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Reduced Serum sRANKL and sTREM2 Levels in High-Grade Gliomas: Association with Prognosis

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ABSTRACT

Introduction: High-grade gliomas (HGG), including Glioblastoma multiforme (GBM), account for the majority of primary brain tumors. Nevertheless, prognostic and diagnostic biomarkers are quite limited for HGG. The objective of this study was to investigate the prognostic value of sRANKL and sTREM2 levels in HGG patients.

Methods: Twelve consecutive patients with HGG, 14 patients with non-glial tumors (non-GT) and 20 age and gender-matched healthy controls were recruited. Overall survival duration of the patients was recorded. Pre-operative serum levels of sRANKL and sTREM2 were measured by ELISA. Tumors of HGG patients were analyzed by immunohistochemical staining for p53 and Ki67 and percentage scores were calculated.

Results: Patients with HGG and non-GT showed lower serum sRANKL and sTREM2 levels than healthy individuals. Levels of sRANKL were inversely correlated with the overall survival of patients (p=0.002, R=0.787), while sTREM2 levels were inversely correlated with p53 score (p=0.018, R=-0.666) but not survival.

Conclusion: Brain tumor patients show suppressed levels of glial activity biomarkers in the peripheral circulation. Serum sRANKL levels may serve as a potential prognostic biomarker for HGG.

Keywords: Glioblastoma multiforme, glioma, soluble receptor activator for nuclear factor-κB ligand (sRANKL), soluble triggering receptor expressed on myeloid cells 2 (sTREM2)

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INTRODUCTION

High-grade gliomas (HGG), including Glioblastoma multiforme (GBM), account for the majority of primary brain tumors (1). Glioblastoma multiforme (GBM) is classified as grade IV glioma and is the most common and invasive primary brain tumor (2). GBMs constitute more than half of all high grade gliomas (HGG) and 16% of all central nervous system tumors. Depending on the location of tumor, the most prevalent symptoms are new-onset seizures, speech deficits, motor and cognitive impairment and recurrent headaches (1–3). Depending on the prognosis, location, size and clinical findings of the tumor, surgical resection, radiotherapy and chemotherapy are applied (3). In addition, although isocitrate dehydrogenase-I (IDH-I) gene mutation analysis provides prediction about disease progression, there are no well-defined and practical serum-based biomarkers available for HGG that can be used for early diagnosis and prognosis (2). The average life expectancy after a diagnosis of HGG is usually less than 2 years (4).

Receptor activator of nuclear factor-κB ligand (RANKL) is a tumor necrosis factor (TNF) family protein, which has a vital role in osteoclast formation for bone remodeling through interaction with the receptor activator of nuclear factor-κB (RANK) and development of lymph nodes by means of activation of different signaling pathways, including NF-κB, c-Jun and serine/threonine kinase Akt/PKB (5, 6). Binding of RANKL to the receptor RANK is blocked by binding of osteoprotegerin (OPG) to RANKL, and this blockade is substantial for preventing excessive bone resorption and protecting arteries from calcification. RANKL can be found as a transmembrane molecule or a soluble form (sRANKL), which is secreted by effector T cells (7). Its expression occurs at different levels in synovial, epithelial and bone marrow stromal cells, lymph nodes, lung, thymus, spleen and activation is directed by pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-11, IL-17 as well as parathyroid hormone (PTH), vitamin D3, progesterone and corticosteroids (5, 7, 8). Alteration in levels of peripheral blood and gene expression of the RANK/RANKL system has been associated with various diseases such as rheumatoid arthritis (RA), coronary artery disease (CAD) (6), lung (9) and breast cancer (8). In vivo studies have revealed that elimination of RANKL expression causes disruption in B lymphocyte and thymocyte development. Moreover, communication of RANK, expressed in dendritic cells (DCs) and RANKL activation from T lymphocytes can lead to disruption of immune tolerance by directing organogenesis in lymph nodes (8, 10). Increased expression of sRANKL has been associated with the risk of sex hormone-stimulated and familial BRCA1 mutation associated breast cancer (11, 12). Increased RANKL activation has also been found in lung, prostate, gastric and cervical cancers, and has been associated with poor prognosis (13, 14).

The triggering receptor expressed on myeloid cells 2 (TREM2) is an innate immune response receptor with an immunoglobulin domain

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which is known to direct inflammation processes by increasing microglial phagocytosis and inflammation-related cascades (12). It has significant anti-inflammatory effect in the cleaning of apoptotic neurons and amyloid plaques, and therefore its mutations or dysfunction lead to neurodegenerative diseases such as Nasu-Hakola disease, Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) (15). Enzymatic processing of TREM2 with proteases generates its soluble form (sTREM2), which is present in human body fluids such as cerebrospinal fluid (CSF), plasma and serum (16). Decreased sTREM2 levels in body fluids have been shown in patients with dementia, while increased levels of sTREM2 were detected in the CSF of other inflammatory neurological diseases, such as multiple sclerosis (17).

The aim of this study was to identify potential alterations in serum levels of sRANKL and sTREM2 molecules in patients with HGG and determine whether they generate a prognostic and/or diagnostic biomarker signature.

METHODS

Participants

Twelve consecutive patients with high-grade gliomas (HGG), 14 patients with non-glial tumors (non-GT) and 20 sex-aged matched healthy individuals were included. All participants met the relevant diagnostic criteria of the 2016 World Health Organization Classification of Tumors of the Central Nervous System for glioblastoma and other tumors (18) as a result of neurological findings, magnetic resonance imaging (MRI), computed tomography (CT), genetic and immunohistochemical examinations. A standard chemoradiotherapy after surgical resection was applied to all patients (19). The study was approved by the institutional review board and all participants gave their written consents. All procedures performed were in accordance with the ethical standards of the institutional review board and with the 1964 Helsinki declaration and its later amendments.

Immunohistochemical Analysis

Immunohistochemical analysis was performed on formalin-fixed and paraffin-embedded tumor specimens using p53 and Ki67 antibodies (DAKO, Denmark) and scored (percentage of labeled nuclei per 100 cells), as decribed previously. Brown nuclear staining was considered positive for both antibodies (20). IDH-1-R132H mutation was also determined by immunohistochemistry, as described previously (21).

ELISA

Blood samples of patients were collected before the neurosurgical intervention, centrifuged at 3000 rpm for 10 minutes and stored at -80°C until use. Serum levels of sRANKL (Elabscience, Wuhan, China) and sTREM2 (MyBiosource Inc., San Diego, USA) were detected with commercial ELISA kits, as per manufacturer's specifications. Optical density (OD) was measured at 450 nm and concentrations were calculated by reference to the standard curve.

Statistical Analysis

ANOVA and Tukey's post hoc test were used for comparison of sRANKL and sTREM2 levels. Correlation studies were accomplished by Pearson's correlation test; p value below 0.05 was defined as statistical significance.

Table 1. Clinical and demographic features of patients with high grade gliomas

Gender/Age	Grade	Survival (months)	Ki67%	p53%
F/73	IV	5	15	1
F/50	IV	15	20	60
F/61	IV	5	20	50
F/25	IV	15	18	5
M/48	IV	12	20	50
M/51	IV	1	50	30
M/70	IV	5	12	60
M/81	IV	8	50	30
M/49	IV	9	20	0
M/17	III	11	24	10
M/79	IV	3	80	0
F/55	III	13	29	26

F, female; M, male.

RESULTS

Clinical and Pathological Features of HGG Patients

All HGG patients (5 women/7 men; mean age at diagnosis ± standard deviation, 54.9±19.8) had isocitrate dehydrogenase 1 mutation negative (IDH1-wildtype) grade III or IV tumors. Non-GT patients (8 women/6 men; 59.6±18.7) were diagnosed with meningioma, adenocarcinoma, schwannoma and lymphoma. Overall survival duration of HGG patients ranged between 1–15 months (8.5±4.7). HGG was predominantly located in frontal lobe (n=5), parietal lobe (n=3), corpus callosum (n=2), thalamus (n=1) and temporo-parieto-occipital junction (n=1). Clinical, demographic and immunohistochemistry data of HGG patients are presented in Table 1.

Association of Clinical Features with sRANKL and sTREM2 Levels

Both HGG and non-GT patients showed trends towards exhibiting decreased pre-operative serum levels of both sRANKL and sTREM2 compared to healthy control group (p=0.1602 and p=0.0158, respectively). However, this trend attained statistical significance only for sTREM2 (Figure 1). Correlation analysis were performed to determine whether

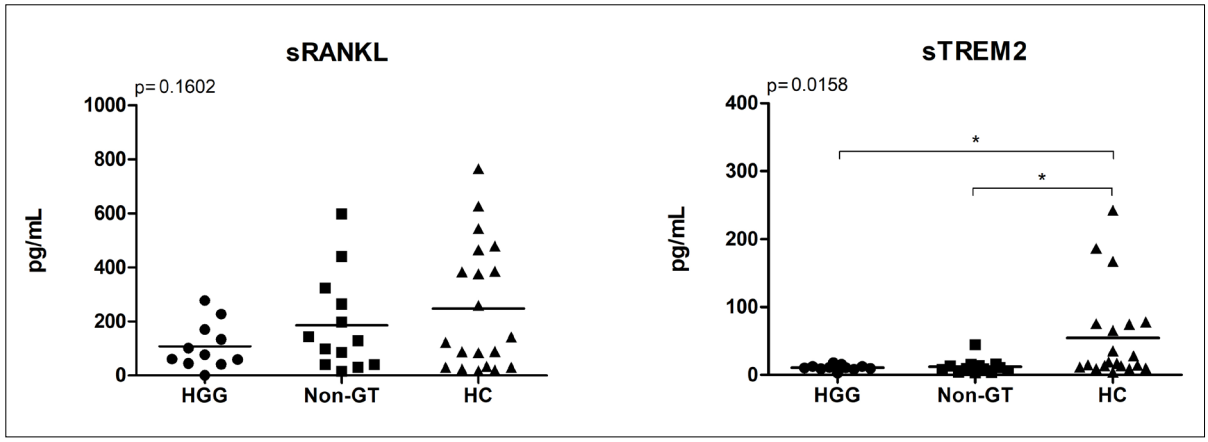

Figure 1. Serum sRANKL and sTREM2 levels of patients with high grade gliomas (HGG), non-glial tumors (non-GT) and healthy controls (HC). Horizontal lines indicate mean values. p values on the upper left corner of the panel are obtained by ANOVA. *p<0.05 by Tukey's post-hoc test.

sRANKL or/and sTREM2 may have a prognostic value for HGG patients. No significant correlation was determined between sRANKL and sTREM2 levels and sRANKL/sTREM2 levels versus age and Ki67 score parameters of the patients. However, serum sRANKL levels showed significant inverse correlation with overall survival time of patients with HGG (p=0.002, R=0.787). By contrast, sTREM2 levels were inversely correlated with p53 score (p=0.018, R=-0.666) but not survival time.

DISCUSSION

sRANKL and sTREM2 are two remarkable molecules known to be associated with the survival of specific cell types such as microglia and lymphocytes and known to play a role in inflammation process in the brain. In present study, we investigated sRANKL and sTREM2 serum levels in sera obtained before the operation in patients diagnosed with HGG and non-GT. Notably, both molecules were found to be suppressed in peripheral circulation of tumor patients.

RANK and RANKL are overexpressed in tumors and may lead to aberrant cell proliferation, survival, tumor invasion and metastasis (22). Moreover, RANKL-induced NF-κB activity has been linked to gliomagenesis and radio/chemotherapy resistance (23). Impaired function of TREM2, which is abundant in microglia, macrophage and dendritic cells, is associated with axonal damage and myelin loss in neurodegeneration (24). Furthermore, both RANKL and TREM2 have been shown to promote glioma cell proliferation and invasion (25). Therefore, decreased levels of these mediators may be a reflection of a counter-protective negative feedback mechanism to avoid further expansion of the tumors.

In line with this assumption, we found that HGG patients with higher RANKL levels were more likely to display lower overall survival durations, indicating that serum levels of RANKL may be used as a prognostic biomarker for HGG. In likeness to our study, sRANKL levels measured in bronchoalveolar lavage fluid were inversely proportional to the survival of the patients with non-small cell lung cancer (9). These results bring forward RANKL as a therapeutic target. Denosumab, an IgG2-type human monoclonal antibody against RANKL has been registered to be used in the treatment of prostate and breast cancer (26). Selective inhibition of RANKL in experimental animal models and human studies has been shown to reduce proliferation of breast epithelial cells and several other cancer types (27). Thus, there is accumulating evidence that RANKL inhibition has an anti-tumor effect (26, 27).

The exposure of TREM2 knockdown microglia cells to apoptotic neurons causes an increased proinflammatory response, indicating that TREM2 has an immune regulatory function (28). Since microglia and macrophages gathered around the tumor in HGG may contribute to tumor development (29), reduced production of sTREM2 in HGG patients may be an effort to increase the activity of the cerebral innate immunity and thereby decrease tumor expansion. Additionally, reduced sTREM2 levels might also diminish proliferation and invasion of glioma cells through direct manipulation of proliferation pathways of tumor cells (25). In this context, p53 prevents cancer formation, functions as a tumor suppressor and regulates TREM2. This association may explain the significant correlation between p53 index score and sTREM2 levels (30). Thus, serum sTREM2 may be utilized as a biomarker of tumor suppressor activity in HGG.

Ki67 is a cellular marker for proliferation and immunohistochemically-determined index of Ki67 may be used to extrapolate the clinical course of HGG (20). Nevertheless, neither sRANKL nor sTREM2 showed significant correlation with the Ki67 index score suggesting that these mediators are not significantly involved in progression of cell cycle.

In conclusion, the assessment of circulating serum levels of sRANKL may be prognostic tool for clinical outcome of patients with HGG. Although RANKL inhibiting agents have not been used in HGG, our results suggest that this molecule may be a potential therapeutic target for gliomas. Overall, alterations in serum levels of sTREM2, which might indicate a possible compensatory mechanism in tumorogenesis, may be useful in understanding microglia behavior in future studies.

Ethics Committee Approval: This study was approved by Haydarpasa Numune Education and Research Hospital Ethical Committee (HNEAH-KAEK 2015/KK/96).

Informed Consent: Written informed consent was obtained from all patients and healthy controls.

Peer-review: Externally peer-reviewed

Author Contributions: Concept – ME, NB; Design – EŞ, ME, NB; Supervision – ME, EŞ, NB; Resource – ME, NB; Materials – EŞ, CT, NB; Data Collection and/or Processing – ME, EŞ, NB; Analysis and/or Interpretation – EŞ, NB; Literature Search – EŞ, ME, CT, NB; Writing – EŞ, ME, CT, NB; Critical Reviews – ME, EŞ, NB.

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