Advances in Medical Sciences 61 (2016) 237–243

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original Research Article

Survivin, caspase-3 and MIB-1 expression in astrocytic tumors of various grades

Agnieszka Lebelt [a], Robert Rutkowski [e], Waldemar Och [d], Kamil Jaczun [b], Dorota Dziemiańczyk-Pakieła [f], Robert Milewski [c], Zenon Mariak [e], Joanna Reszeć [b,*]

[a] Department of Anatomy, Medical University of Bialystok, Bialystok, Poland
[b] Department of Medical Pathomorphology, Medical University of Bialystok, Bialystok, Poland
[c] Department of Statistics and Medical Informatics, Medical University of Bialystok, Bialystok, Poland
[d] Neurosurgery Department, Regional Hospital, Olsztyn, Poland
[e] Department of Neurosurgery, Medical University of Bialystok, Bialystok, Poland
[f] Department of Maxillofacial and Plastic Surgery, Medical University of Bialystok, Bialystok, Poland

ARTICLE INFO

Article history:
Received 1 April 2015
Accepted 2 February 2016
Available online 12 February 2016

Keywords:
Caspase-3
Survivin
MIB-1
Gliomas

ABSTRACT

Purpose: Gliomas are the most common primary brain tumors. The etiology is still unclear and the progression from low to high-grade gliomas is frequent. The molecular mechanisms are quite established, however the heterogeneity of glioblastomas force the scientist to look for the new therapeutic targets. The aim of the study was to evaluate the caspase-3 and survivin expression in correlation with MIB-1 expression in gliomas of various grade to assess the apoptosis in gliomas and to determinate new possible targets for the future therapy.

Material and methods: We identified 131 patients with a histopathological diagnosis of astrocytic tumors (diffuse astrocytoma, anaplastic astrocytoma and glioblastoma). The evaluation of caspase-3, survivin and MIB-1 expression was done using immunohistochemical methods.

Results: Caspase-3 and survivin expression was observed both in low- and high-grade astrocytomas. The differences in expression were the most evident in glioblastoma group. All primary glioblastomas (31 cases) expressed caspase-3. In secondary glioblastoma group only 17 out of 30 specimens were positive for caspase-3. Survivin expression was observed in 80.6% primary glioblastomas and in all examined secondary glioblastomas and the staining was strong and diffuse in all cases. MIB-1 expression was low in diffuse astrocytomas (DA) and ranged between 1 and 5%. In anaplastic astrocytoma group it was ranged between 5 and 10% and the highest percentage of the positive cells was observed in glioblastoma cases and ranged from 10% even to 30%. The most evident MIB-1 expression was observed in the cells surrounding the pathological blood vessels and necrosis.

Conclusions: The high incidence of survivin and caspase-3 expression in diffuse and anaplastic astrocytoma cases may suggest, that the regulation between pro- and antiapoptotic proteins may play an important role in tumor growth and progression. The overexpression of survivin and MIB-1 expression in glioblastoma cases also may confirm the theory about the important role of anti-apoptotic and proliferation processes in glioblastoma progression and as such may be potential therapeutic targets.

1. Introduction

Glial tumors are the most common primary brain tumors. The WHO classification includes low grade gliomas (including pilocytic and diffuse astrocytomas (DA)) and high grade gliomas (anaplastic astrocytoma (AA) and glioblastoma (GB)). Glioblastoma is the most malignant type of primary brain tumor, with a worse prognosis and high mortality. The survival ranges to 50 weeks after the diagnosis. This is due to the heterogeneity of glioblastoma cells and the yet poorly understood mechanisms of the neoplastic cells infiltration into the brain parenchyma. Genetic alterations of gliomas include p53 mutation, EGFR amplification, PTEN mutation, PDGFR overexpression, Loss of Heterozygosity (LOH) and many others. Glioblastoma may arise the novo from stem cells (primary

* Corresponding author at: Department of Medical Pathomorphology, Medical University of Bialystok, Waszyngtona 13, 15-269 Bialystok, Poland.
Tel.: +48 85 748 5945; fax: +48 85 748 5990.
E-mail address: joannareszec@gmail.com (J. Reszeć).

http://dx.doi.org/10.1016/j.advms.2016.02.001
1896-1126/

glioblastomas) or from the more differentiated glial tumors (DA, AA). The biology, prognosis and treatment often differ between these two groups, but the histology features are very similar. Therefore, scientists try to establish factors which allow them to distinguish these two lesions in routine pathological practice [1–3].

Apoptosis is a process of natural cell death, observed both in physiological and pathological circumstances. It is regulated by two pathways: intrinsic and extrinsic, leading to the activation of anti- or proapoptotic signals. Proapoptotic proteins include Bax and Bak, as well as a cascade of enzymes called caspases (including caspase-3). Bcl-2, Bcl-Xl and survivin belong to a group of antiapoptotic proteins which promote cell survival. In many types of malignant neoplasms apoptosis is inhibited by the activation of antiapoptotic proteins, as is the case in colorectal and breast cancers, which may lead to tumor growth and progression [4]. Functionally, survivin plays regulatory functions on cell division and inhibition of apoptosis, induces angiogenesis and plays a pivotal role in cancer progression. This protein is highly expressed in the G2/M phase of the cell cycle and directly inhibits caspase-3 and caspase-7 activity. It is also expressed during embryogenesis and fetal development, but is absent in most normal adult differentiated cells other than the thymus, placenta, endometrium, bone marrow stem cells and basal colonic epithelial cells [5–7]. However, survivin seems to be expressed extensively in common human cancers, such as lung, breast, pancreatic, colon, gastric, prostate and bladder carcinomas, hematologic malignancies, brain tumors and others [4]. Survivin expression is associated with the regulation of mitosis in cancer cells, where it counteracts a default induction of apoptosis during mitosis. When overexpressed in cancer cells, it also permits an aberrant proliferation through mitosis. In the central nervous system survivin is expressed in normal ependymal cells growth and neural stem cell differentiation [8–10].

Also several reports have shown mRNA and protein expression levels of survivin to be markedly increased in gliomas [11,12]. Survivin overexpression is also correlated with the pathologic grade and poor prognosis for the malignancy. An increase in the level of survivin expression was found to predict reduced survival times for all glioma patients, and markedly so for glioblastoma patients [5,11,13]. In cancer cells, elevated survivin is commonly associated with enhanced proliferative index [14–16], reduced levels of apoptosis, resistance to chemotherapy, and increased rate of tumor recurrence. Retrospective studies have evaluated the correlation between survivin, disease variables, and clinical outcomes. The mechanisms whereby survivin regulates cancer cell proliferation is poorly understood; however, survivin can regulate apoptosis, cell cycle, or cytokinesis through functional or physical interactions with heat shock protein 90, Smac/Diablo, X-linked inhibitor of apoptosis protein, p21WAF1/Cip1, Cdk4, Cdc2, retinoblastoma/E2F, nuclear factor κB, signal transducers and activators of transcription-3, or p53 [14,17]. Survivin regulates the activation apoptosis in human cancer cells by binding to activated caspase-3, inhibition of Bax and Fas-induced apoptosis [18], therefore may regulate cell survival or cell death. Survivin interferes with activation of caspase-3 and caspase-7, other apoptotic proteins like Smac/DIABLO and proteins required for cell division [17,19].

Caspase-3 is an apoptosis executor caspase and when its activated leads to the programmed cell death. Caspases are synthesized as inactive proteins (pro-caspases) and are activated by many signals. Release of mitochondrial cytochrome c as a consequence of opening of the Bax/Bak channels results in the assembly of the Apaf-1/caspase-9, apoptosome and activation of caspase-9 within this complex and then it activates the procaspase-3 to the active caspase-3 [1,20]. Based on their proapoptotic functions, the caspases have been divided into two groups: initiators and effectors (including caspase-3). The effector caspases are able to directly degrade multiple substances including the structural and regulatory proteins in the nucleus, cytoplasm and cytoskeleton of the cell [19].

Caspases also cleave and activate some protein kinases including MEKK-1, PAK2 in late events in apoptosis; a number of protein kinases (Akt-1 and Raf-1) which are crucial for cell division and survival and cleave the antiapoptotic proteins Bcl-2 and Bcl-xl [19,20]. Many recent data confirm deregulation of apoptosis in cancer cells, resulting in cancer development and progression. Inactivation of proapoptotic and/or activation of antiapoptotic components of cell death machinery have been found in many type of cancers (including breast cancer, prostate, cervical carcinoma) [19]. Several pieces of evidence suggest that loss of caspase-3 correlates with resistance to drug-induced apoptosis in a variety of human cancer cell lines, including breast cancer cells and some data show, that expression of caspase-3 and -7 does not correlate with the extent of apoptosis in primary breast carcinomas [17,21].

The aim of the present study was to evaluate the expression of caspase-3 and survivin in correlation with MIB-1 expression in astrocytic tumors of various grades to determinate the role of apoptotic proteins in the tumor growth and progression.

2. Material and methods

We identified 131 patients with a histopathological diagnosis of astrocytic tumors obtained in the Department of Neurosurgery at the Medical University of Bialystok in the years 2000–2013. The study was approved by the Bioethical Committee at the Medical University of Bialystok, Poland (No R-I-002/202/2014) and was performed in accordance with ethical standards laid down in the 1964 Declaration of Helsinki. We retrospectively waived the 10% buffered formalin-fixed paraffin embedded sections. The evaluation of caspase-3 and survivin expression was done using immunohistochemical methods. Following deparaffinison and rehydration, epitope retrieval was carried out in the EnVision Flex Target Retrieval Solution (DAKO), in high pH. Endogenous peroxidases were blocked by incubating the sections in methanol and 3% hydrogen peroxide for 20 min. Next, slides were incubated with polyclonal antibody against the active form of caspase-3 protein (Anti-Caspase-3 antibody [E87]) (ab32351) in 1:75 dilution for 30 min at room temperature and against survivin protein (DAKO Survivin Clone 12C4) in 1:100 dilution for 30 min at room temperature. Visualisation reagent EnVision Flex (DAKO) was applied for 30 min and followed by DAB solution for 10 min. To determinate the mitotic activity of the glial tumors we used the monoclonal mouse anti-human antibody against Ki-67 protein (clone MIB1 DAKO Code GA626). The Ki-67 antigen is a large nuclear protein (345, 395 kDa) preferentially expressed during all active phases of the cell cycle (G1, S, G2 and M-phases), but absent in resting cells (G0-phase). The slides were than counterstained with hematoxylin and examined under a light microscope. An immunohistochemical evaluation of each protein expression was performed by a pathologist. The intensity of immunostaining was evaluated in random 10 fields under 20× magnification. The staining score was obtained as proportion of imunopositive cells in each tumor. The expression of the studied proteins was scored as (−) for negative expression or expression in less than 10% of cells, (+) for positive expression in less than 50% of cells and (++) for positive expression in more than 50% of cells [22]. MIB-1 expression was evaluated as the percentage of the positive cells within the tumor.

Appropriate positive and negative controls were performed. The negative control was done using a nonimmunized IgG replacing the primary antibody. We used lymph node specimens