Apoptosis phenomenon in the selected neoplasms of the glial origin

Szkudlarek M[1], Lebelt A[1], Klim B[1], Pawlak J[1], Lemancewicz D[1], Bogusłowicz W[1], Sulima D[1], Zimnoch L[2], Dzięcioł J[1]

[1]Department of Human Anatomy, [2]Department of Clinical Pathomorphology,
Medical University of Bialystok, Poland

Abstract

Gliomas cause a therapeutic problem because of their localization and asymptomatic growth in the initial phase. Neoplastic growth is connected with disturbance between proliferation and apoptosis. In the study, we assessed the Bcl-2 family proteins involved in apoptosis in gliomas. The study comprised 61 patients with gliomas and based on tissue material sent for the diagnosis. Apoptosis was assessed in various types of gliomas and was defined by the apoptotic index (IA) and shown immunohistochemically with using Bcl-2, Bax and Bcl-x antibodies. The data were statistically analyzed.. We found an increased percentage of the Bax (+) cells in less matured gliomas. A reverse dependence was revealed for Bcl-x. It was found that, probably in gliomas, the assessment of the Bcl-2 family proteins may serve only as an additional parameter for the evaluation of the disease course and the therapeutic success.

Key words: gliomas, apoptosis, Bcl-2, Bax, Bcl-x.

Introduction

The main principle of homeostasis in the multicellular organism is the maintaining of a quantitative balance between cell proliferation and death. Apoptosis, as a type of the physiological suicidal cell death, takes place during ontogenesis and cell replacement in constantly proliferating tissues. During apoptosis, morphological changes, observed within the cell, are the manifestation of intracellular biochemical reactions. Their key point is the cascade activation of the proteolytic enzymes known as caspases. Chain activation of caspases results in an origin of the active form of caspase 3, regarded as an enzyme responsible for proteolysis, observed during apoptosis [1]. The extrinsic mechanism, activating caspase 3, is connected with the stimulation of the outer cell membrane receptors of TNF family. In the intrinsic mechanism of apoptosis, the crucial role is played by mitochondria from which cytochrome c is released. In the cytoplasm, cytochrome c connects to Apaf-1 protein. Indirectly, this connection leads to an activation of caspase 3. The intrinsic apoptosis mechanism is controlled by the Bcl-2 family genes and proteins, comprising about twenty pro- and anti-apoptotic genes and proteins [1, 2]. Anti-apoptotic Bcl-2 protein co-operates in the regulation of apoptosis with pro-apoptotic Bax protein. Another homologue of the Bcl-2 family genes and proteins is the anti-apoptotic bcl-x gene. Its products are two antagonistic proteins: anti-apoptotic Bcl-X_L (consisting of 233 amino acids) and pro-apoptotic Bcl-Xs (consisting of 170 amino acids) [3]. In mitochondrial membranes, Bcl-2 and Bax proteins form ionic channels, regulating their permeability for cytochrome c and Apaf-1, either inhibiting (Bcl-2, Bcl-X_L) or facilitating (Bax, Bcl-Xs) their release. Thanks to the protein sections, called BH domains, Bcl-2 and Bax proteins form either Bcl-2/Bax heterodimers or homodimers. An overexpression of Bcl-2 leads to formation of anti-apoptotic Bcl-2/Bcl-2 homodimers. The overexpression of Bax results in forming pro-apoptotic Bax/Bax homodimers [4]. Disturbances of the balance between the pro- and anti-apoptotic factors of the Bcl-2 family proteins are observed in neoplasms. The overexpression of Bcl-2 and Bcl-X_L proteins prevents apoptosis and elimination of cells with abnormal genome. An insufficient expression of Bax protein influences the inhibition of apoptosis in cells of various neoplasms [5]. Disturbances between proliferative cell activity and apoptosis occur also in gliomas. The aim of the study was an assessment of the Bcl-2, Bax, Bcl-x proteins in gliomas of various histological types and malignancy grades.

ADDRESS FOR CORRESPONDENCE:
Magdalena Szkudlarek
Department of Human Anatomy
Medical University of Bialystok
Mickiewicza 2A; 15-230 Białystok, Poland
e-mail: anatomia@amb.edu.pl

Table 1. Apoptosis in various types of human gliomas.

Group	Number of cases	Bcl-2		Bax		Bcl-x	
		number of cases with positive reaction	percentage of cells with positive reaction (x ± SD)	number of cases with positive reaction	percentage of cells with positive reaction (x ± SD)	number of cases with positive reaction	percentage of cells with positive reaction (x ± SD)
Astrocytoma	14	11/14 (79%)	82.3±11.4	12/14 (86%)	39.5±8.3	9/14 (64%)	69.1±10.4
Anaplastic astrocytoma	18	12/18 (67%)	67.2±8.4	15/18 (83%)	47.8±10.2	10/18 (56%)	54.9±7.6
Glioblastoma multiforme	12	8/12 (67%)	70.3±8.7	10/12 (83%)	80.4±6.9	5/12 (42%)	65.4±9.8
Oligodendroglioma	10	9/10 (90%)	67.5±8.8	7/10 (70%)	38.9±5.3	8/10 (80%)	52.7±6.1
Anaplastic oligodendroglioma	7	5/7 (71%)	45.0±9.1	6/7 (86%)	56.0±9.8	4/7 (57%)	41.1±8.2

Figure 1. Oligodendroglioma. Different forms of neoplastic cells with positive immunohistochemical reaction for Bcl-2 antigen.

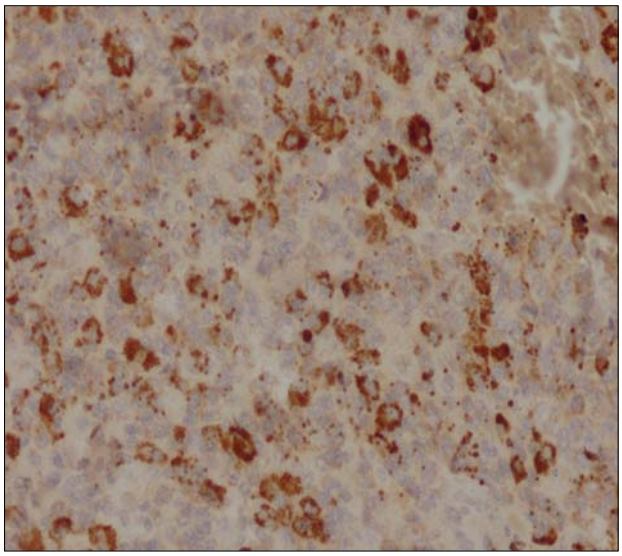
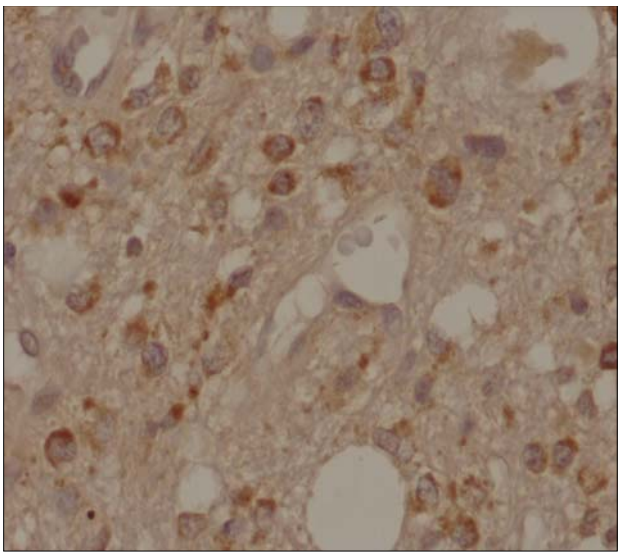


Figure 2. Glioblastoma multiforme. Different forms of neoplastic cells with positive immunohistochemical reaction for Bcl-x antigen.



Material and Methods

The tissue material, used in the study, was obtained during operations of brain tumours, performed at the Neurosurgery Clinic of the Medical University of Białystok. Next, it was routinely sent for diagnostic evaluation to the Department of Clinical Patomorphology of the Medical University of Białystok. The tissue material was then used for scientific research after determining the histopathological diagnosis of brain tumour. The study comprised 61 patients, aged from 18 to 72 years, with brain tumours. An assessment of the incidence of apoptosis was performed in gliomas of various histological types and malignancy grades: astrocytomas (14), anaplastic astrocytomas (18), glioblastomas multiforme (12), oligodendrogliomas (10) and anaplastic oligodendrogliomas (7). Apoptosis was immunohistochemically demonstrated with the use of monoclonal Bcl-2 and Bax antibodies, and the policlonal Bcl-x antibodies (DAKO), employing an LSAB+Kit detective set and DAB as a chromogen. For the evaluation of the cells with indicating antigens, a computer software for image analysis - MicroImage

Olympus - was applied. Apoptosis was defined by the apoptotic index (IA), determined as the percentage of neoplastic cells with a positive reaction for the antigen. For particular IAs, means and standard deviations (SDs) were determined. Statistical evaluation of the data was performed, using the Statistica Pl software.

Results

Table 1 summarizes detailed results of the analysis of the Bcl-2, Bax and Bcl-x antibodies in 61 gliomas included in the study. Figures 1 and 2 illustrate immunohistochemical reactions with the Bcl-2 and Bcl-x antibodies

Discussion and Conclusions

The incidence of the positive reaction for the Bcl-2 protein was 70% in the study of Rieger et al. [6]. Stirk et al. [7] revealed - both in initial and recurrent glioblastomas - a high percentage of the Bcl-2 positive reaction (92% and 97%, respectively) and, in both cases, 100% positive reaction for Bcl-x. Whereas the percentage of the Bax positive reaction amounted to 84% and 73%, respectively. Krajewski et al. [8] revealed higher Bcl-2, Bcl-x and Bax expressions (92%, 98 and 98%, respectively) in gliomas. Similarly to our study, the Bcl-2 expression in astrocytomas was higher than that in glioblastomas multiforme. They also obtained an increased percentage of Bcl-x positive cells in tumours of higher differentiation grade but the Bax positive reaction was observed in less differentiated tumours. Krishna et al. [9] indicated the highest percentage of Bcl-2 positive cells in low- grade gliomas and a lack of significant dependence between IA and malignancy grade, while such a dependence was present in the case of proliferative activity. Wharton et al. [10] came to similar conclusions in the case of oligodendrogliomas. However, Deininger et al. [11] considered that overexpression of the anti-apoptotic Bcl-2 family proteins was connected with the progression of oligodendrogliomas and anaplastic oligodendrogliomas. Despite difficulties with the interpretation of the results, many authors consider that an evaluation of apoptosis by determining the expression of the Bcl-2 family proteins may be a prognostic factor, serving for the assessment of chances for successful radio- and (or) chemotherapy [12], of the recurrence risk after surgical treatment [7] and also, in the case of glioblastoma multiforme, for the assessment of the neoplastic growth rate and time of the patients' survival [13].

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