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Clinical case

Adult recurrent pilocytic astrocytoma: Clinical, histopathological and molecular study

Astrocytome pilocytique récidivant de l'adulte : étude clinique, histopathologique et moléculaire

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ABSTRACT

Background. – PA is a grade I glial tumor that mostly occurs in children. However, although apparently similar to paediatric PA, adult PA presents a different clinical follow-up that could arise from specific molecular alterations. A variety of genetic alterations have been identified as diagnostic or prognostic glioma molecular markers.
Material and methods. – We describe a right infratentorial tumor that occurred in a 58-year-old man. Neuroimaging and neuropathological examination suggested PA as an initial diagnosis. The tumor was completely resected. Unexpectedly, two years later, a rapidly growing tumor on the operative site was observed with a second location in the pineal region. Immunohistochemical reactions (IHC), Multiplex ligation probe amplification (MLPA) and fluorescence *in situ* hybridization (FISH) was performed in both primary and relapse tumor.
Results. – Neuroimaging and neuropathological examinations suggested an unusual diagnosis for adult patients: a recurrent PA. Both MLPA and FISH analysis contribute to diagnostic confirmation by *KIAA1549:BRAF* fusion detection. Additional genetic results revealed interesting findings that justified the tumor aggressivity.
Conclusion. – Molecular analysis of adult PA cases should be routinely combined with histopathological and neuroimaging examination to further refine prognostic diagnoses.

Mots clés :
Astrocytome pilocytique
Adulte
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RÉSUMÉ

Introduction. – L'astrocytome pilocytique (AP) est une tumeur gliale de grade 1, survenant le plus souvent à un âge pédiatrique. Chez les adultes, en dépit de la similarité histologique, l'AP est de moins bon pronostic. Cette évolution défavorable est sous-tendue par un profil génétique spécifique. En effet, un grand nombre d'altérations moléculaires de valeurs diagnostiques, pronostiques et théranostiques seraient à l'origine de cette différence pronostique.
Matériels et méthodes. – Dans cette étude, nous rapportons un cas de tumeur gliale infratentorielle chez un patient adulte de 58 ans. Les données d'imagerie et de neuropathologie suggèrent l'AP comme premier diagnostic. Deux ans après une résection complète, nous avons observé une récidive bifocale au siège initial de la tumeur avec une évolution rapide, accompagnée d'une deuxième localisation

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dans la région pinéale. Aussi bien la tumeur initiale que la récidive ont été explorées par techniques d'immunohistochimie (IHC), d'amplification et ligation multiplexes de sondes (MLPA) et d'hybridation in situ fluorescente (FISH).
Résultats. – Les données d'imagerie et neuropathologie suggèrent un diagnostic rare d'AP récidivant de l'adulte. Les analyses moléculaires par MLPA et FISH apportent la confirmation diagnostique en révélant la présence du gène de fusion *KIAA1549:BRAF*. D'autres altérations génétiques pouvant justifier l'agressivité de la tumeur ont également été détectées.
Conclusion. – L'analyse moléculaire en matière d'AP de l'adulte s'avère complémentaire de l'histopathologie et de l'imagerie. En plus de sa valeur diagnostique connue, elle serait d'un grand apport pronostique. Elle devrait ainsi intégrer le protocole routinier du diagnostic des PA adultes.

1. Introduction

Pilocytic astrocytoma (PA) is a circumscribed glioma that primarily occurs in the cerebellum in children [1]. Clinical presentation depends on tumor location. Headache, neck pain, vomiting and visual abnormalities are usually reported with the cerebellum location [2]. Histopathological description includes biphasic pattern, elongated and rounded cells, Rosenthal fibers, and eosinophilic granular bodies [2]. PA is classified as grade I by the World Health Organization (WHO) [3] and usually reported with a good prognosis. In most cases, only surgical resection is considered to be curative [4].

Very few recurrent adult PA cases have been reported with malignant progression [4–6]. In such unusual presentations, features like microvascular proliferation, necrosis and nuclear atypia may occur. Differential diagnoses as high-grade gliomas could then be suspected with a considerably worse prognosis [7]. Furthermore, unlike paediatric PA, the adult cases seem to present a poor prognosis [8]. The correct diagnosis has great importance in the treatment choice and prognosis of patients.

2. Material and methods

2.1. Patient

A 58-year-old man with no previous family medical history of cancer was referred for severe headache, neck stiffness and difficulty in walking. The physical examination showed signs of cerebellar dysfunction. MRI revealed a well-circumscribed lesion extended into the 4th ventricle (Fig. 1a, b). The patient underwent a complete tumor resection without evidence of any residual or recurrent mass at 6-months follow-up by MRI.

Two years later, the patient was readmitted with increased intracranial pressure, a cerebellar syndrome, vertical/horizontal strabismus and VI and IX right nerve palsy. There was no previous family medical history or clinical signs of neurofibromatosis [9]. The MRI showed a bifocal tumor located in the cerebellar region at the operative site with a second location in the pineal region (Fig. 1c, d). The masses presented with cystic and intensely enhanced solid components. The patient was readmitted for a second tumor resection. The pineal component was unresectable and the tumor was partially removed in the cerebellar location alone. The patient died shortly after the operation.

2.2. Histological Immunohistochemical study

Routine sections (4 mm) of both primary and relapse tumor were stained with haematoxylin and eosin. Immunohistochemical analysis was performed on 4 mm of formalin-fixed, paraffin-embedded sections using a panel of polyclonal antibodies (Table 1). Slides were dewaxed in xylene and rehydrated through a descending ethanol series. Antigen retrieval was performed at 98 °C with DAKO antigen retrieval solution during 20 min (Table 1). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol. The detection system used was Novolink Polymer (Leica Microsystems, Newcastle Ltd.) with diaminobenzidine as chromogen. Slides were counterstained with Mayer's haematoxylin. HE and IHC results were separately reviewed by two pathologists.

2.3. DNA extraction

Primary tumor Genomic DNA was purified from FFPE sections using an Qiagen kit (Qiamp DNA FFPE Tissue Kit). Recurrent tumor genomic DNA was purified from fresh tumoral tissue according to phenol–chloroform protocol.

2.4. Multiplex ligation probe amplification (MLPA)

Both primary and recurrent tumoral DNAs were analysed using a multiplex ligation probe amplification (MLPA) reaction through SALSA MLPA Kits P105, P370 and P088 (MRC Holland, Amsterdam, The Netherlands) according to manufacturers protocol. DNA from healthy tissue was adopted as MLPA control sample.

MLPA kits enabled us to analyse multiple chromosomal aberrations located at 1p36, 7q3, 9p21, 10q23, 11p11, 17p13 and 19q13 (Table 2). Additional point mutations may be detected using the P370 MLPA kit (*IDH1* R132H, *IDH1* R1321C, *IDH2* R172K, *IDH2* R172M and *BRAF* V600E).

PCR products were analysed on ABI 310 (Applied Biosystems, Foster City, CA) using as an internal size standard the ROX-500 "Genescan®" (ABI 401734). Data analysis was performed with "GeneMarker®" software V1.91 (SoftGenetics). Intra normalization for sample data was initially performed on control probes, and then on healthy control samples. Normal ratio limits were set at 0.75 and 1.3 [10]. For more accuracy, we considered that displaying 2 or more probes, adjacent to each other on a chromosomal region, exhibiting the ratio below 0.45 or above 2 were accepted respectively as deleted or gained.

2.5. *Fluorescence* in situ *hybridization (FISH)*

FISH assay was performed in both the primary tumor and relapse to look for presence of the *KIAA1549:BRAF* fusion gene. A dual-colour FISH probe set was designed to identify the chromosomal duplication that generates the *KIAA1549:BRAF* fusion, using human genomic sequences from the RP4-726N20 clone that encompass *BRAF* gene labelled in Spectrum Red and the using human genomic sequences from the RP11-355D18 clone that encompass *KIAA1549* labeled in Spectrum Green. Probe preparation and FISH assays were performed as reported elsewhere [11].