Pediatric Brain Tumor Grading Based on CD56 Quantification

INTRODUCTION

Brain tumors constitute the most common solid neoplasms in children and the second most common cancer after leukemia. Astrocytomas are the most frequent, followed by medulloblastomas (MB), the most aggressive tumor, and ependymomas.[1,2] In a previous study, we performed cell cycle analysis by propidium iodine staining of CD56+ (gated) cells by flow cytometry and found that this method could accurately differentiate between neoplastic and nonneoplastic tissue, as well as high-grade from low-grade tumors.[3] An important observation during that study has been the great variability in the expression of CD56, a neural cell adhesion molecule (NCAM), among tumors. On the basis of this observation, we set out to quantify the expression of CD56 in pediatric
brain tumors and to investigate its correlation with clinicopathologic parameters.

**Materials and Methods**

Herein, we quantified CD56 expression in tissues obtained from 46 pediatric brain tumor cases. The study population has been as reported previously, and it included 17 MB, 12 anaplastic ependymomas, 9 atypical teratoid/rhabdoid tumors, 1 primitive neuroectodermal tumor, 1 glioblastoma, 2 low-grade astrocytomas, 1 atypical meningioma, and 2 atypical papillomas. We have used samples of brain tissue from three patients as controls, obtained during surgery for epilepsy (mean age, 2.8 years).

**CD56 Quantification Protocol and Immunohistochemical Analysis**

In all samples, quantitative assessment of the cell surface expression of CD56 was performed. Tumor samples (0.5–2 mm²) were minced (Medimachine System, BD Bioscience, San Jose, USA) for 1 min in phosphate-buffered saline (Ca²⁺ and Mg²⁺ free, with 0.5 mg/mL RNase) and a cell suspension was obtained. The cell suspension was then filtered (Consult No. 10; Medicons, BD Bioscience, San Jose, USA). In order to get a final concentration of 1.0 × 10⁶ cells/mL the cells were counted using an automated hematology analyzer. Then, 20 μL of CD56 FITC antibody, which recognizes an extracellular immunoglobulin-like domain common to three molecular weight forms—20, 140, and 180 kDa—of the NCAM, were added to 100 μL of the cell suspension and incubated for 15 min at room temperature (in the dark). Flow cytometric analysis was then performed. All the stained samples were analyzed within 1 h on a FACS Calibur (Becton-Dickinson) flow cytometer, using CellQuest software (Becton-Dickinson), for at least 10,000 cells/sample. From the histograms, the geometrical mean was calculated and used for quantification. Quantitative measurement of bound anti-CD56 antibodies was achieved using the flow cytometry-based QIFIKIT assay (Dako, Glostrup, Denmark) according to the manufacturer’s instructions. Immunohistochemical staining of Ki-67 and P-53 was performed using the Bond-Max Autostainer (Leica Microsystems, Buffalo Grove, Illinois) as reported previously.

**Statistical Analysis**

The CD56 molecules/cells between grade I/II, grade III, and grade IV tumors were compared using the two-sided, nonparametric Mann–Whitney U test. Correlation between Ki-67, P-53, and CD56 molecules/cell were analyzed using Spearman’s rho test. A two-sided P-value <0.05 was considered statistically significant.

**Results**

We found a significant negative correlation between Ki-67 index and CD56 molecules/cells (r = -0.493, P = 0.0008) and a significant negative correlation between P-53 index and CD56 molecules/cells (r = -0.368, P = 0.038) [Figure 1]. Using the Mann–Whitney U test, normal brain tissue could be differentiated from all tumor groups based on CD56 molecules/cell. Grade I/II tumors could be differentiated from grade III tumors (39.004 ± 4.584 vs. 24.721 ± 12.638 molecules/cell, P = 0.035) and grade III from grade IV tumors (24.721 ± 12.638 vs. 16.380 ± 783 molecules/cell, P = 0.037).

**Discussion**

This study showed that quantification of CD56 expression in tumor cells might be a novel indicator of pediatric brain tumor’s grade and aggressiveness and could be an adjunct to the standard histopathological evaluation of tumor samples. To the best of our knowledge, no previous study quantified CD56 expression in pediatric brain tumors by flow cytometry.

![Figure 1: Correlation between number of CD56 molecules/cell and Ki-67 (A) and P-53 (B) expression. (C) Relationship between histological grade and number of CD56 molecules/cell.](image-url)
The NCAM, also known as CD56, is involved in the intercellular junctions of neurons and glial cells and is also expressed on the surface of a subset of lymphocytes, the natural killer cells.[4] CD56 has three main isoforms (NCAM-120, NCAM-140, and NCAM-180).[5] Furthermore, several brain tumors, such as gliomas, MB, and ependymomas, express CD56.[6-8] As a cell surface glycoprotein, CD56 is involved in cell-to-cell adhesion. Furthermore, there is evidence that CD56 plays the additional role of a signaling receptor having an impact on cells’ several functions such as proliferation, differentiation, migration, and survival.[6-9] On the basis of immunohistochemical methods, Todaro et al.[4] reported NCAM immunopositivity, in tissues from brain tumors, being inversely correlated with the histological grade of malignancy. In glioblastoma, CD56 expression was correlated with tumor chemoresistance.[10] In a rat model, transfected glioma tumor cells with the 140-kDa isoform of the NCAM became less invasive and destructive than control cells.[11] Blaheta et al.[12] after studying 11 neuroblastoma cell lines, showed an inverse correlation between CD56 expression and cell adhesion, thus promoting tumor metastases. Contrary to immunohistochemistry, flow cytometry is able to provide objective and quantitative results, even on very small samples, within minutes. Furthermore, for the diagnosis, staging, classification, and monitoring response to the therapy of hematologic malignancies, it has been proven to be superior to immunohistochemistry.[13]

As quantification of CD56 in brain tumors can be performed within 20 min, this technique could be also used during intraoperative consultation. To date, frozen section analysis is the gold standard; however, this procedure has several shortcomings (for e.g., there might be freezing artifacts, morphology of cells might be changed by the freezing process, and sections might be of poor quality).[14,15] Thus, a technique that could provide additional information to pathologists would be of great value.

In this study, we found that tumor aggressiveness inversely correlated with CD56 expression. On the basis of the results so far, assessment of CD56 molecules/cell by flow cytometry in pediatric brain tumors provides important information for the assessment of tumor grade and aggressiveness. Thus, this method could be a novel adjunct to the standard histopathological evaluation of tumor samples. Further studies with larger number of cases are needed to verify our preliminary results and investigate possible prognostic significance.

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Conflicts of interest
There are no conflicts of interest.

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