Original Article

The association between calreticulin and glucagon-like peptide-1 expressions with prognostic factors in high-grade gliomas

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ABSTRACT

Objective: The aim of this study is to present the expressions of Calreticulin (CALR) and Glucagon-like peptide-1 (GLP-1) in high-grade gliomas and to further show the relation between the levels of these molecules and Ki-67 index, presence of Isocitrate dehydrogenase (IDH)-1 mutation, and tumor grade.

Patients and Methods: A total of 43 patients who underwent surgical resection due to high-grade gliomas (HGG) (grades III and IV) were included. The control group comprised 27 people who showed no gross pathology in the brain during the autopsy procedures. Adequately sized tumor samples were removed from each patient during surgery, and cerebral tissues were removed from the control subjects during the autopsy procedures. Each sample was stored at −80°C as rapidly as possible until the enzyme assay.

Results: Patients with high-grade gliomas showed significantly higher levels of CALR and significantly lower levels of GLP-1 when compared to control subjects ($P = 0.001$). CALR levels were significantly higher, GLP-1 levels were significantly lower in grade IV gliomas than those in grade III gliomas ($P = 0.001$). Gliomas with negative IDH-1 mutations had significantly higher CALR expressions and gliomas with positive IDH-1 mutations showed significantly higher GLP-1 expressions ($P = 0.01$). A positive correlation between Ki-67 and CALR and a negative correlation between Ki-67 and GLP-1 expressions were observed in grade IV gliomas ($P = 0.001$).

Conclusions: Our results showed that higher CALR and lower GLP-1 expressions are found in HGGs compared to normal cerebral tissues.

KEY WORDS: Calreticulin, glioma, glucagon-like Peptide-1, surgery, tumor immunity

INTRODUCTION

Gliomas are the most common type of primary brain tumors, and despite advances in radiotherapy and chemotherapy in addition to surgery, prognosis still remains poor particularly in cases with a higher Ki-67 index and absence of IDH-1 mutation. The median survival of patients with high-grade gliomas (HGGs), such as glioblastoma (grade IV astrocytoma), is less than 2 years.[1] The management of HGGs is challenging for both neurosurgeons and patients considering that the side effects of radio-chemotherapy and chemotherapy are detrimental. The development of an effective treatment at the cellular level requires an improved understanding of the molecular mechanisms behind these tumors.

Studies have shown that the immune system is most likely involved in the pathophysiology of brain tumors.[2] Thus, personalized and immune cell-based treatments seem to be promising. Dendritic cell-mediated antitumor antigens have been studied in recent years, and it has been demonstrated that a high number of tumor-infiltrating dendritic cells are associated with better survival in several types of cancers.[3] Dendritic cell activations can be promoted by necrotic tumor cells and the release of some danger signals, such as calreticulin.[4]

Calreticulin (CALR) is a multifunctional calcium-binding protein that is abundant in the endoplasmic reticulum.[5] Its main function is to prevent misfolded proteins and the current literature showed that immunogenic cell death is dependent on the exposure of CALR on the cell

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surface. The engulfment of dying tumor cells is performed by dendritic cells which need the action of CALR. Although, this protein has been investigated in recent studies, some studies reported contradictory findings with respect to its expression in several cancers. Higher expressions of CALR were associated with poor outcomes in esophageal squamous cell carcinoma,[6] breast ductal carcinoma,[7] and gastric cancers.[8] They found that higher CALR was associated with tumor growth, increased angiogenesis, metastasis, and decreased survival. Moreover, in prostate cancer,[9] higher CALR levels suppressed tumor growth and prevented distant metastasis, whereas in colon cancer.[10] higher CALR expressions were associated with increased tumor infiltration by suppressor T-cells and increased overall survival. Furthermore, in neuroblastoma, the absence of CALR was associated with poorer survival.[11] More importantly, some studies suggested that CALR could be used as a marker for anti-tumor immune responses which may lead us to develop a strategy for immunogenic chemotherapy.[12]

Glucagon-like peptide-1 (GLP-1), which is the receptor expression in the neurohypophysis, meninges, hypothalamus, brain stem, and hippocampus in the healthy human brain, is an important member of the incretin hormone family.[13-16] GLP-1, which is normally released from the intestinal L cells into the circulation and plays a role in glucose homeostasis, is also a neuropeptide produced by preproglucagon neurons in the brain and has neuroprotective effects on cerebral glucose balance.[16,17] According to previous studies, GLP-1 or GLP-1 analogs have proliferative and antiapoptotic effects on insulinoma cells and the exact opposite effects on prostate cancer.[18-20] Moreover, the fact that GLP-1 receptor (GLP-1R) agonists have autophagic/apoptotic effects on endometrial cancer and growth-reducing effects on breast cancer, along with varying degrees of GLP-1R expression in gastrointestinal endocrine and embryonal tumors and carcinomas, is an indication that GLP-1 is an important molecule in the field of oncology.[21-23] Also, the fact that GLP-1 analogs have proliferative and anti-apoptotic effects on the central nervous system cells[24] and inhibitory effects on cell migration/invasion in glioblastoma[25] and varying degrees of GLP-1R expression in various brain tumors, such as meningioma, astrocytoma, glioblastoma, and ependymoma[23] makes GLP-1 an important molecule. Furthermore, the increasing use of G protein-coupled receptors in cancer treatment and the fact that GLP-1R is also a G protein-dependent receptor make GLP-1 even more valuable in this field.[26]

Therefore, the aim of this study is to present the expressions of CALR and GLP-1 in resected gliomas and to further show the relation between the levels of these molecules and Ki-67 index, presence of IDH-1 mutation, and tumor grade.

MATERIALS AND METHODS

This clinical study was performed with the collaboration of the Department of Neurosurgery, Cerrahpaşa Medical Faculty, Istanbul University-Cerrahpasa, Istanbul, Turkey and Biochemistry Clinics of Istanbul Training and Research Hospital. Patients who presented with histopathologically proven HGG and underwent surgery and signed the informed consent were included. All patients or their next of kin were fully informed, and the ethical approval for this study was obtained from the local ethics committee.

Patient population

A total of 43 patients who underwent surgical resection due to HGG (grades III and IV) were included. All the patients were discussed in our local preoperative case meetings, and routine pre-surgical measurements were taken. Moreover, all the patients underwent contrasted cranial magnetic resonance imaging (MRI) scans and routine blood work-up. After the completion of pre-surgical measurements, the risk/benefit ratio of the surgery was explained to all the patients and if they approved, the surgery was performed. All the patients were followed up after surgery at regular intervals with respect to surgical and neurologic outcomes. All the patients diagnosed with HGG were consulted to radiation and medical oncology for further treatment.

Control group

The control group composed of 27 people who died in traffic accidents or falls from height. All of them underwent autopsy procedures in the Department of Forensic Medicine. No subject showed gross pathology in the brain during the autopsy procedures. Any case with a suspicion of a neoplastic disease or intoxication was excluded from the control group.

Specimen handling

Adequately sized tumor samples were removed from each patient during surgery. Normal cerebral tissues from the control subjects were removed during the autopsies, which were performed within 4 hours of death. Tissue samples stored at −80°C were thawed, and their temperature was increased to room temperature. The tissues were weighed on a precision scale. 1:9 phosphate buffer (PBS, 0.1 M, Ph = 7.4) was added to each sample. The samples were homogenized using a homogenizer (MICCRA, Germany) and were stored in ice to prevent heat-induced protein loss during and after the homogenization process. The homogenate was centrifuged at 5000×g for 5 min. The supernatant was split in half for CALR and GLP-1.

Assay of calreticulin

Protein and CALR measurements were performed in the supernatant. Protein was measured using the colorimetric method in the biochemistry autoanalyzer (Beckman Coulter, AU5800, USA). Calreticulin was studied in accordance with the Sandwich ELISA principle using the Human Calreticulin ELISA kit (Elabscience, Texas, USA, Cat. No. E-EL-H0627). The measurements were performed in line with the manufacturer's recommendations regarding the study procedures. The samples were studied twice and verified. The concentration of calreticulin was given per mg of protein to exclude the

error that may be associated with possible protein loss. The detection range of the ELISA kit was 0.16–10 ng/mL. The coefficient of variation of the test was less than 10%.

Assay of GLP-1

Protein and GLP-1 were measured in the supernatant. Protein was measured using the colorimetric method in the biochemistry autoanalyzer (Beckman Coulter, AU5800, USA). GLP-1 was studied in accordance with the Sandwich ELISA principle using the Human GLP-1 ELISA kit (Elabscience, Texas, USA, Cat. No. E-EL-H0148). The measurements were performed in line with the manufacturer's recommendations regarding the study procedures. The samples were studied twice and verified. The concentration of GLP-1 was given per mg of protein to exclude the error that may be associated with possible protein loss. The detection range of the ELISA kit was 0.31–20 ng/mL. The coefficient of variation of the test was less than 10%.

Statistical analysis

The NCSS (Number Cruncher Statistical System) statistical software (Utah, USA) was used for statistical analysis. While evaluating the study data, in addition to the descriptive statistical methods (mean, standard deviation, median, frequency, ratio), Shapiro–Wilk test and box plot graphs were used for the normal distribution of variables. Student's *t* test was used for comparing normally distributed variables according to groups. Mann–Whitney U test was used to compare the variables that did not show a normal distribution. Spearman's correlation coefficient was used for evaluating the relationships between the variables. Pearson's Chi-square test was used to compare the qualitative data. Furthermore, significance was evaluated at the $P < 0.05$ level.

RESULTS

Demographic and clinical characteristics

Table 1 summarizes the clinical characteristics of the groups. The mean age of the HGG group was 49.09 ± 12.61, whereas the mean age of the control group was 46.04 ± 13.58 years. Moreover, the tumor group consisted of 28 (65.1%) male and 15 (34.9%) female patients [Table 2], whereas the control group included 14 (51.9%) and 13 (48.1%) male and female patients, respectively. No statistically significant differences were observed regarding the mean age and sex between the groups ($P > 0.05$). Headache was the most common presenting symptom among the patients (46.5%) and other complaints were as follows: Seizure (20.9%), motor weakness (9.3%), loss of consciousness (9.3%), speech disorder (7.0%), and vision problems (7.0%). Radiological imaging scans showed the right-side involvement in 26 of the patients (60.5%) and the left side involvement in 17 (39.5%). The majority of the tumors were located in the frontal lobe (n = 10; 23.3%) followed by those in the temporal lobe (n = 9; 20.9%), fronto-parietal lobe (n = 5; 11.6%), parietal lobe (n = 5; 11.6%), temporo-parietal lobe (n = 4; 9.3%), parieto-occipital lobe (n = 2; 4.7%), and occipital lobe (n = 2; 4.7%). The preoperative neurological examinations showed that the majority of the patients showed normal neurological examinations (n = 26; 37.2%) and the remaining had several other signs depending on the location of the tumors. In 16 patients (37.2%), subtotal surgical resections had to be performed because of the proximity of the

Table 1: Distribution of characteristics of tumor cases

	Tumor	
	n	%
Symptoms		
Headache	20	46,5%
LOC*	4	9,3%
Visual problems	3	7,0%
Speech disorder	3	7,0%
Weakness	4	9,3%
Seizure	9	20,9%
Tumor localization		
Frontal	10	23,3%
Fronto-insular	2	4,7%
Fronto-parietal	5	11,6%
Fronto-temporal	2	4,7%
Occipital	2	4,7%
Parietal	5	11,6%
Parieto-occipital	2	4,7%
Temporal	9	20,9%
Temporo-insular	2	4,7%
Temporo-parietal	4	9,3%
Lesion side		
Right	26	60,5%
Left	17	39,5%
Neurological exam		
Language deficit	5	11,6%
Low GCS**	4	9,3%
Visual field defect	2	4,7%
Motor deficit	6	14,0%
Normal	26	60,5%
Number of surgeries		
1	31	72,1%
2	9	20,9%
3	3	7,0%
Pathology		
Anaplastic astrocytoma	4	9,3%
Anaplastic oligoastrocytoma	1	2,3%
Anaplastic oligodendroglioma	7	16,3%
Glioblastome Multiforme	31	72,1%

*LOC: Loss of consciousness, **GCS: Glasgow Coma Scale

Table 2: Evaluation of descriptive characteristics according to groups

	Group		*P*
	Tumor (*n*=43)	Control (*n*=27)	
Age			
Mean±SD	49,09±12,61	46,04±13,58	[a]0,342
Sex			
Male	28 (65,1)	14 (51,9)	[b]0,270
Female	15 (34,9)	13 (48,1)	
CALR (ng/mg protein)			
Median (Min-Max)	0,07 (0,03-0,14)	0,01 (0,002-0,019)	[c]0,001**
Mean±SD	0,07±0,025	0,010±0,004	
GLP-1 (ng/mg protein)			
Median (Min-Max)	0,03 (0,01-0,059)	0,11 (0,08-0,18)	[c]0,001**
Mean±SD	0,029±0,016	0,12±0,03	

[a]Student *t*-test. [b]Pearson Chi-Square Test. [c]Mann Whitney U test **P<0,01

tumor to the crucial areas, whereas in 14 patients (27.9%), multiple surgeries were performed. The pathology of 31 patients who had single surgery (72.1%) was glioblastoma multiforme, 7 patients (16.3%) anaplastic oligodendroglioma, 4 patients (9.3%) anaplastic astrocytoma, and 1 patient (4.3%) anaplastic oligoastrocytoma. The second pathology results indicated that in patients who underwent two surgeries, six patients had glioblastoma multiforme, two had anaplastic oligodendroglioma, and one had anaplastic astrocytoma. The third pathology of the three patients who had surgery three times was reported as glioblastoma multiforme.

Comparisons of the groups

The results showed that the tumor group had significantly higher CALR and lower GLP-1 levels compared to the controls. Table 2 summarizes the statistical results. Regarding CALR, the mean levels were 0.07 ± 0.025 ng/mg protein and 0.010 ± 0.004 ng/mg protein in the patients and controls, respectively, and the difference was significant ($P = 0.001$) [Figure 1]. The GLP-1 level in the tumor group was found to be significantly lower (0.029 ± 0.016 ng/mg protein) than that in the control group (0.12 ± 0.03 ng/mg protein) ($P = 0.001$) [Figure 2].

Comparisons with the tumor grades and IDH-1 mutation

The mean levels of CALR and GLP-1 also showed a difference according to the tumor grade. CALR levels were significantly higher in grade IV gliomas than those in grade III gliomas (0.08 ± 0.02 ng/mg protein versus 0.04 ± 0.01 ng/mg protein; $P = 0.001$). On the other hand, GLP-1 levels were significantly lower in grade IV gliomas than those in grade III gliomas ($P = 0.001$). Table 3 and Figure 3 show the summary of the statistical results.

As expected, grade IV gliomas had higher Ki-67 index than grade III gliomas. In grade III gliomas, the Ki-67 index was 11.33 ± 5.21%, and in grade IV gliomas 26.87 ± 14.49%, indicating a significant difference ($P = 0.001$). Positive IDH-1 mutations were observed in 7 of the 12 cases with grade III glioma and in 6 of the 31 cases with grade IV glioma, indicating a significant difference ($P = 0.02$). Moreover, the incidence of IDH-1 mutations was higher in grade III gliomas [Table 3].

The effect of IDH-1 mutations on CALR and GLP-1 tissue expressions was also investigated. Statistical analysis showed that gliomas with negative IDH-1 mutations had significantly higher CALR expressions than gliomas with positive IDH-1 mutations ($P = 0.01$). On the other hand, gliomas with positive IDH-1 mutations showed significantly higher GLP-1 expressions than gliomas with negative IDH-1 mutations ($P = 0.01$) [Table 4].

Table 3: Evaluation of CALR and GLP-1 expressions according to tumor grade

	WHO		*P*
	Grade 3 (n=12)	Grade 4 (n=31)	
CALR (ng/mg protein)			
Median (Min-Max)	0-0,1 (0)	0-0,1 (0,1)	[c]0,001**
Mean±SD	0,04±0,01	0,08±0,02	
GLP-1 (ng/mg protein)			
Median (Min-Max)	0,04-0,06 (0,05)	0,01-0,05 (0,02)	[c]0,001**
Mean±SD	0,048±0,006	0,022±0,014	
Ki-67 (%)			
Median (Min-Max)	4-20 (10)	8-60 (30)	[c]0,001**
Mean±SD	11,33±5,21	26,87±14,49	
IDH-1; *n* (%)			
Positive	7 (58,3)	6 (19,4)	[a]0,024*
Negative	5 (41,7)	25 (80,6)	

[b]Pearson Chi-Square Test. [c]Mann Whitney U test **P<0,01

Table 4: Evaluation of CALR and GLP-1 expressions according to IDH-1 mutation

	IDH-1 mutation		*P*
	Negative (*n*=30)	Positive (*n*=13)	
CALR (ng/mg protein)	0-0,1 (0,1) 0,08±0,02	0-0,1 (0) 0,05±0,01	0,001**
GLP-1 (ng/mg protein)	0,01-0,05 (0,02) 0,023±0,015	0,02-0,06 (0,04) 0,045±0,01	0,001**

[c]Mann Whitney U test. **P<0,01

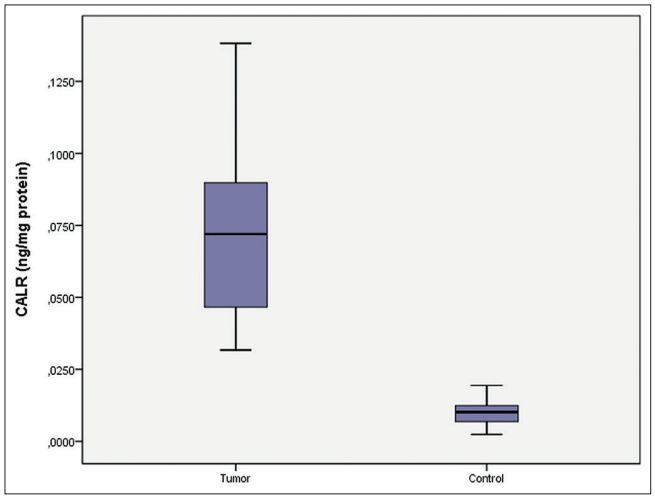

Figure 1: CALR measurements of the groups

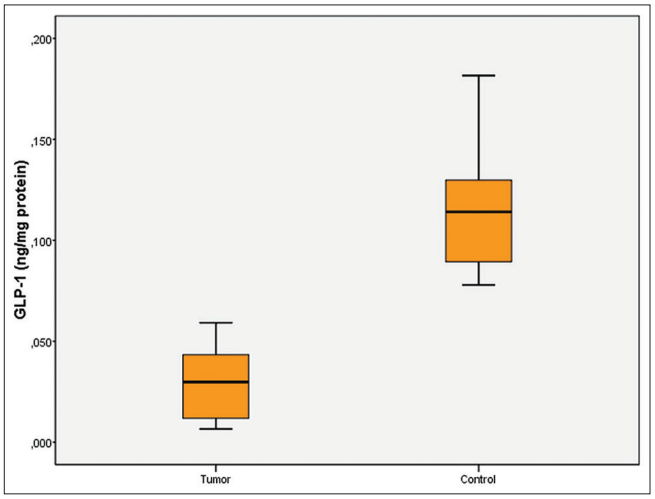

Figure 2: GLP-1 measurements of the groups

Correlations

The correlation analysis showed no correlation among Ki-67, CALR, and GLP-1 in grade III gliomas. However, a positive correlation between Ki-67 and CALR ($r = 0.90$; $P = 0.001$) and a negative correlation between Ki-67 and GLP-1 ($r = -0.88$; $P = 0.001$) expressions were observed in grade IV gliomas [Table 5 and Figures 4 and 5].

DISCUSSION

Despite the great advances in the management of gliomas, the 5-year survival of patients with HGGs has not been improved, and the treatment methods of these devastating diseases are still being developed. These tumors have a very aggressive behavior owing to their high invasive nature. Therefore, early diagnosis and effective treatment in addition to surgical resection is a very important task which requires the understanding of molecular biology and immunogenic properties of these tumors. Investigating the protein profile in normal and tumor cells may be a starting point to explain a novel diagnostic and therapeutic target. CALR has been studied in recent years to better understand tumor immunogenicity. The expression of this protein has been studied in several cancers with contradictory results. In some cancers, such as neuroblastoma[11] and prostate cancers,[9] CALR level has been observed to be associated with good prognosis, but in others, such as gastric[8] and esophageal cancers,[6] a higher CALR level was correlated with tumor growth and increased angiogenesis.

A limited number of studies have focused on brain tumors and the expression of CALR by Western blotting and immune-histochemical analysis using glioma cell lines.[5,10,27-31] Like the studies that investigated other types of cancers, studies that included glioma cell lines also provided contradictory results. In the present study, for the first time, we provided tissue expressions of CALR in HGGs and normal cerebral tissues using resected human gliomas. Our results showed that CALR expressions were higher in HGGs (grades III and IV) than in normal cerebral tissues. This result may be due to the fact that HGGs are aggressive, invasive, and more angiogenic.

By Western blotting and immune-histochemical analysis using glioma samples, Gao *et al.*[27] found that CALR expression was lower in grade III and IV gliomas than those in grade II and suggested that CALR downregulation was associated with the malignant phenotype of gliomas. More importantly,

Table 5: Analysis of CALR and GLP-1 expressions with Ki-67 measurements in WHO Grade 3 and Grade 4 glial tumors

	WHO	
	Grade 3	Grade 4
Ki-67/CALR (ng/mg protein)		
r	0,571	0,902
P	0,052	0,001**
Ki-67/GLP-1 (ng/mg protein)		
r	−0,237	−0,887
P	0,459	0,001**

r: Spearman korelasyon katsayısı, **P<0,01

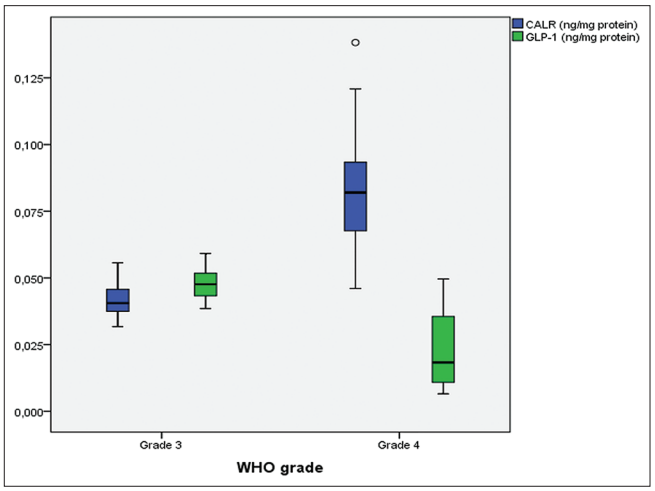

Figure 3: CALR and GLP measurements according to tumor grade

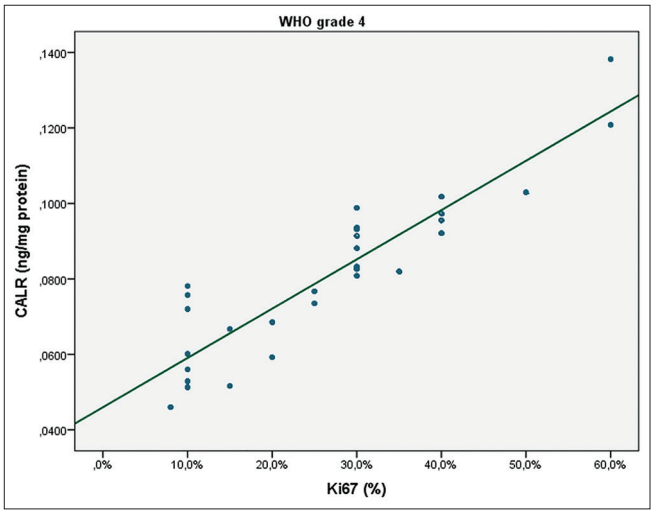

Figure 4: Relationship between Ki-67 and CALR in grade 4 tumors

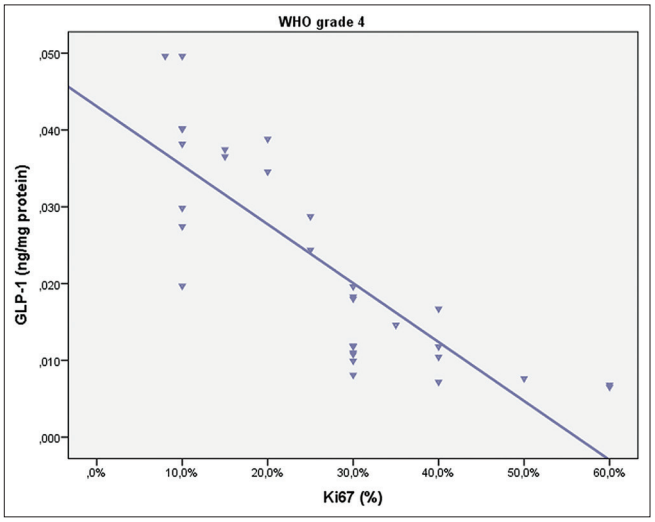

Figure 5: Relationship between Ki-67 and GLP-1 in grade 4 tumors

they found that decreased CALR expression was found to be a predictor for poor survival among patients with gliomas. Another immune-histochemical study by Muth *et al*.[29] with a very small sample of nine grade IV patients revealed that the tissue expressions of CALR were higher in relapse than those of the primary tumor and the level of the tumor surrounding the normal tissue was comparable to that of the primary tumor. Using glioblastoma cell lines, Nair *et al*.[30] found that the CALR expression was, on average, fourfold lower than that of normal human astrocytes, but after administering dexamethasone, an increased expression of CALR was observed which in turn inhibited the primary human glioblastoma dispersal. Moreover, our results are contrary to those of the abovementioned studies in which HGGs showed significantly higher expressions of CALR than the normal tissues. Although this difference may be due to the methodological differences, a common notion is that HGGs have a higher expression of CALR. It is well known that radio-chemotherapy induces the surface expression of CALR which enhances the phagocytosis of dying tumor cells by dendritic cells and thus fosters anti-tumor immune reactions.[12] These findings suggest that tumors expressing a high CALR level may be more immunogenic and targeting this mechanism may be a promising treatment strategy. Studies have shown that CALR facilitates the biological processes in several tumors by mediating anti-angiogenic properties and immune response. In gliomas, CALR expression is increased with high radiation sensitivity. Okunaga *et al*.[32] found that CALR modulates the radiosensitivity of human glioblastoma U251MG cells by affecting the cell survival pathway of Akt signaling through alterations of calcium homeostasis. Another study showed that childhood brain tumor cells can respond to anti-neoplastic agents by exposing CALR.[31] It has been shown that intratumoral injection with Ad-CALR/melanoma antigen gene-A3 (MAGE-A3) suppressed the tumor growth of glioblastoma *in vivo*. Moreover, it was suggested that the overexpression of CALR and MAGE-A3 in glioblastoma cells suppressed the proliferation and apoptosis by suppressing the Erk-1/2 MAPK and PI3K/Akt signaling pathways.[28] A recent study using human glioma cell lines indicated that resistance to bortezomib, a proteasome inhibitor, was modulated by arginylated CALR and the insertion of CALR into glioma cells undergoing bortezomib treatment resulted in the activation of apoptosis.[5] It was then concluded that the susceptibility of glioma cells to bortezomib treatment was strongly associated with the expression of CALR.[5] A limited number of glioma studies suggested that CALR exposure may be a prognostic marker of glioma treatment and the use of strategies targeting or promoting CALR pathways may contribute to the improvement of cancer treatments.

Currently, several studies on GLP-1 have been reported in Neurology and Oncology literature. In amyotrophic lateral sclerosis,[33] GLP-1 analogs have been found to be neuroprotective and have been shown to improve motor functions and prolong survival in Huntington's disease[34] through the suppression of mutant huntingtin inclusion bodies. Furthermore, they have the following functions:

(1) Protection against metabolic and oxidative trauma in Parkinson's disease and stroke[33]
(2) Protection of neurons against the lack of oxygen and glucose with antiapoptotic mechanism[34]
(3) Prevention against cell death in traumatic brain damage and correction of cognitive deficits[35]
(4) Restoring of behavioral disorders occurring upon traumatic brain damage[36]
(5) Prevention of hippocampal gene expression changes induced by traumatic brain damage or such changes in Alzheimer's disease (AD)[37,38]
(6) Prevention of blood–brain barrier impairment occurring upon traumatic brain damage and also of cortical neuronal damage[36]
(7) Correction of neurological function disorders due to tauopathy[39]
(8) Correction of memory related to the increase of the neuron count in hippocampal CA1 region in AD.[40]

Moreover, numerous studies in the literature have reported the neuroprotective effects of GLP-1 agonists.

GLP-1 analogs also have many effects on neoplastic diseases. Such numerous studies focused on the inhibition of tumor formation and metastasis in ovarian cancer,[41,42] inhibition of growth in breast cancer and prostate cancer,[20,22,43] inhibition of cell migration and invasion in glioblastoma,[25] better prognosis due to autophagic and apoptotic effects on endometrial cancer cells,[21] apoptotic and growth inhibitory effects on colon cancer cells,[44] and antiproliferative/apoptotic effects on pancreatic cancer cells along with decelerating growth and inhibition of tumor development and metastasis.[45,46] The fact that the most commonly observed brain tumors were metastases in adults[47] and, as noted above, that GLP-1 analogs have anti-cancer effects in numerous cancer types and in glioblastoma and neuroprotective effects in conditions other than cancer has led us to investigate this molecule. Contrary to the other previous studies, the effects of GLP-1 on oncological and neurological diseases and the studies in literature that focused on GLP-1R and GLP-1R agonists have led to the idea of conducting studies on GLP-1.

Although several studies reported the relation between GLP-1 and other types of cancers, only a limited number of studies focused on brain gliomas in the current literature. In this respect, Nie *et al*.[25] investigated the effects of exenidin-4 (a GLP-1R agonist which has a considerable amount of constructional similarity to the GLP-1) on the glioma cell lines. They demonstrated the GLP-1R expression in all four glioma cell lines but selected two of them, which had lower levels of expression, for further investigations. After the exendin-4 treatment, these cell lines showed less cell survival, proliferation, migration, and invasion. To explain the mechanism of cell migration and invasion, they

measured the expression levels of E-cadherin and vimentin which are the markers of epithelium and mesenchyme, respectively. They found that the exendin-4 treatment promoted the vimentin expression and suppressed the E-cadherin expression remarkably. As a result, they suggested that the alterations of these contents led to the inhibition of epithelial-to-mesenchymal transition, which is involved in the invasion and migration, in glioma cell lines. When considered the lower levels of GLP-1 in gliomas, particularly higher grades, than the control group in our study; it seems the endogenous GLP-1 have similar inhibitory effects like exendin-4, which had been applied as treatment previously in cell lines.

Körner *et al*.[13] investigated the GLP-1 in terms of the receptor expression in various human tumor and normal tissues. They pointed out that the glioblastomas had low levels of GLP-1 receptors. Moreover, they found that neurohypophysis had the most striking receptor expression in the studied tissues but the brain tissues adjacent to the brain tumors were rarely receptor positive. But they emphasized that the brain samples were collected during the brain tumor surgeries. Regarding the higher levels of GLP-1 in cadaver-derived normal brain samples and lower levels in high-grade glial tumors in our study, the decaying of GLP-1 or its receptor content in the normal tissue may be one of the reasons for the emerging tumors. Thus, investigating the exact and further mechanisms can be helpful to identify new diagnostic and treatment options.

From another perspective, the design of our study does not allow the conclusion that the aberrant calreticulin and GLP-1 levels are indeed involved in tumor progression. Thus, the main focus of treatment goals may vary. But even if assumed that calreticulin and GLP-1 are involved in glioma progression, their targeting would be hard to achieve. One of the main reasons why it is difficult is that the inhibition of calreticulin can be challenging because it is not an enzyme.

Limitations

However, the present study has some limitations. The first and the most important limitation is that we included less number of patients. This is because the majority of patients operated during the study period did not consent to participate in the study. The second limitation is that the differences between the different grades of glioma were not examined. Future studies should include a larger cohort of patients with different grades of glioma and also explain how the levels of CALR and GLP-1 change after treatment with surgery and/or radio-chemotherapy in relapse.

CONCLUSION

High-grade gliomas are highly aggressive and invasive tumors. Moreover, studies on the molecular mechanisms in the biology of tumors are needed. Overall, our results showed that higher CALR and lower GLP-1 expressions are found in HGGs compared to normal cerebral tissues.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, *et al*. Cancer statistics, 2005. CA Cancer J Clin 2005;55:10-30.
2. Weathers S-P, Gilbert MR. Current challenges in designing GBM trials for immunotherapy. J Neurooncol 2015;123:331-7.
3. Gutkin DW, Shurin MR. Clinical evaluation of systemic and local immune responses in cancer: Time for integration. Cancer Immunol Immunother 2014;63:45-57.
4. Spel L, Boelens J-J, Nierkens S, Boes M. Antitumor immune responses mediated by dendritic cells: How signals derived from dying cancer cells drive antigen cross-presentation. OncoImmunology 2013;2:e26403.
5. Comba A, Bonnet LV, Goitea VE, Hallak ME, Galiano MR. Arginylated calreticulin increases apoptotic response induced by bortezomib in glioma cells. Mol Neurobiol 2019;56:1653-64.
6. Du X-L, Yang H, Liu S-G, Luo M-L, Hao J-J, Zhang Y, *et al*. Calreticulin promotes cell motility and enhances resistance to anoikis through STAT3-CTTN-Akt pathway in esophageal squamous cell carcinoma. Oncogene 2009;28:3714-22.
7. Lwin Z-M, Guo C, Salim A, Yip GW-C, Chew F-T, Nan J, *et al*. Clinicopathological significance of calreticulin in breast invasive ductal carcinoma. Mod Pathol 2010;23:1559-66.
8. Chen C-N, Chang C-C, Su T-E, Hsu W-M, Jeng Y-M, Ho M-C, *et al*. Identification of calreticulin as a prognosis marker and angiogenic regulator in human gastric cancer. Ann Surg Oncol 2009;16:524-33.
9. Alur M, Nguyen MM, Eggener SE, Jiang F, Dadras SS, Stern J, *et al*. Suppressive roles of calreticulin in prostate cancer growth and metastasis. Am J Clin Pathol 2009;175:882-90.
10. Peng R-Q. Expression of calreticulin is associated with infiltration of T-cells in stage IIIB colon cancer. World J Gastroenterol 2010;16:2428-34.
11. Hsu WM, Hsieh FJ, Jeng YM, Kuo ML, Chen CN, Lai DM, *et al*. Calreticulin expression in neuroblastoma--a novel independent prognostic factor. Ann Oncol 2005;16:314-21.
12. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini J-L, *et al*. Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med 2007;13:54-61.
13. Körner M, Stockli M, Waser B, Reubi JC. GLP-1 receptor expression in human tumors and human normal tissues: Potential for *in vivo* targeting. J Nucl Med 2007;48:736-43.

14. Schick RR, Zimmermann JP, vorm Walde T, Schusdziarra V. Peptides that regulate food intake: Glucagon-like peptide 1-(7-36) amide acts at lateral and medial hypothalamic sites to suppress feeding in rats. Am J Physiol Regul Integr Comp Physiol 2003;284:R1427-435.
15. Larsen PJ, Tang-Christensen M, Holst JJ, Ørskov C. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. Neuroscience 1997;77:257-70.
16. Koshal P, Jamwal S, Kumar P. Glucagon-like Peptide-1 (GLP-1) and neurotransmitters signaling in epilepsy: An insight review. Neuropharmacology 2018;136:271-9.
17. Lerche S, Brock B, Rungby J, Botker HE, Moller N, Rodell A, *et al*. Glucagon-like peptide-1 inhibits blood-brain glucose transfer in humans. Diabetes 2008;57:325-31.
18. Hui H, Nourparvar A, Zhao X, Perfetti R. Glucagon-like peptide-1 inhibits apoptosis of insulin-secreting cells via a cyclic 5′-Adenosine monophosphate-dependent protein kinase A- and a phosphatidylinositol 3-kinase-dependent pathway. Endocrinology 2003;144:1444-55.
19. Buteau J, Foisy S, Joly E, Prentki M. Glucagon-like peptide 1 induces pancreatic -cell proliferation via transactivation of the epidermal growth factor receptor. Diabetes 2003;52:124-32.
20. Li X-n, Bu H-m, Ma X-h, Sh Lu, Sh Zhao, Cui Y-l, *et al*. Glucagon-like peptide-1 analogues inhibit proliferation and increase apoptosis of human prostate cancer cells *in vitro*. Exp Clin Endocrinol Diabetes 2016;125:91-97.
21. Kanda R, Hiraike H, Wada-Hiraike O, Ichinose T, Nagasaka K, Sasajima Y, *et al*. Expression of the glucagon-like peptide-1 receptor and its role in regulating autophagy in endometrial cancer. BMC Cancer 2018;18:657.
22. Iwaya C, Nomiyama T, Komatsu S, Kawanami T, Tsutsumi Y, Hamaguchi Y, *et al*. Exendin-4, a glucagonlike peptide-1 receptor agonist, attenuates breast cancer growth by inhibiting NF-κB activation. Endocrinology 2017;158:4218-32.
23. Körner M, Christ E, Wild D, Reubi JC. Glucagon-like peptide-1 receptor overexpression in cancer and its impact on clinical applications. Front Endocrinol (Lausanne) 2012;3:158.
24. Brubaker PL, Drucker DJ. Minireview: Glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. Endocrinology 2004;125:2653-9.
25. Nie Z-J, Zhang Y-G, Chang Y-H, Li Q-Y, Zhang Y-L. Exendin-4 inhibits glioma cell migration, invasion and epithelial-to-mesenchymal transition through GLP-1R/sirt3 pathway. Biomed Pharmacother 2018;106:1364-9.
26. Brubaker PL, Drucker DJ. Structure-function of the glucagon receptor family of G protein-coupled receptors: The glucagon, GIP, GLP-1, and GLP-2 receptors. Recept Channels 2002;8:179-88.
27. Gao H, Yu B, Yan Y, Shen J, Zhao S, Zhu J, *et al*. Correlation of expression levels of ANXA2, PGAM1, and CALR with glioma grade and prognosis. J Neurosurg 2013;118:846-53.
28. Liu X-L, Zhao D, Sun D-P, Wang Y, Li Y, Qiu F-Q, *et al*. Adenovirus-mediated delivery of CALR and MAGE-A3 inhibits invasion and angiogenesis of glioblastoma cell line U87. J Exp Clin Cancer Res 2012;31:8.
29. Muth C, Rubner Y, Semrau S, Rühle P-F, Frey B, Strnad A, *et al*. Primary glioblastoma multiforme tumors and recurrence: Comparative analysis of the danger signals HMGB1, HSP70, and calreticulin. Strahlenther Onkol 2016;192:146-55.
30. Nair M, Romero J, Mahtabfar A, Meleis A, Foty R, Corbett S. Dexamethasone-mediated upregulation of calreticulin inhibits primary human glioblastoma dispersal *ex vivo*. Int J Mol Sci 2018;19:572.
31. Sonnemann J, Greßmann S, Becker S, Wittig S, Schmudde M, Beck JF. The histone deacetylase inhibitor vorinostat induces calreticulin exposure in childhood brain tumour cells *in vitro*. Cancer Chemother Pharmacol 2010;66:611-6.
32. Okunaga T, Urata Y, Goto S, Matsuo T, Mizota S, Tsutsumi K, *et al*. Calreticulin, a molecular chaperone in the endoplasmic reticulum, modulates radiosensitivity of human glioblastoma U251MG cells. Cancer Res 2006;66:8662-71.
33. Li Y, Perry T, Kindy MS, Harvey BK, Tweedie D, Holloway HW, *et al*. GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and parkinsonism. Proc Natl Acad Sci U S A 2009;106;1285-90.
34. Wang M-D, Huang Y, Zhang G-P, Mao L, Xia Y-P, Mei Y-W, *et al*. Exendin-4 improved rat cortical neuron survival under oxygen/glucose deprivation through PKA pathway. Neuroscience 2012;226:388-96.
35. Eakin K, Li Y, Chiang Y-H, Hoffer BJ, Rosenheim H, Greig NH, *et al*. Exendin-4 ameliorates traumatic brain injury-induced cognitive impairment in rats. PLoS One 2013;8:e82016.
36. Hakon J, Ruscher K, Romner B, Tomasevic G. Preservation of the blood brain barrier and cortical neuronal tissue by liraglutide, a long acting glucagon-like-1 analogue, after experimental traumatic brain injury. PLoS One 2015;10:e0120074.
37. Tweedie D, Rachmany L, Rubovitch V, Lehrmann E, Zhang Y, Becker KG, *et al*. Exendin-4, a glucagon-like peptide-1 receptor agonist prevents mTBI-induced changes in hippocampus gene expression and memory deficits in mice. Exp Neurol 2013;239:170-82.
38. Tweedie D, Rachmany L, Rubovitch V, Li Y, Holloway HW, Lehrmann E, *et al*. Blast traumatic brain injury-induced cognitive deficits are attenuated by preinjury or postinjury treatment with the glucagon-like peptide-1 receptor agonist, exendin-4. Alzheimers Dement 2016;12:34-48.
39. Hansen HH, Barkholt P, Fabricius K, Jelsing J, Terwel D, Pyke C, *et al*. The GLP-1 receptor agonist liraglutide reduces pathology-specific tau phosphorylation and improves motor function in a transgenic hTauP301L mouse model of tauopathy. Brain Res 2016;1634:158-70.
40. Hansen HH, Fabricius K, Barkholt P, Niehoff ML, Morley JE, Jelsing J, *et al*. The GLP-1 receptor agonist liraglutide improves memory function and increases hippocampal CA1 neuronal numbers in a senescence-accelerated mouse model of Alzheimer's disease. J Alzheimers Dis 2015;46:877-88.
41. He W, Yu S, Wang L, He M, Cao X, Li Y, *et al*. Exendin-4 inhibits growth and augments apoptosis of ovarian cancer cells. Mol Cell Endocrinol 2016;436:240-9.
42. Kosowska A, Gallego-Colon E, Garczorz W, Kłych-Ratuszny A, Aghdam MR, Woz Niak M, *et al*. Exenatide modulates tumor-endothelial cell interactions in human ovarian cancer cells. Endocr Connect 2017;6:856-65.
43. Ligumsky H, Wolf I, Israeli S, Haimsohn M, Ferber S, Karasik A, *et al*. The peptide-hormone glucagon-like peptide-1 activates cAMP and inhibits growth of breast cancer cells. Breast Cancer Res Treat 2012;132:449-61.
44. Koehler JA, Kain T, Drucker DJ. Glucagon-like peptide-1 receptor activation inhibits growth and augments apoptosis in murine CT26 colon cancer cells. Endocrinology 2011;152:3362-72.
45. Zhao H, Wei R, Wang L, Tian Q, Tao M, Ke J, *et al*. Activation of glucagon-like peptide-1 receptor inhibits growth and promotes apoptosis of human pancreatic cancer cells in a cAMP-dependent manner. Am J Physiol Endocrinol Metab 2014;306:E1431-41.
46. Zhao H, Wang L, Wei R, Xiu D, Tao M, Ke J, *et al*. Activation of glucagon-like peptide-1 receptor inhibits tumourigenicity and metastasis of human pancreatic cancer cells via PI3K/Akt pathway. Diabetes Obes Metab 2014;16:850-60.
47. Gállego Pérez-Larraya J, Hildebrand J. Brain metastases. Handb Clin Neurol 2014;121:1143-57.