BRAIN CANCER: A DIFFERENT APPROACH USING NUCLEOLAR ORGANIZING REGIONS

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Summary

Argyrophilic staining of nucleolar organizing regions (NOR) is a representation of the ribosomal RNA transcription, giving an image on cell proliferation. The silver-stained nucleolar organizing regions (AgNOR) may be useful to reflect the degree of malignancy.

The present study aimed to assess the correlation between the features of nucleolar organizing regions (AgNOR) in tumour cells and the degree of cell differentiation and cell proliferation. Eighteen gliomas diagnosed at the Emergency County Hospital in Cluj-Napoca, Neurosurgery Clinic between 2007 - 2009 were studied. They were graded according to the World Health Organization grading system. The fragments were silver stained using the Crocker and Nar technique. For every tumour piece one hundred consecutive nuclei were studied, considering the morphology and the average number of AgNOR/case. The data was analysed using T-test. Data having p-value < 0.05 was considered statistically relevant. As the degree of malignancy increased, the number of visible AgNORs was significantly elevated, their location became peripheral and their shape was inconstant in contrast with low-grade tumours. Differences (p<0.05) have been seen between the low and high-grade astrocytomas. Significant dissimilarity has been noticed between the grade II astrocytoma and the anaplastic astrocytoma (p<0.05) and also between the grade III astrocytoma and glioblastoma (p<0.05).

The obtained numbers of AgNOR/nucleus/case in the 18 studied gliomas corresponds with other studies cited in the literature, showing a positive correlation with the histological malignancy degree. Statistical analysis allows a clear distinction between the two main categories: high-differentiated astrocytomas (grades I si II) and the low-differentiated ones (grades III and IV). The data obtained by studying AgNOR can provide useful information in order to appreciate the malignancy degree and also a different approach to the prognosis and management of patients with brain cancer.

Keywords: astrocytoma, nucleolar organising region, glioblastoma

Introduction

In the last decade, the field of neuro-oncology has evolved, by finding new methods in the area of epidemiology, molecular biology, histological techniques and neurosurgery protocols, thus increasing the quality of life and brain functions for severely ill patients.

Even though they are not very frequent, the tumours that appear in the central nervous system are extremely important, given their complexity (from a histological and morphoathological point of view). Gliomas, being derived from the neuroepithelial tissue and its precursors, are the most frequently found primary tumours of the central nervous system (CNS), having an average annual incidence of 4,9:100 000 (Minesh, 2011).

Astrocytomas are the most frequently found tumours of the CNS, astrocytes being the origin of approximately 60% of all glial cell
tumours. An important characteristic of these tumours is the fact that the glioblastoma, the major type, has a rather unfortunate prognosis, having a survival rate after 5 years of only 3%.

According to the World Health Organization (WHO)/2007, astrocytomas are classified by taking into consideration the degree of malignancy: Low-grade astrocytoma (grade I and II) and high-grade astrocytoma (grade III and IV-multiform glioblastoma). Parallel with the increase of the degree of malignancy, there is a rise in the number of undifferentiated cells, having nuclear atypia, hypercromasia and more cell mitosis. In the multiform glioblastoma newly-formed blood vessels can be easily observed, along with areas where necrosis and haemorrhage are obvious.

In the nuclei, the nucleolar organizing regions are chromosomal regions which, at the end of the telophase, will contribute for the organizing of the nucleoli. The nucleoli are the cellular structures that coordinate the functioning of the ribosomal protein synthesis, and thus, their increase in number can be used as a marker of cellular proliferation. Because of the argyrophilic properties of the non-histonic proteins associated with the nucleolar organising regions, using a silver-staining technique we can easily observe the regions as little black spots, which will be further called silver-stained nucleolar organizing regions (AgNOR).

Many recent studies have suggested that there is a connection between the number and the shape of the AgNOR/nuclei, cell differentiation and cellular proliferative activity (Pintea et al, 2002). This finding might be useful in the process of diagnosing and grading brain lesions (Mao - 1995). Two types of analysis of AgNOR can be used in this process: the direct counting of the black dots (AgNOR), and the morphometric analysis (which uses an automatic analysis for the images, with the measuring of the silver-stained area in each nucleus). (Adams, 2011). Generally, the tumours with a high number of AgNOR are low-differentiated, with a high metabolism rate and a high proliferation rate, all these indicating a malignant phenotype.

Because there are some issues related to observing the differences between reactive gliosis and astrocytomas, and also because of the difficulty of the grading process for brain lesions, the study of AgNOR might be a step forward regarding the diagnosis and prognosis of gliomas.

Material and methods

Eighteen glial tumours were used in this study, all of them diagnosed at the Emergency County Hospital in Cluj-Napoca, Neurosurgery Clinic between 2007 – 2009. They were graded according to the World Health Organization/2007 grading system: 3 grade I astrocytomas, 3 grade II astrocytomas, 4 grade III astrocytomas and 8 multiform glioblastomas. After the formaldehyde fixation, the tissue samples were embedded in Disterine Plasticiser Xylene (DPX) medium. AgNOR were counted in tumour cells, being visible as black dots on a pale yellow background.

Using optical microscopy 100 nuclei/case were studied by directly counting the AgNOR. The following parameters were taken into consideration: the average number of AgNOR/case, the size and distribution of AgNOR within the nuclei.

The data was processed using the Data Analysis Toolpack for Microsoft
Excel 2007. T-test was used, and the data having a p-value lower than 0.05 was considered as being statistically relevant.

Results and Discussion

From a histological point of view, AgNOR can be described as well-differentiated dots, being highly argyrophilic compared with cell’s nucleolus. In low-grade astrocytomas, AgNOR are scarce, usually uniform and located in the centre of the nucleolus. As the degree of malignancy increases, the number of AgNOR also increases, but there is a shift in their morphology: now they can be found in peripheral areas, they are asymmetric and they have an irregular shape. (Fig. 3 and 4).

Unusual morphological aspects can be seen though both low-grade and anaplastic tumours.

Statistically significant differences can be observed between the low-grade and high-grade astrocytomas. Statistically significant differences were also found after comparing the average numbers of AgNOR between grade II and the anaplastic astrocytoma (p<0.05) and grade III and glioblastoma (p<0.05). (Table 1)

Insignificant differences were observed when grade I astrocytoma and grade II astrocytoma where compared, and the same thing was proven comparing grade I astrocytoma and anaplastic astrocytoma. The average number of AgNOR/cases was analysed, and some
overlays between different types of astrocytomas was observed. (Table 2) Thus we have an argument to sustain the fact that, although there is an important correlation between the characteristics of nucleoli and the degree of malignancy in brain tumours, the study of AgNOR cannot be used as a unique method for diagnosing “AgNOR” parameter has now become a trustful tool in the process of defining the clinical evolution for an oncologic disease.

The current knowledge in the field of neuro-oncology supports the theory regarding the fact that AgNOR, along with the analysis MIB-1 antibody (antibody for the Ki-67 antigen) are considered the most efficient and simple methods for prognostic purposes. (Quiñones-Hinojosa et al, 2005).

<table>
<thead>
<tr>
<th>Table 1. Comparison of average numbers of AgNOR</th>
<th>Table 2. Analysis of the average number</th>
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<tbody>
<tr>
<td>First grade Astroctoma: 2.5</td>
<td>First grade Astroctoma: 2.5</td>
</tr>
<tr>
<td>Second grade Astroctoma: 2.0</td>
<td>Second grade Astroctoma: 2.0</td>
</tr>
<tr>
<td>Third grade Astroctoma: 1.8</td>
<td>Third grade Astroctoma: 1.8</td>
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<tr>
<td>Glioblastoma: 3.0</td>
<td>Glioblastoma: 3.0</td>
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Many studies in medical literature have proven that evaluating the quantitative distribution of AgNOR while the studied cell goes through interphase has been used in oncology both for diagnosis and prognosis purposes. (Derenzini, 2000). The
Conclusions

In astrocytomas, the study of AgNOR revealed a direct proportionality between the number and morphology of AgNOR and the degree of malignancy. Thus, it has been proven that there is a correlation between the diagnosis of astrocytomas based on HE slides and the analysis of AgNOR. Because of the overlays that were found when analysing the average number of AgNOR/cases between different types of astrocytomas, this method cannot be taken into consideration as a unique parameter for the diagnosis and prognosis of brain neoplasia.

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