The Problems of Astroglial Brain Tumours

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Summary

Astroglial tumours are a group of tumours derived from the astroglial cells. They belong to a group of most frequently diagnosed tumours of the nervous system. This group of tumours is variable in many aspects. The tumours differ not only morphologically, but also with their growth potential, the extent of invasive growth, their localization within the CNS and the clinical course of the disease due to their progressive features. The variability of these neoplasms is given by the sequence and type of genetic alteration in the course of the neoplastic transformation. According to the currently used classification, it is possible to differentiate between benign I.-II. forms (low grade glioma-I. pilocytic astrocytoma, II. diffuse astrocytoma) and malignant form III.-IV. (high grade glioma- III. anaplastic astrocytoma, IV. glioblastoma) of astroglial tumours. The number of vasoproliferative and necrotic changes, together with the number of cell divisions are the key factors for distinction between benign ad malignant tumours from the histological point of view. From the molecular-genetic point of view, it is the types and numbers of cumulating genetic alterations which are of the greatest importance. One type of significant alterations occurs in the p53, PTEN and EGFR genes.

Keywords: astrocytoma, glioblastoma, imunohistochemistry, molecular-biology methods, mutations, tumorsuppressor genes, amplifications, p53 gene, PTEN gene, EGFR amplification

1. Introduction

Astroglial tumours are one of the most frequent primary brain tumours of the intracranial area, responsible for, according to the literature, more than 60 % of primary brain tumours. They are most often classified on the basis of internationally recognized criteria defined by the World Health Organisation (WHO). The tumours may be divided into several types due to the variability of the histological findings and variable biological behaviour. The astroglial tumours may be localised anywhere within the CNS, they most frequently appear in the brain hemispheres. The growth characteristic of these tumours is mostly infiltrative, and they pose a danger mostly due to their tendency to move towards more malignant forms. Tumours of this group are more often diagnosed in adult patients, some types have been found to have a higher incidence in males [1], [2].

The degree of malignancy and the tumour differentiation may be defined according to a grading system, which is a significant prognostic indicator and is important for the definition of further treatment procedures. The criteria important for the grading determination include proliferation activity of the cells and the degree of cellular differentiation (in the histological picture we follow the maturity of the tumour tissue the degree of differentiation), the presence of cellular and nucleic atypias, angiogenesis and the presence of necrosis [1], [2]. According to their biological features, the astroglial tumours may be differentiated into relatively benign and malignant forms (Tab.1.).

Tab. 1. Grading of astroglial tumours.

Grade	Name of the brain tumour	Tumour characteristics
I	Pilocytic astrocytoma	relatively benign
II	Diffuse (Low-grade) astrocytoma	relatively benign (with a tendency to malignant progression)
III	Anaplastic astrocytoma	malignant
IV	Glioblastoma	highly malignant

To determine the features of the tumour from the histopathology point of view, the following characteristics are of vital importance: the number of vasoproliferative and necrotic changes, the degree of cellular differentiation, the number of cell divisions and the presence of cellular and nucleic atypias. From the molecular-genetic point of view, it is important to look for the presence of characteristic genetic alterations and their number. Some of the significant changes occur in p53, PTEN, EGFR, Rb and p16 genes.

1.1 The present classification of astroglial tumours

Pilocytic astrocytoma is a Grade I tumour according to the WHO classification. This type of tumour is most frequently observed in children and young adults in various localisations within the CNS, it frequently affects the area of cerebellum and the optic nerve. It is a circumscribed, slowly-growing tumour of rather benign characteristics, with favourable prognosis. The histological picture shows biphasic tumour pattern with areas of densely concentrated GFAP (glial fibrillary acidic protein) positive bipolar astrocytes and eosinophilic Rosenthal threads. Further present are sparsely cellular areas, spumous or even microcystic with GFAP weakly positive protoplasmatic astrocytes without fibrils. Regressive changes in the form of hyalinised vessels and calcification are also frequently observed. Nucleic pleomorphias may be also present, however these are less frequent and less prognostically significant. Necrotic changes, angiogenesis or mitosis are not usually observed in pilocytic tumours [1], [2].

The second tumour type, according to the grading, is a diffuse or low-grade astrocytoma. This tumour is usually well-differentiated, slowly growing, however with the features of infiltrative growth and tendencies of malignant progression. It affects especially younger adults. The mitotic activity of the diffuse astrocytoma is low, when compared to higher grades.

Morphologically observed are distinct tumour populations formed by astrocytary cells, which are difficult to differentiate from non-tumour cells. Nevertheless, it is possible to differentiate among three types of low-grade astrocytomas [1], [2].

The most frequent one is fibrillary astrocytoma, where we find nucleic atypias in cells with a low mitotic activity. The typical signs are a formation of microcysts with muccoid contents and the presence of lymphocytary infiltrate in the proximity of vessels. On immunohistochemical demonstration, the cells express GFAP (glial fibrillary acidic protein), S100 and vimentin. The Ki67 proliferation marker shows a low expression.

The second type is gemistocytary astrocytoma. The tumorous cells are usually larger, with plentiful eosinophilic cytoplasm and the presence of perivasal lymphatic infiltrate. The nuclei with small nucleoli are located eccentrically. Immunophenotype and the proliferation activity are the same as with the previous type.

The last type of a low-grade astrocytoma, according to the histological picture, is a less frequently observed protoplasmic astrocytoma. The population of tumour cells is sparse, the cells are small with oval-shaped nuclei and a low content of glial filaments. Formation of microcysts and muccoid degeneration are typical signs of this astrocytoma type, it is possible to focally determine GFAP.

Low-grade astrocytomas are favourable from the prognostic point of view, the average survival period varies between six and eight years. One of the prognostic signs is the Ki67 proliferation activity, when the prognosis may be defined as worse at expression of tumour cells greater than 5 %. The presence of perivascular lymphoid infiltrate and microcysts is a sign of a more favourable prognosis [1], [2].

Anaplastic astrocytoma presents a high proliferation activity and its biological characteristic is malignant. The tumour is diagnosed in adults, more frequently in male population, between the fourth and fifth decade of life. According to the grading it is a III grade tumour. These tumours are significantly more cellular that the previous types, the cellular nuclei are of various sizes (anizonucleosis), the cell nucleoli are very distinctive. The mitotic activity is elevated, with the presence of typical mitoses. The angiogenesis (formation of new vessels) becomes very apparent, however, in comparison with glioblastomas, the necroses are usually not present. It is possible to determine the expression of S100 protein and vimentin with immunohistochemical determination, the expression of GFAP is variable. The expression of the Ki67 proliferation marker is usually between 5-10 %. The prognosis in this type of tumour is bad, the average survival period varies between two and five years. The tumour may originate from an originally diagnosed diffuse astrocytoma, or my appear de novo. The cells lose their differentiation ability and have a tendency towards non-differentiated stages and non-regulated proliferation. The outcome is usually associated with a transfer to the more malignant stage, glioblastoma [1], [2], [3].

Glioblastoma is the most frequently observed type of a malignant brain tumour; it is usually diagnosed in adults between 45 and 75 years of age, with a prevalence in males [2], [4]. We may differentiate between two types of tumours, with regard to the onset mechanism. The primary glioblastoma is more frequent, it appears de novo, without any prior tumorous lesions. The onset and clinical course of the disease is usually very rapid. The second type, a secondary glioblastoma, develops gradually, through a malignant progression from anaplastic astrocytoma of another type of a glial tumour. The glioblastomas vary greatly histologically and topographically. Glioblastoma is a Grade IV astrocytoma, the cells show a low degree of maturity with manifestations of anaplasia, the tumour is highly cellular, formed by non-differentiated pleomorf elements. Extra large cells with large nuclei and typical mitoses are present. We may typically observe necrotic nidi (local and massive), especially in the centre of the tumour, where the tumorous cells form typical palisade structures at the periphery of the necrosis. Formation of new blood vessels is vast and plentiful. The immunohistochemical markers are vimentin and CD99, the expression of GFAP is variable. The proliferation marker Ki67 manifests a positive expression in approximately 15 to 20 % [1], [2], [3], [4].

The average period of survival in patients with a diagnosed glioblastoma is one year from the diagnosis. From the prognostic point of view, the age of the patients, the proliferation activity of the tumour and the level of EGFR expression are significant. The higher the age, the proliferation activity and the expression of EGFR, the worse the prognosis [1], [2], [3], [4].

1.2 Genetic alteration of the astroglial tumours

Thanks to molecular-biological methods, a number of genetic alterations have been defined in the astroglial tumours. These are related to the onset and development of these tumours. The onset of the tumour is usually preceded by an initiation damage of a gene, together with further accumulating defects which lead to the progression of the tumour.

The most frequent initiation point in the astrocytary tumours is a mutation of the p53 gene. This gene is localised on a short arm of the chromosome 17 (17p13.). The defects of this gene are the most frequently observed in human tumours. In most cases mutations of both alleles of the gene are present, however cases of a hereditary mutations of one allele are also described in the literature. The p53 gene produces a transcription factor which, under normal circumstances, stimulates expression of other genes. It is an important tumour suppressor gene participating upon the regulation of the cellular cycle and playing an important role in cellular stress. It blocks the cellular cycle and gives the cell the time to assess the degree of damage and possible subsequent DNA reparation. In case the defects cannot be repaired, the p53 gene initiates the mechanisms of apoptosis. The defects of this gene, together with an abundant expression of PDGF (Platelet Derived Growth Factor) are considered to have a connection with an onset of diffuse astrocytomas, which may further progress into anaplastic astrocytoma, or even secondary glioblastoma [2], [3], [6].

The progression of astrocytoma towards a higher degree of malignity is further caused by a loss of the Rb gene. The retinoblastoma gene (Rb) is localised on the long arm of the chromosome 13 (13q14). The product of the gene is a nucleic phosphoprotein, which, under normal circumstances, controls the transcription of other genes participating upon the regulation of cellular division. As in the case of p53, it possesses a tumour-suppressor function [2], [3].

The pilocytic astrocytoma is a Grade I tumour with a relatively benign features which develops through an independent genetic pathway after the loss of the NF1 gene. The NF1 gene is localised on the long arm of chromosome 17 (17q11). The product of the gene is protein-neurofibromin which is a negative regulator of the $p21^{ras}$ protein (it plays a significant role in the process of cellular communication and signalling) [2], [3], [6].

In cases of primary and secondary glioblastomas, the defects of genes are different. A primary glioblastoma originating *de novo* has a separate evolution path, with a direct transformation into highly malignant phenotype without interstages. The specific defects of the primary glioblastoma are deletion of the p16 gene, mutation of the PTEN gene, loss of heterozygosity on the chromosome 10, mutation and amplification of the gene for the receptor of epidermal growth factor (EGFR) and MDM2 amplification [1], [2], [3]. The p16 gene codes the inhibitor of cyclin-dependant kinases 4 and 6 and is located on the short arm of the chromosome 9 (9p21). The suppressor gene PTEN is localised on the long arm of the chromosome 10 (10q23.3). Its functions are the blockade of the cellular cycle in the G1 stage. It is especially disorders of this gene which are linked to the progression of lower-stage astrocytomas into more malignant forms. The presence of such mutation is connected with a worse prognosis of the tumour [2], [3], [6], [7], [8], [9], [10]. The gene for EGFR receptor lies on the short arm of the chromosome 7 (7p12-14) and is the most frequently amplified oncogene in primary glioblastomas. Its amplification most probably plays a role in diffusion infiltration of glioblastomas into the surrounding tissues. The high numbers of amplifi-cations are linked with a worse tumour prognosis [2], [3], [4], [5], [6], [11].

Another amplified gene MDM2 is localised on the long arm of the chromosome 12 (12q13-14). This gene is responsible for coding of protein, the function of which is the negative regulation of apoptosis through p53 and Rb proteins [6].

In cases of the secondary glioblastoma which develops through a diffuse astrocytoma and anaplastic astrocytoma, the detected mutations are identical with the mutations in lower stages of these tumours. Less frequently, we may also observe amplification of the gene for EGFR receptor, deletion of the p16 gene or mutations in the PTEN gene. Identically with the primary glioblastoma, the loss of heterozygosity on the chromosome 10 is present to a large extent [2], [3], [6].

2. Current diagnostic approaches

The brain tumorous tissue is usually harvested for bioptic examination by the clinician during the harvesting procedure, with the use of a cannula. The acquired material may be submitted for histology examination in a negative form (being sent to the pathologist immediately after harvesting, placed in a moist chamber closed box with a piece of gauze sodden in the physiological solution), or in a fixation solution. A correct harvesting procedure is an important factor for the diagnostics, in cases of an incorrect localisation, a non-tumorous tissue may be harvested. The consequences of such a mistake include expensive, not necessary and time consuming examinations being performed. Another critical step is the processing of the material, with the maximum effort to prevent depreciation of the harvested tissue and prevention of artefacts.

The molecular-biological methods enable us to examine a "fresh" tissue harvested during a biological harvesting procedure, as well as tissue already processed with the method of paraffin blocks.

The main diagnostic methods for the determination of astroglial tumours are histological and immunohistochemical examinations. The histological exami-nation gives us a morphological picture of the tumour (Fig. 1.),
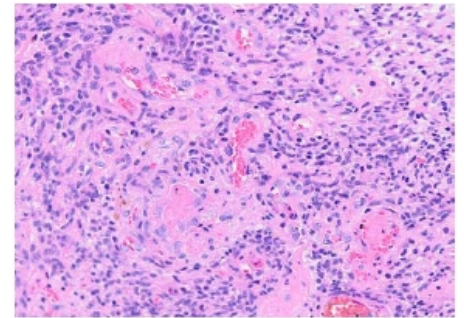

Fig. 1. Histology of glioblastoma (HE, 400x, Department of pathology, University hospital Ostrava).
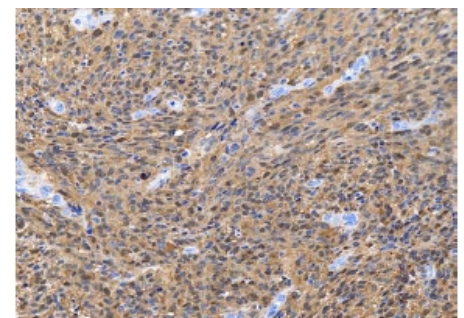

Fig. 2. Expression of S100 protein in glioblastoma tissue (S100, 200x, Department of pathology, University hospital Ostrava).
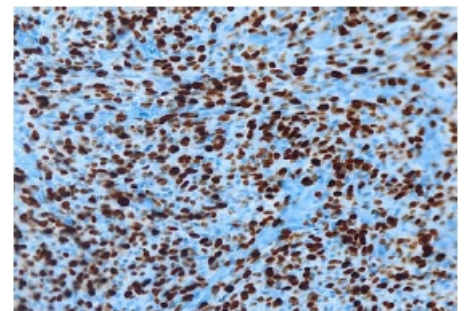

Fig. 3. Expression of Ki67 marker in glioblastoma tissue (Ki67, 200x, Department of pathology, University hospital Ostrava).
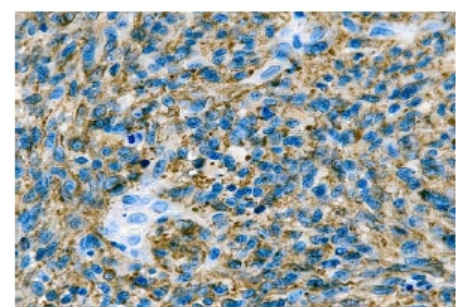

Fig. 4. Expression of GFAP protein in glioblastoma tissue (GFAP, 400x, Department of pathology, University hospital Ostrava).

immunohistochemical examination is a complementary examination, however presents an inevitable part of the bioptic diagnostics.

The most commonly used markers in the problems of astroglial tumours are vimentin, S100 protein (Fig. 2), glial fibrillary acidic protein-GFAP (Fig. 3) and proliferation marker Ki67 (Fig. 4). The markers of connective tissue, vimentin and S100 protein are the proteins of intermediary filaments. Vimentin is expressed in embryonic cells and tissues during the evolution, and in adult mesenchymal cells. It is a predecessor of glial fibrillary acidic protein (GFAP) in immature glial elements and, together with GFAP, is co-expressed in mature astrocytes and their tumorous derivates. S100 protein is an acidic protein of the central and peripheral nervous system, however it cannot be considered as a specific protein of the nervous tissue as its presence has been shown also in other tissues [12].

Ki67 is a marker of the speed of cellular proliferation. It reacts with the human nucleic antigene expressed in proliferating cells of all stages of the cellular cycle, except the G_0 stage [12]. Immuno-histochemical methods enable to carry out the basic typization of tissue and its derivates.

Together with the discovery and evolution of new methods, namely molecular-biological ones, the possibilities of diagnostics have been moved even further. These new methods present another supplement in the diagnostic process and enable us to obtain further information important for a more specific and precise definition of the disease. The methods can be used for the analysis of known mutations and alterations in the genes the damage of which is know to be related with an onset and development of the tumour. Assessment of these mutations becomes an important prognostic factor which is very important for determination of the further course of the disease and for possible initiation of appropriate and adequate treatment. At the same time, the methods make possible the discovery of new, presently unknown mutations, alterations and other factors, which may participate upon the onset of the disease. The most frequently used molecular-biological methods include hybridization, electrophoresis, amplification methods (PCR- polymerase chain reaction), sequenation, microarray analyses, et cetera.

3. Possibilities of evidence and processing of the acquired data

Realization of every research project or a clinical trial is connected with a production of a vast amount of information; this information needs to be processed in some way. In case of astroglial tumours we may obtain information related to the course of the disease, genetic and histological data, etc. However, as this disease is always closely linked to a particular person, we also receive a large amount of personal data regarding this patient. We may use database systems to process this type of data, as these enable us to generate various outcomes.

By the term "database" we mean an exactly defined data file used to describe a real world. In the case of astroglial tumours such a file may contain a record of patients with astroglial tumours, where the patient presents the element from the real world, so called entity, and this element is further described by its specific features - *attributes* [13], [14]. The usually used attributes are name and surname, residence address, birth number, etc. In relation to the disease, such attributes may be the type of the tumour, the degree of grading, detected mutations, and so on.

One of the means of describing a database is a database model. The most frequently used models are relational databases, which may be imagined as a system of tables consisting of lines and columns, where the columns correspond with individual attributes (e.g. name, surname, residence address, etc.) and the lines describe the current state of the patient. In that respect, a very important part of the database model is formed by a so called primary key, the feature of which is having a unique value. The unique value of the primary key prevents a situation when for example two lines in the table could have an identical value of the primary key, which is an unwanted situation. Such a key in case of patients may be presented by the patient's birth number. The problem is, however, that, in relation to the Law concerning the protection of personal data, the birth number has been defined as a classified information and as such it cannot be used. In case we are not able to use a certain column of the table with data as the primary key, or possibly a combination of columns, it is usually possible to add another, so called "id (identification) column", which is a column with unique values of gradually rising numbers. It is also possible to create a primary key by compounding the values of all columns, however such a solution is not very suitable, due to the fact that it increases the computing requirements of the database system. That is why so called "normal forms" have been defined for the database systems, which is a list of rules according to which the database should be defined for an optimal use of the database system. [13]. The existing relations among the tables with certain columns create certain connections which may be found also in the real world. For example a patient (as a table with the columns titled name, surname, birth number, name of the tumour) has a large tumour diagnosed. This relation is defined in the *tumour* column from the table *patient* with a relation to the primary key of the column *name of the tumour* from the table types of tumours with concrete values *large tumour*, *small tumour*, *little tumours*.

The most frequently used language for a formation of databases in the world of information technologies is the SQL (Structured Query Language) and the database systems based upon this language. The language presents a tool for formation of databases (tables) and also enables the users a further manipulation with data, like data storage, update, erasing, searching for specific information, etc. [14].

When defining a database, it is necessary to evaluate every step because every mistake or a wrong step may be critical and these mistakes may cause a malfunction of the database in the future, during transferring and editing the stored data, etc.

Another possibility of editing the acquired data is the use of statistical methods. There exists a wide range of these methods, the choice of the appropriate method needs to be based upon the type of data which we need to process.

The most frequently observed topic in relation to the astroglial tumours is the effectiveness of prognostic factors on the survival of patients with glioblastomas. To assess the mean survival period in patients we use the Kaplan-Meier method and the effect of the variable on the survival is evaluated with the Log-Rank test. The variable is often the age of the patient, sex, Karnofsky score, localization of the tumour in brain, postoperative radiotherapy, chemotherapy and others [15]. Other statistical methods frequently used in the area of astroglial tumours are Chi-Quadrant-Test, Student's t-test, Mann-Whitney U test or Spearman's correlation coefficient [16].

4. Conclusion

The problems of astroglial tumours are very vast and complex, even though a large number of clinical trials have been conducted in that respect, many questions remain unanswered. The most important point is looking for new prognostic and predictive factors which would have a significance for choosing suitable therapeutic procedures. This problem is mostly related to the glioblastomas, where the period of survival from the time of diagnosis is very short. From the overall number diagnosed glioblastomas, around 90% are primary ones. This type of tumour develops very quickly, without any previous clinical or histological manifestation. The primary and secondary glioblastomas vary, apart from different mutations, also in the profiles of expression on the RNA and protein levels in the methylations of promoter areas.

These differences are responsible for a different susceptibility to radio- and chemotherapy [17], [18]. A large percentage of the present clinical trials are aimed at the use of EGFR receptor as the molecular target in cancer therapy. It has also been proved that methylation of O^6-Methylguanin DNA methyltranspherase (MGMT), a key DNA-reparatory enzyme, increase the sensitivity of secondary glioblastomas to alkylating agents used in chemotherapeutic treatment [18], [19].

Obtaining new and more specific information regarding the pathogenesis of astroglial tumours which would result in an improved and more quality diagnostics and treatment of these tumours is a long-term task which will require a willing cooperation among clinicians, pathologists and molecular biologists.

Acknowledgment

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