CASE REPORT

GLIOBLASTOMA MULTIFORME CLASSIFIED AS MESENCHYMAL SUBTYPE

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ABSTRACT

INTRODUCTION: Recently, researchers have been considering as adverse prognostic factors in primary glioblastomas not only clinical indicators but also various cellular, genetic and immunological markers. The aim of the present article was to report a case of primary glioblastoma multiforme with poor survival in a patient after surgical intervention, and to determine the unfavorable prognostic markers. CASE REPORT: We present a 71-year-old man with histologically verified glioblastoma multiforme and a postoperative survival of 48 days. The patient did not receive any radiotherapy and adjuvant therapy with temozolomide because of the short survival. Serum and transcription levels of TNF-α, CD44, YKL-40 and IL-6 were determined by molecular-biological and immunological analyses. We found very high transcription levels of the genes CD44, YKL-40 and IL-6, increased gene expression of TNF-α, and elevated serum concentrations of TNF-α, YKL-40 and IL-6 and reduced serum concentration of CD44. CONCLUSION: Molecular-biological and immunological analyses support the hypothesis that glioblastoma multiforme is presented by a heterogeneous group of glial tumors with different clinical course and prognosis. The high expression levels of TNF-α, CD44, YKL-40, and IL-6 indicate that the tumor can be categorized as mesenchymal subtype of glioblastoma multiforme, which accounts for the rapid clinical course and lethal outcome of the condition.

Key words: glioblastoma multiforme, gene expression, proinflammatory cytokines, YKL-40, CD44
INTRODUCTION

Glioblastoma multiforme (GBM) is the most common glial tumor in adults accounting for 65% of high-grade neoplasms of the central nervous system. It has an adverse clinical course and median post-operative survival of about 12 months. As unfavorable prognostic factors in glioblastoma are discussed: age over 70 years, preoperative Karnofsky performance score less than 70 points, ASA status more than 3 points, tumor location in the vicinity of midbrain structures and sprouting in corpus callosum, inability of using radical surgical resection, various cellular, genetic and immunological markers.

The etiology of GBM remains still unknown, but it is accepted the malignant development of neuroglial cells is triggered by accumulation of genetic mutations in the cells. Oncogenes and tumor suppressor genes known to cause other types of cancer such as the epidermal growth factor receptor (EGFR) and the tumor suppressor gene TP53 have been studied. Overexpression of oncogenes and mutations in tumor suppressor genes are thought to be the key to studying the tumor growth biology. The most important signaling pathways that are thought to be implicated in the genetics of GBM are Ras and PI3K-Akt oncogenic signaling pathways, and the p53 and RB tumor-suppressor pathways. The role of aberrant cellular metabolism in GBM related to mutations of isocitrate dehydrogenase genes 1 and 2 (IDH1 and IDH2) is also examined.

The idea that glioblastomas are a heterogeneous group of glial tumors with different clinical course and prognosis was put forward in 1938 for the first time (Scherer 1938, 1940). The two main subtypes of GBM were defined - primary (de novo) and secondary (progressing from lower-grade glial tumors), that were histologically undifferentiable but very different biologically and clinically.

Primary GBM develops in elderly patients and usually shows EGFR gene overexpression, PTEN (MMAC1) mutations, CDKN2A (p16) deletions and, rarely, MDM2 amplification. Secondary GBM develops in younger patients where TP53 mutations are often found.

Categorizing GBM into subtypes is a problem, now much in the focus of research, that offers potential opportunities to individualize therapy. The rapid advance of genomic techniques allowed researchers to generate a database (The Cancer Genome Atlas, TCGA) that permits the systematization of GBM subtypes and determination of the potential diagnostic and prognostic biomarkers using a variety of mathematical, statistical and computational methods.

The commonly accepted classification of gliomas divides them into four subtypes depending on the frequency of the detected genetic mutations:

CLASSICAL SUBTYPE

Almost 100% of the tumors in the classical subtype show aberrations in chromosomes 7 and 10, combined with EGFR amplification (97%), and no mutation in the gene TP53 (which is very often mutated in GBM). A deletion in 9p21.3 leading to a loss of the gene CDKN2A (encoding the p16INK4A and p14ARF) and abnormalities in RB tumor suppressor pathway are found.

MESENCHYMAL SUBTYPE

This subtype is characterized by a deletion in chromosome 17q which contains the gene NF1 (> 53%). Low expression of the genes NF1 and PTEN have been demonstrated. Elevated expression levels have also been found of astrocyte markers (CD44 and MERTK), of mesenchymal markers CHI3L1 (known as YKL-40) and MET, and of the TNF genes. There are very pronounced necrotic changes and inflammatory infiltration in the tumor tissue in the mesenchymal subtype. These genetic aberrations probably lead to activation of the AKT-oncogenic pathway followed by increased cell division, migration, angiogenesis and resistance to therapy.

PRONEURAL SUBTYPE

The proneural subtype shows amplification in the gene 4q12 and high levels of PDGFRA gene expression. Frequent mutations in the genes IDH1 and TP53 are also detected.

NEURAL SUBTYPE

High expression levels of neural markers such as NEFL, GABRA1, SYT1 and SLC12A5 have been found in this subtype.

In more recent studies researchers have been trying to identify the glioma subtypes by analyzing transcription levels of some genes and the levels of expression of the proteins encoded by them. The results show that the expression of EGFR, p53, CD44, MERTK and OLG2 can classify the GBM subtypes similar to those defined by DNA microarrays analysis. Assessing the tissue expression of these markers is more cost-effective and takes considerably less time compared with the time needed to perform molecular genetic analysis. It has been suggested that glioma diagnosis should include these subtyping markers, besides the criteria in the WHO classification, so that therapy can be
individualized and tumors subtyped.6

And yet, prognostic biomarkers for each subtype have not been systematically studied.

We report a case of a primary GBM which was studied using conventional clinical and non-conventional molecular markers to get a better understanding of the rapid and aggressive lethal clinical course of the condition.

CASE REPORT

A 71-year-old man presented with a six-week history of impaired memory and unsteady gait. The patient received regular therapy for arterial hypertension and chronic ischemic heart disease. The patient did not report any allergic disorders, autoimmune and cancer diseases. Family history was not indicative of any hereditary predisposition. Blood count was normal. The ECG showed a normal sinus rhythm and a left anterior hemiblock.

Additional studies were needed because of the headache and the muscle weakness reported by the patient. Neurologically, we found right-sided central hemiparesis of second degree, more expressed in the arm. The Karnofsky performance score was 70. Brain CT showed a tumor mass located in the left parietal lobe and corpus callosum, growing into the right hemisphere. The medial parts of the lesion had low density, probably caused by necrotic degradation. The peritumoral edema was poorly expressed and there was no distinct caption of contrast matter in both arterial and venous phases of examination. Pre-operative planning of surgery was done on a 3D model generated on images acquired using CT (Fig. 1).

Partial ultrasound-assisted resection of the tumor was performed microsurgically (about 70% of the tumor has been removed). Intraoperatively, we found pale-gray neoplasm infiltrating the brain tissue; it was moderately vascularized and showed multiple necrotic areas. A diagnosis of GBM was histologically verified (Fig. 2).

The routine paraclinical studies before surgery were normal.

The serum levels of the cytokines TNF-a and IL-6 (Biolegend, Cat No 430201), the new biomarker YKL-40 (Quidel Corporation, Cat No 8020) and the

(A,B) - pre-operative CT of the patient, identifying the tumor mass located in the left parietal lobe and corpus callosum, and growing into the right hemisphere; (C,D) - 3D reconstruction and visualization of the brain tumor.

Figure 1. Pre-operative neuroimaging diagnostics.

(A) - Necrosis (arrows) surrounded by hyperchromatic, polymorphic tumor cells with pseudo-palisades (x10); (B) - Multinucleated giant cells (x40).

Figure 2. Histological study of glioblastoma multiforme. HE staining.
adhesion molecule CD44 (Abcam, Cat No ab45912) were measured preoperatively using ELISA. They were compared with the values assessed in healthy age-matched individuals (n = 20). The expression levels of the genes responsible for the synthesis of these proteins were determined in brain tissue isolated during the surgical removal of the tumor and post mortem in controls (n = 4). The gene expression of TNF-α, CD44 and IL-6 was studied applying qPCR with RT2 Profiler PCR array (SABiosciences), and GAPDH was used as a reference gene. In another experiment, the relative gene expression of YKL-40 was measured using the gene UBC as reference. Triplicate measurements were taken for each sample in accordance with MIQE guidelines. In both experiments, ddCt method was used in the analysis of the results, wherein the expression of the examined genes is compared with that of the control group (the calibrator) and then with the level of the reference gene. Preliminary studies of GBM patients showed that the transcription levels of the examined genes were higher than those in the controls (by 60 times for CD44, by 30 times for TNF-α, by 17 times for IL-6 and by 90 times for YKL-40) (Fig. 3).

In the presented case, we found extremely high transcription levels of CD44, IL-6 and YKL-40, increased expression of TNF-α (Fig. 3), elevated serum concentrations of IL-6, TNF-α and YKL-40 and reduced serum concentration of CD44 (Table 1). Four weeks after surgery the patient reported increasing muscle weakness of the right limbs, general weakness, impaired memory, headache, and drowsiness. The patient died 48 days after the surgical intervention.

**DISCUSSION**

High expression of YKL-40 in GBM is associated with resistance to therapy, rapid progression of the disease and death. There is evidence that this glycoprotein takes part in angiogenesis by modulating the morphology of vascular endothelial cells – it stimulates their migration and reorganization. YKL-40 expression has been demonstrated to correlate with the expression of VEGF. This finding explains the association between the glycoprotein and angiogenesis. The gene expression of YKL-40 and of the corresponding protein serum level were found in our study to be considerably higher than these in healthy individuals.

CD44 is a transmembrane glycoprotein that serves as a hyaluronic acid receptor in the extracellular matrix. ADAM metalloproteinases cleave the receptor in cancer cells, which facilitates the cell separation from the matrix and assists cell migration and the respective tumor invasion. It has been demonstrated that CD44 inhibits the Hippo signaling pathway, thus reducing the stress-induced apoptosis. Over a 200-fold increase of the transcription level was found in the studied patient, which supports the hypothesis that the higher levels correlate with the histopathologic grade of the tumor and tumor invasion. In contrast to the results of the transcription analysis, the serum levels of CD44 in the studied patient were found to be much lower than those in the controls, and even than those in other patients we analyzed for the same marker. The prognostic value of CD44 serum levels in tumors is contradictory. Compared with healthy controls, there is no significant difference

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**Table 1. Serum levels of the examined proteins in the patient and in healthy individuals**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Patient</th>
<th>Controls</th>
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<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>37.24</td>
<td>3.9 **</td>
</tr>
<tr>
<td>CD44 (ng/ml)</td>
<td>29.2</td>
<td>120 *</td>
</tr>
<tr>
<td>YKL-40 (ng/ml)</td>
<td>112.8</td>
<td>84.19</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>42.96</td>
<td>7.8 **</td>
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* CD44 concentration in the controls (n = 20) is 120 ng/ml (range 61 to 314 ng/ml). As normal concentration is accepted 175.89 ± 38.88 ng/ml (according to the instructions of the manufacturer - Abcam). ** The TNF-α and IL-6 serum concentrations in the controls are below the lowest reference value.
in colorectal adenocarcinoma and squamous cell carcinoma.\textsuperscript{13,14} In patients with breast cancer the high concentrations of the soluble form of CD44 are associated with an increased tumor size and metastases in the lymph nodes. There are no similar studies for gliomas, and our initial results suggest that the lower CD44 serum concentration could be indicative of the extent of tumor progression.

The study on the cytokine profile of the patient demonstrated a significant change in the concentration of proinflammatory cytokines - TNF-\(\alpha\) and IL-6 compared to healthy individuals. Neoplastic cells have the ability to affect cytokine production. There is data that drastic alterations in the expression of cytokines and their receptors are most observable in more malignant gliomas.\textsuperscript{15} In accordance with the high serum levels, increased transcription rates in the tumor site were observed, with IL-6 being elevated ever 1000 times.

We believe that the combination of high expression levels of CD44, TNF-\(\alpha\), IL-6 and YKL-40 is indicative of subtyping the tumor as mesenchymal subtype of GBM and probably determines the rapid clinical course, and the lethal outcome.

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REFERENCES