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Expression of Bcl2 proto-oncogene in primary tumors of the central nervous system.

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Abstract

The present study was addressed to find out the expression of Bcl2 proto-oncogene in tumor tissues derived from 25 patients with primary central nervous system tumors. Brain parenchyma in 8 cases, with deeply located tumor, was also examined for Bcl2 expression which served as control. Both benign and malignant tumors (confirmed by histopathological examination) expressed Bcl2 gene product. Tumors exhibited 2-6 fold increase in Bcl2 expression as compared to the normal parenchyma adjacent to some of these tumors studied. However, no correlation was found between the histopathological types of tumor, glial fibrillary acidic protein positivity and degree of Bcl2 expression. Based on this study, we propose that the overexpression of Bcl2 gene product found in primary CNS tumors may be an important molecular event which is known to make the various types of tumor resistant to chemotherapy or radiotherapy.

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Full Text

:: Introduction

The cellular compartment in a tissue is maintained by a finely orchestrated balance between input (proliferation) and output (differentiation and apoptosis) processes. Abnormalities in any of these processes can lead to initiation of cancer. Apoptosis is a mechanism of active removal of superfluous cells that have served their purpose.[1],[2] This programmed cell death is an indispensable part of development of the nervous system. Survival of developing cells in the nervous system depends on survival signals from neurotrophic factor like nerve growth factor, by their target cell.[3],[4],[5],[6] Bcl2 gene family is composed of a group of related genes that either promote or prevent apoptosis.[7],[8] It includes genes such as Bcl2 which is ntiapoptotic, and BAX which is proapoptotic. The ratio of expression of pro and anti-apoptotic genes determines whether a cell lives or dies after an insult. Excess of BAX leads to death and excess of Bcl2 leads to life.[9] Bcl2 proto-oncogene is located on chromosome 18. It functions as an apoptosis suppressor and can promote neoplastic transformation.[5] Molecular analysis has detected Bcl2, in adult and embryonic neuronal and ependymal cells of the central nervous system

(CNS).[10],[11] Several authors have examined the relationship between expression of Bcl2 and prognosis in medulloblastoma and neuro-blastoma.[12],[13] Oka et al reported expression of Bcl2 oncogene protein in patients with embryonal tumors of CNS.[11]

The present study was undertaken to study Bcl2 expression in primary CNS tumors and its correlation with tumor type.

:: Material and methods

Tumor tissue was taken from 25 patients operated for intracranial tumors in the department of neurosurgery, Postgraduate Institute of Medical Education and Research, Chandigarh. Histopathological examination was performed in all 25 cases. Small portion of each tissue was collected in vial containing glutaraldehyde solution (pH-7.4) and immediately transferred to the department of pathology, where routine Hematoxylin and Eosin staining was done. Glial fibrillary acidic protein (GFAP) staining was also done in 14 cases. Brain parenchyma was taken from 8 cases with deeply located tumors, which required corticectomy to approach the tumor for excision. They served as controls.

Brain tumor tissue and brain parenchyma samples were transported to experimental medicine department in an ice box (0oC) for Bcl2 expression determination, using enzyme linked immunosorbent (ELISA) technique described by Forrest.[8] Antibodies specific for Bcl2 were obtained from Santa Cruz Biotechnology (USA). Tissue was homogenized in carbonate-bicarbonate buffer (pH 9.6). 20 痢 protein from each sample was added to the wells of the microtiter plate. The plate was kept at 4oC overnight. After overnight incubation the micro titer plate was washed 4-5 times with phosphate buffer saline (PBS) (pH 7.4) enriched with 0.5% tween-20. After thorough washing, 100 痞 of 2% bovine serum albumin was added in each well and the plate was kept at 37oC for 1 hour. At the end of this incubation period the plate was washed twice with PBS containing tween-20. 100 痞 of diluted antibody against Bcl2 was added in horizontal wells and plate was kept at 37oC for two hours. After this incubation period, the plate was washed at least 5 times by PBS containing tween-20 thoroughly. 100 痞 of horse radish peroxide conjugated lgG, diluted to 1:1000 with PBS (a concentration that gave optimal sensitivity in trial studies) was added. The plate was again incubated at 37oC for 2 hours containing secondary antibody conjugate. At the end of this incubation the plate was washed 5 times. 100 痞 of orthophenylenediamine (OPD) (0.05%) dissolved in citrate buffer (pH 5.0) and H2O2 (1.5 痞/ml) was added into each well and kept at room temperature in dark for 30 minutes. Exactly after 30 minutes the reaction was stopped by adding 1N H2SO4. The intensity of color developed was read with ELISA reader at 492 nm and the corresponding value for Bcl2 expression in each well was recorded.[14]

:: Results

Tumors were classified into benign and malignant groups. Details of these tumors are shown in [Table I]. In glial tumors Bcl2 expression ranged from the lowest 0.5 (in a anaplastic astrocytoma) to highest 1.80 (glioblastoma multiforme) [Table I]. In our study youngest patient was 7 years female with pilocystic astrocytoma (case no 1 [Table I] with a Bcl2 expression of 0.52. Eldest patient was 64 years male with glioblastoma multiforme and Bcl2 expression was 1.80 (case no. 14). Sixty year male patient with anaplastic astrocytoma had Bcl2 expression of 0.50 (case no. 6). This suggests that age possibly does not affect Bcl2 expression. Bcl2 expression is not affected by sex and was expressed equally in both the sexes. Bcl2 expression ranged from 0.42 to 2.40 in meningiomas and 0.85 to 0.997 in schwannomas. Bcl2 expression was from 0.85 to 1.40 in pituitary adenoma and 0.89 in ependymoma [Table I].

The Bcl2 expression was 2 to 6 fold higher in the tumor tissue as compared to normal brain tissue. Both the benign and malignant tumors expressed the Bcl2 gene. There was no correlation between the histopathological

type of tumor, glial fibrillary acidic protein (GFAP) positivity and the degree of Bcl2 expression. These results suggest that all CNS tumors, whether benign or malignant, with proliferative potential can express Bcl2 gene.

:: Discussion

In normal tissue development, a balance between cell proliferation and cell death is maintained. Certain cells have a built-in programme for cellular death. If this programme is lost or altered, cells may continue to grow indefinitely. Bcl2 is a proto-oncogene which enhances cell survival by blocking apoptosis or programmed cell death. It delays the rate of cell death that occurs upon the removal of neurotrophic factors. Bcl2 probably allows more time for secondary genetic damage to be inflicted upon a particular cell,[13] which deregulates proliferation. The inhibition of apoptosis can promote neoplastic transformation.[5]

Over expression of Bcl2 has been reported to induce a plethora of apoptosis associated changes, such as alterations of cellular redox state (depletion of glutathione and generation of oxygen free radicals), and plasma membrane changes (loss of phospholipid bilayer asymmetry, changes in subcellular ions distribution (Ca+,H+), activation of caspases and disruption of mitochondrial transmembrane potentially.[15] Bcl2 might act as an antioxidant, regulator of intra-cellular ion fluxes,[16] protease inhibitor and mitochondriotropic agent.[17]

Bcl2 protein resides in the nuclear envelope, in the outer mitochondrial membrane and endoplasmic reticulum.[6] The endoplasmic reticulum and mitochondria are major intracellular storage sites for calcium ions. Calcium plays an important role in apoptosis, primarily through activation of endonucleases. Bcl2 overexpression appeared to inhibit calcium dependent endonuclease activity and then resulted in DNA fragmentation and apoptosis.[18] Bcl2 also blocks apoptosis in thymocytes caused by calcium ionophores. Zhang et al however, reported that over production of Bcl2 did not prevent the rise in intracellular calcium levels. Because Bcl2 blocks apoptosis in these cells, it must do so by blocking signal downstream from cytosolic rise in calcium. Recent work on Bcl2 family indicates that these polypeptides can also prevent radiation induced cell death, regardless of p53 status.[5] Over expression of Bcl2 did not augment tumor progression in p53 null mice.[5],[19] It indicates that p53 may act, at least in part, via down regulating Bcl2 and upregulating BAX expression.

Expression of Bcl2 proto-oncogene was first seen in B-cell Hodgkin's lymphoma.[20] Direct evidence that Bcl2 directly contributes in oncogenesis was originally shown by McDonell et al.[5] Elevated levels of Bcl2 are seen in almost all patient with chronic lymphocytic leukemia. Bcl2 expression has been reported in prostatic carcinoma and small cell lung carcinoma.[5],[21] In node negative breast cancer, expression of Bcl2 gene correlates with estrogen receptor positivity and also with favourable prognosis.[2]

Bcl2 expression has been reported to drop in metastatic melanoma.[16] Reed et al found enhanced expression of Bcl2 in cases of neuroblastoma and other neural crest derived tumors and some neuroepitheliomas, neurofibroma and melanoma.[6] Expression of this proto-oncogene correlates with differentiation characteristics of the tumor cell lines.[22] Reeds reported that Bcl2 gene was not expressed in medulloblastoma in his immunoblotting study of 39 human tumor cell lines of neural origin. Reed et al surveyed a variety of tumor cell lines for the presence of Bcl2 protein and found very high levels in three of the 9 neuroblastoma cell lines examined.[6] These cell lines were composed primarily of neuron like cells, suggesting that expression of this protooncogene correlates with the differentiation characteristics of these tumor cell lines. Bcl2 expression was absent from most medulloblastomas but was detected at low levels in some glioblastoma cell lines.

We could come across only one report where Bcl2 expression was studied in brain tumors.[11] These authors examined the incidence and significance of Bcl2 expression in embryonal tumors of the central nervous system, which have the possibility of neuronal differentiation. Mature cells like ganglionic cells strongly expressed Bcl2 gene. They demonstrated that tumors positive for Bcl2 belonged to early differentiated and neuronal types and most of the Bcl2 negative tumors belonged to the undifferentiated type. It was suggested that the tumor cells begin expressing Bcl2 along with their neuronal differentiation from premature cells. In the present study, Bcl2 expression was studied in a wide histopathological spectrum of brain tumors and also in the adjacent brain tissue in some cases. The detection of Bcl2 expression in both benign and malignant tumors suggests that Bcl2 expression is not linked directly only to neuronal differentiation, but all tumors with proliferative potential can

express this protooncogene. The Bcl2 expression was significantly higher in tumor tissue as compared to normal brain tissue suggested that the tumor cells are less susceptible to apoptosis as compared to cells of normal brain tissue. We did not find any correlation between the degree of Bcl2 expression and the grade of malignancy. There was correlation with Bcl2 expression and glial fibrillary acidic protein (GFAP) positivity.

Involvement of Bcl2 in the regulation of apoptosis has led to the hypothesis that dysregulation of apoptosis may be responsible for chemoresistance. Bc12 related proteins constitute prime target for therapies directed at altering the levels of expression of these genes and may mediate and/or potentiate commonly used chemotherapeutic agents. The gliomas are sensitive to the manipulations of the levels of Bcl2 gene expression. [23]

Inactivation of Bcl2 is achieved by chemotherapeutic agents acting on microtubulies such as taxal and colchicine, which provoke Bcl2 inactivation via hypophosphorylation during G1-M phase. The importance of apoptosis in mediating cell death from potential antitumoral therapy in glioma was noted by Weller et al[24] who showed that over expression of Bcl2 prevented Fas/APO/antibody mediated apoptosis. The manipulation of P53 expression may also serve to increase BAX expression because P53 is transcriptional activator of BAX.[11] However, the association between the level of expression of member of Bcl2 gene family and response to therapy or outcome in gliomas remains to be determined. Manipulation of the relative levels of Bcl2 family members may be a useful strategy for increasing the sensitivity of glioma to conventional ionizing radiations and chemotherapeutic agents. [25] In this context, the results reported here assume importance because Bcl2 gene expression was significantly higher in both benign and malignant tumors as compared to that in normal tissue adjacent to these tumors.

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