumstances.[11] Sakuma *et al.* identified that radiation can be a possible source of DDR1 expression in rat astrocytes.[33] Therefore, in patients receiving this type of treatment, upregulation of the receptor in tissue samples could be possible. The results of the study by Yoshimura *et al*. stated that the tissue-infiltrating macrophages, which have increased DDR1b levels, played a role in the increased production of proinflammatory cytokines and chemokines in the tissue microenvironment and may contribute to the development of inflammation.[16] Thus, increased levels of DDR1 in serum may play a role in the development of edema due to the increased number of leukocytes present in the body.

Valiathan *et al*. reported the complex role of DDR1, in either suppressing or promoting tumor cell behavior, especially by affecting the migration and invasion of tumor cells.[14] In the literature, it was shown that either independent or co-expressed DDR1 with DARPP32 suppressed migration in hepatocellular carcinoma and aggressive triple-negative and double-positive breast cancer lines.[34,35] However, this regulation differs according to the cell type and receptor isoforms.[36] Therefore, the higher survival rate observed in patients with higher DDR1 tissue expression levels may also be related to the changing role of DDR1 and the patients with low-grade but larger tumors. The localization of the tumor, as well as IDH1 positivity, is also thought to affect these results.[30,37]

CONCLUSION

This study can be a starting point, since it investigated and showed, for the first time, the relative association of DDR1 mRNA levels in both tissue and serum samples of patients. However, further molecular studies on the differential

Table 4: Evaluation of discoidin domain receptor 1 tissue and discoidin domain receptor 1 serum measurements according to characteristics of patient group

Patient group (*n*=34)	*n*	DDR1 tissue		DDR1 serum	
		Minimum–maximum (median)	Average±SD	Minimum–maximum (median)	Average±SD
Gender					
Woman	15	0.1–9.9 (0.8)	1.44±2.38	22.3–252 (33.9)	55.72±62.92
Man	19	0.1–9.3 (0.9)	2.42±2.92	13.7–166 (36.1)	56.06±48.50
P		0.445		0.286	
Smoking					
No	23	0.1–9.9 (0.8)	2.01±2.80	13.7–252 (35.5)	55.56±57.69
Yes	11	0.3–9.3 (1)	1.94±2.61	23.7–166.1 (36)	56.60±50.53
P[a]		0.740		0.939	
Localization					
Frontal	9	0.3–9.9 (0.9)	2.90±3.82	25.7–152 (37.8)	50.25±41.48
Occipital	2	0.7–1.2 (0.9)	0.92±0.35	26.4–33.5 (29.9)	29.94±4.97
Parietal	7	0.1–5.9 (0.5)	2.21±2.59	13.7–252 (34.1)	81.14±90.74
Temporal	16	0.1–9.3 (0.9)	1.51±2.17	22.3–166.1 (36)	50.94±42.81
P[b]		0.824		0.977	
Grade					
Grade 2	12	0.1–9.9 (0.6)	2.11±3.54	22.3–164.1 (36)	63.55±55.73
Grade 3‡	1	1–1 (1)	0.96±0	166–166 (166)	166.06±0
Grade 4	21	0.2–9.1 (1)	1.97±2.26	13.7–252 (34.5)	45.81±49.41
P[a]		0.178		0.572	
Pathological subtype					
GBM (−)	13	0.1–9.9 (0.8)	2.02±3.40	22.3–166.1 (36)	71.44±60.46
GBM (+)	21	0.2–9.1 (1)	1.97±2.26	13.7–252 (34.5)	45.81±49.41
P[a]		0.202		0.387	
Therapy					
KRT	26	0.1–9.9 (0.9)	2.00±2.67	13.7–252 (36)	58.82±58.49
RT‡	2	0.8–9.3 (5.1)	5.07±6.02	35–150 (92.5)	92.51±81.33
None	6	0.3–1.4 (0.9)	0.91±0.43	22.3–39.5 (31.8)	31.56±7.02
P[a]		0.866		0.134	
Resection					
Total	7	0.3–1.4 (0.9)	0.85±0.44	22.3–40.1 (33.5)	32.52±6.69
Subtotal	27	0.1–9.9 (0.9)	2.29±2.96	13.7–252 (36)	62.20±60.10
P[a]		0.496		0.194	
Degree of edema					
Locally minimal	10	0.3–9.9 (0.9)	2.54±3.76	22.3–152 (37.8)	57.10±49.95
Smaller than mass	12	0.1–9.1 (0.7)	1.55±2.49	25.7–252 (36)	66.06±73.38
Similar to mass	10	0.1–5.9 (1.1)	1.68±1.70	23.7–164 (36.4)	50.16±41.20
Bigger than mass‡	2	1.2–5.6 (3.4)	3.44±3.12	13.7–31.9 (22.8)	22.78±12.83
P[b]		0.642		0.985	
IDH1 (*n*=27)					
Negative	15	0.3–9.3 (0.9)	1.95±2.48	25.7–252 (35)	58.55±61.48
Positive	12	0.2–9.9 (1.4)	2.93±3.40	13.7–152 (31.9)	41.57±37.74
P[a]		0.435		0.204	
Status					
Alive	19	0.1–9.1 (1)	2.07±2.36	13.7–252 (36)	55.03±58.44
Exitus	15	0.1–9.9 (0.8)	1.89±3.17	22.3–166.1 (35)	56.96±51.64
P[a]		0.252		0.651	
		r	*P*	*r*	*P*
Age (years)		0.057	0.748	−0.076	0.675
Size (mm)		0.028	0.876	0.370	0.034*
Ki-67		−0.245	0.327	−0.210	0.419
Follow-up time (months)		−0.057	0.751	−0.162	0.368

*P<0.05. [a]Mann–Whitney U-test, [b]Kruskal–Wallis test. r=Spearman's correlation coefficient, SD=Standard deviation, DDR1=Discoidin domain receptor 1, GBM=Glioblastoma multiforme, KRT=Kidney replacement therapy, IDH1=Isocitrate dehydrogenase 1, RT=Radiotherapy, P<0.05. the ‡ sign was provided to indicate the low sample size

expression of key genes in specific pathways are required to gain in-depth knowledge about the exact pathophysiology surrounding the phenomenon. Therefore, the limitations of the study, such as small sample size, and tumor and treatment heterogeneity, verification of DDR1 expression with another method, should be overcome by larger study groups with similar characteristics.

Although new and exciting findings in the DDR1 field have raised considerable interest in these receptors as potential therapeutic targets and prognostic biomarkers, several questions remain unanswered. The results of this study showed that DDR1 expression may have a significant relationship with survival of patients with brain tumors; therefore, DDR1 can be considered as a novel prognostic marker, especially

Table 5: Survival analysis in patient group depends on the discoidin domain receptor tissue levels

DDR1 tissue	*n*	Exitus	Alive	Survival rate (%)	Average survival time
<1	20	10	10	50.0	40.66±1.92
>1	14	5	9	64.3	47.36±1.04

Kaplan–Meier analysis. DDR1=Discoidin domain receptor 1

Table 6: Survival analysis in patient group depends on the discoidin domain receptor serum levels

DDR1 serum	*n*	Exitus	Alive	Survival rate (%)	Average survival time (months)
<36	16	8	8	50.0	42.88±2.57
>36.1	17	7	10	58.8	43.41±1.82

Kaplan–Meier analysis. DDR1=Discoidin domain receptor 1

in high-grade gliomas. By recognizing DDR1's role, further studies may help clinicians to better identify, manage, and treat patients with brain tumors.

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Conflicts of interest

There are no conflicts of interest.

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