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Case report

The molecular pattern of histopathological progression to anaplastic meningioma – A case report

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ABSTRACT

Meningiomas (MGs) are the most frequent primary tumours of the central nervous system (CNS) and exhibit a large spectrum of histological types and clinical phenotypes. The WHO classification of CNS tumours established strict diagnostic criteria of the benign (Grade 1), atypical (Grade 2) and anaplastic (Grade 3) subtypes. Combined with the resection rate, WHO grading has the most crucial role as the prognostic factor. Additionally, such biomarkers as Ki-67/MIB-1, progesterone receptors and phosphor-histone H3 were correlated with MG progression. Recently, it was suggested that the aggressive behaviour of some MGs is attributed to molecular alterations, regardless of their histopathology. The analysis of loss of heterozygosity (LOH) at chromosomes 1, 9, 10, 14 and 22 was performed. The presented case of WHO Grade 2 MG initially exhibited LOH at chromosomes 10, 14 and 22. In the first recurrence, the tumour genetic profiling revealed additional LOH at chromosome 1p and atypical histopathology. During the second recurrence, an aggressive phenotype was observed and tumour progressed to an anaplastic form. Considering the appearance of the tumour relapses, the set of molecular changes overtook the histopathological progression. The genetic and histopathological imbalance in the tumour progression in secondary anaplastic MGs has not been previously described. The evolution of genetic and histopathological changes was presented in the same patient. In the future, the individualised therapy of potentially more aggressive forms of MGs could be based on certain chromosome aberrations.

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1. Introduction

Meningiomas (MGs) are the most common intracranial tumours. They comprise 25–30% of the primary central nervous system (CNS) neoplasms [1]. The origin of MGs is unclear and arachnoid granulations with secondary invasion of the dura mater is the most convincing hypothesis [2]. The meningothelial hyperplasia of dural cells as a transitional form of MG was regarded as a reason for multifocal occurrence [3]. Histological diagnosis of a benign MG (Grade 1) is more clear for the pathologist than diagnosis of atypical (Grade 2) or anaplastic (Grade 3) types of this tumour. Microscopically, the anaplastic form may under a microscope resemble a carcinoma, sarcoma or melanoma, thus making an additional immunohistochemistry necessary for establishing a final diagnosis [2]. According to the 2007 revision of the criteria for CNS tumours [4], the World Health Organization (WHO) classified some MGs to an 'invasive' subtype. Regarding tumour recurrence, both the WHO grading and the resection rate are the most powerful prognostic factors. Some authors suggested other prognostic biomarkers, including Ki-67/MIB-1, progesterone receptors and phosphor-histone H3 [5–7]. However, some MGs present with invasive behaviour, regardless of their histopathology [2]. Their aggressive behaviour may be attributed to the alterations at the molecular level, similar to those described in other brain neoplasms, e.g. glioblastomas. Accordingly, low- and high-grade gliomas can be either of primary or of secondary origin. A question arises whether this pattern be easily translated to anaplastic MGs? De novo or secondary anaplastic origins of MGs are at least debatable [4] and the molecular findings illuminate the issue. Early reports suggested monosomy of 22/22q as the most frequent aberration and the mutation underlying the formation of MGs [8]. The alteration of several chromosomes [1,6,9,10,14,18] is commonly found in Grade 2 but less often in Grade 1 MGs [8]. On the other hand, the progression-associated loci were sparsely reported and limited to losses of genetic material on 6q, 9p, 10q, 14q, or amplification of 17q23 [2,8]. We report an MG case in which the evolution of genetic alterations explains the relapsing clinical course. The molecular findings were unrelated to the histopathology.

2. Case report

A 56-year-old male presented with persistent headache and left hemiparesis. Medical history was significant for diabetes type 2 and hyperlipidaemia. Contrast-enhanced computed tomography (CT) revealed a 5 cm meningioma in right frontal convexity, with sagittal sinus invasion and apparent brain oedema surrounding the lesion (Fig. 1a). Hemiparesis withdrew within days after the operation and the whole postoperative period was uneventful. Nevertheless the pathologist diagnosed an atypical, grade 2 meningioma MG (Grade 2) (Fig. 1b) and CT performed 6 months after the surgery revealed a multifocal tumour relapse in the parasagittal area (Fig. 2a). The tumour was removed together with its multidirectional extensions during the second-look surgery (Simpson grade 2) and again HP confirmed atypical features of recurrent MG (Fig. 2b). After 4 months the neoplasm relapsed again, this time as a tumour disseminated from its primary location along the falx (Fig. 3a). Third surgery was undertaken to result in Simpson grade 2 resection of all numerous round-shaped tumour extensions. HP examination revealed anaplastic transformation (Fig. 3b), therefore adjuvant radiotherapy was applied (unknown total dose). The anaplastic MG recurred again two times: two rescue partial resections were performed but the patient died at 22 months after initial diagnosis.

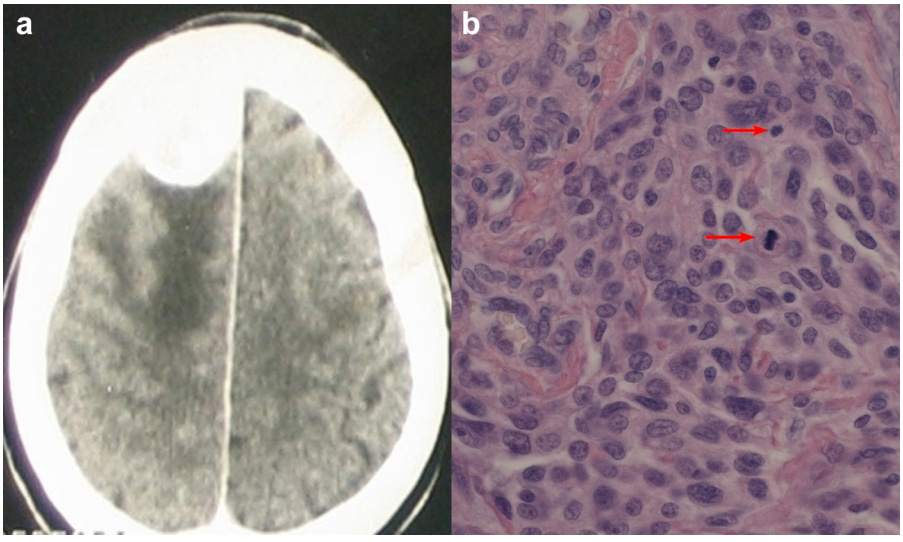

Fig. 1 – Imaging (a) and histopathological findings (H&E staining, magnification 400×) (b) of primary tumour (1a-b), first (2a-b) and second (3a-b) relapse. (a) Computer tomography (CT) imaging of the primary meningioma (MG) with surrounding oedema. (b). Atypical MG. Enlarged nuclei or prominent nucleoli, two mitoses (red arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

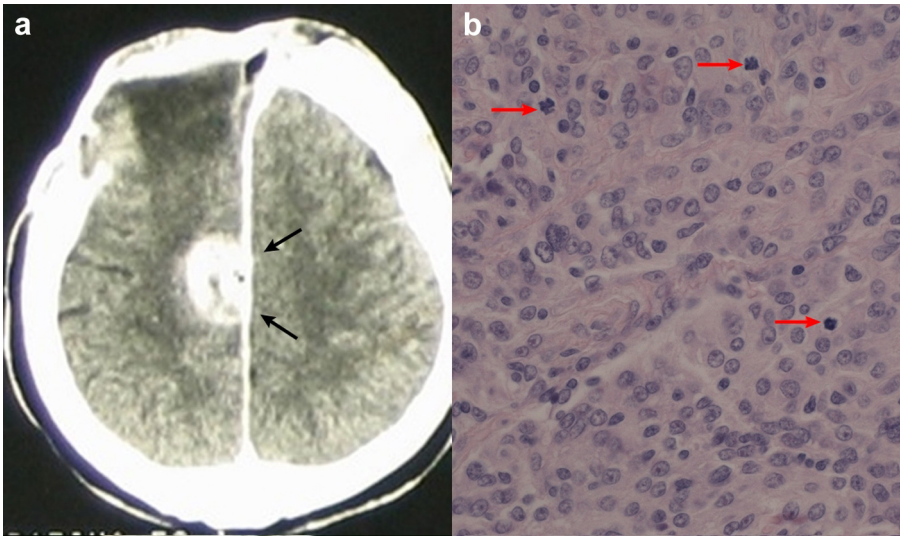

Fig. 2 – (a) 6-months postoperative CT; the relapsed MG (black arrows). (b) Features of atypia including enlarged nuclei and prominent nucleoli, three mitoses (red arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

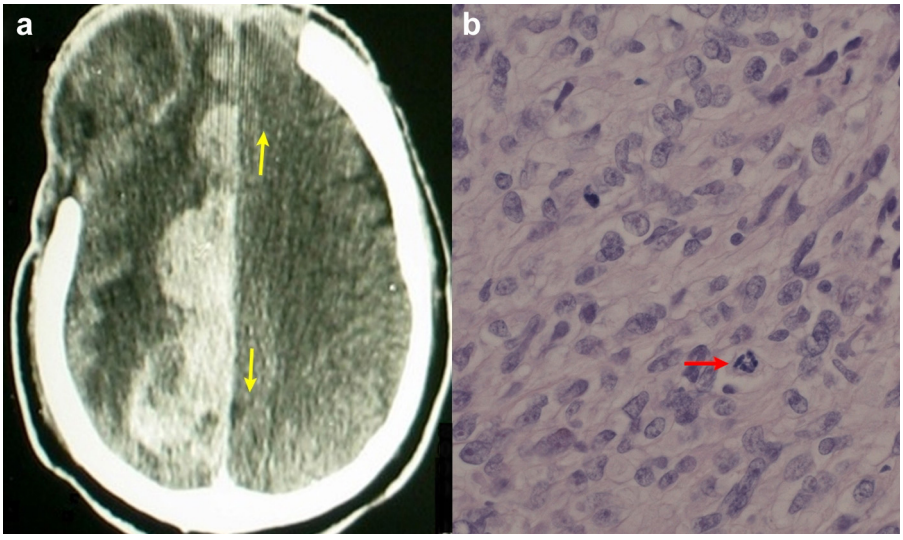

Fig. 3 – (a) Parasagittal spread of MG (yellow arrows). (b) Anaplastic MG. Clear-cut features of cellular atypia, two mitoses, one of them atypical (red arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Methods of genetic evaluation

3.1. *Genetic evaluation*

Tumour samples were collected intraoperatively for the histopathological and molecular analysis. The genomic DNA was isolated from the tumour tissues (stored in −80 °C) and peripheral blood leukocytes. Using the chloroform phenol method, the quantification and the analysis with respect to purity and protein content was performed and the markers were selected from NCBI database (ncbi.nlm.nih.gov). Paired DNA samples were analysed for loss of heterozygosity (LOH) with 24 microsatellite markers (HVD Holding AG; Ebersberg, Germany). The following microsatellite loci were tested: D1S508, D1S199, D1S197, D1S162, D9S156, D9S162, D9S319, D9S1748, D10S197, D10S209, D10S587, D10S1709, D14S292, D14S1010, D22S257, D22S258, D22S268, D22S298, D22S303, D22S449, D22S609, D22S1150, and D22S1163. The polymerase chain reaction (PCR) was performed according to a standard protocol. The products were electrophoresed on 6% denaturing polyacrylamide gel (containing 7 mol/L urea); for visualisation LiCor automatic sequencer (LiCor Biotechnology, Lincoln, NE, USA) was used. A reduction intensity of >50% in the tumour lane compared to the corresponding blood lane was regarded as LOH and was repeatedly analysed. Histopathological examination: 4% solution of buffered formaldehyde; dehydrated after 10–24 h of fixation; cleared and impregnated with paraffin in tissue

Table 1 – Genetic profile of the tumour: initial (0), during first relapse (I) and second relapse (II). Loss of heterozygosity at chromosome 1 was marked with an exclamation mark (!) to emphasise that molecular progression to anaplastic meningioma actually proceeded histopathological progression.

LOH at chromosome		1	9	10	14	22	Histopathology
Recurrence	0	–	–	+	+	+	Atypical
	I°	+ (!)	–	+	+	+	Atypical
	II°	+	–	+	+	+	Anaplastic

Abbreviation: LOH, loss of heterozygosity.

processor and embedded in paraffin blocks; 4 μm thick slices were prepared; stained by haematoxylin and eosin. Immunostains with progesterone receptor antibodies, epithelial membrane antigen (EMA) and MIB-1 were obtained. The tumour was classified according to the WHO criteria [4,10].

4. Results

The loss of heterozygosity (LOH) at chromosomes 10, 14 and 22 was confirmed in the primary tumour sample. An additional aberration occurred, LOH at chromosome 1 even though the histopathology was atypical. The genetic profile of the tumour during the second surgery has not changed, but the tumour progressed histologically (Table 1).

5. Discussion

At least 20% of MGs recur. The relapsed tumours pose a clinical challenge as they are more difficult to remove safely. However, atypical (Grade 2) MGs recur 8 times more often than Grade 1 MGs. Atypical MGs not only tend to recur but also dedifferentiate to the more malignant, anaplastic (Grade 3) MG. According to WHO classification Grade 2 MGs are defined by one or more of the following four criteria: (1) chordoid or clear cell histologic subtype, (2) 4–19 mitoses per ten high-power field (HPF), (3) brain infiltration, and (4) three or more of the following five histologic features: small cell change, increased cellularity, prominent nucleoli, sheet-like growth, or necrosis. Grade 3 (anaplastic/malignant) MGs are defined by rhabdoid or papillary subtypes, a histological picture of frank malignancy resembling that of carcinomas, melanomas, or high grade sarcomas, or 20 or more mitosis per ten high-power field (HPF). The risk of death in 2 years from diagnosis is greatly increased if a malignant tumour is diagnosed [2,11,12]. The sentence is not clear for me. Tumours that fulfil the strict WHO criteria of atypical meningioma can clinically behave either as a benign Grade 1 tumour or malignant tumour Grade 3 in WHO scale [13]. This can be explained in terms of molecular alterations, which supplement the histopathological and immunohistochemical diagnosis. On the contrary, the cytogenetic studies indicated areas of various molecular patterns in a single lesion [14]. The existence of these heterogeneous regions in the MG can partially explain LOH at chromosome 1, which overtook the histological progression to the anaplastic form. The evolutional mechanism resembles glioma behaviour; some are de novo tumours while some are secondary. In fact, these two forms of gliomas differ in genetic profile and require different treatment [15]. Unfortunately, MGs have not been widely studied in terms of de novo and secondary occurrence. Based on the results of comparative genomic hybridisation method and gene expression investigations, the diagnosis of either low- or highly proliferative MG relies on the molecular signature, regardless of the tumour's histopathological type [2,16]. Patients with atypical MGs can benefit the most from the molecular evaluation. Riemenschneider et al. summarised the cytogenetic changes associated with the progression of atypical MG [17]. It was attributed to LOH at chromosomes 6, 9, 10, 14 and 17. On the other hand, 1p losses were found in up to 26% of Grade 1, <76% of Grade 2 and in almost every anaplastic MG [8]. Moreover, LOH on 1p was associated with malignant progression of the recurrent MG, even if no specific gene was found [8,18,19].

In the presented a case, the initial diagnosis was Grade 2 MG with a typical clinical and radiological picture. The molecular findings were also typical for Grade 2 tumour, including LOH at chromosome 14. That alteration lead to the loss of suppressor genes such as *MEG3* and *NDRG2* [20,21]. The another initially diagnosed aberration was LOH at chromosome 10, which correlate with shorter survival time and higher recurrence rate [22]. On the contrary, the cytogenetic role of the chromosome 22 anomaly is not linked with aggressive behaviour [23]. The investigations of *BCR*, *TIMP3*, and *ELAVL4* did not confirm their role in MG progression [24–27].

The examination of the recurred tumour revealed LOH on 1p, although without histopathological progression. The anaplasia was found until the second relapse was recognised. The delay of anaplastic transformation was evident. Furthermore, in the presented case the LOH at chromosome 1 was probably required to complement other alterations. The complex mutations together with their sequence underlies the progression mechanism [28]. Al-Mefty et al. found complex genetic aberrations in 3 of 4 MG samples prior to anaplastic transformation. He suggested that a certain subpopulation of benign MGs are 'programmed' to progress to the anaplastic form [29]. Krayenbuhl et al. compared cytogenetic profile of de novo and secondary MGs (that progressed from atypical) [30]. In his study, the alterations on chromosomes 1, 10, 14, 18 and 22 were more frequent in progressed MGs than in the de novo form. Moreover, 9p deletion as well as amplification of 17p23 are characteristic for anaplastic MG [8], though not present in our case nor examined routinely. The question whether the above alterations characterise only de novo MGs remains still unanswered.

6. Conclusions

Anaplastic MGs can progress from the atypical form. The molecular progression can precede histopathological progres-

sion. The analysis of alterations on several chromosomes of the original tumours and of the recurrences in the same patient can lead to a better understanding of the MG transformation.

Conflict of interest

None declared.

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None declared.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pjnns.2016.03.008.

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