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Histomolecular characterization of intracranial meningiomas developed in patients exposed to high-dose cyproterone acetate: an antiandrogen treatment

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Abstract

Background. Meningiomas are the most common primary intracranial tumors in adults. The relationship between meningiomas and exogenous sex hormones such as cyproterone acetate (CPA) is well documented, yet the underlying mechanisms remain unknown. Defining the histomolecular status of meningiomas developed on CPA would help us to better understand the oncogenesis of these tumors.

Methods. We identified 30 patients operated for a meningioma after long-term high-dose CPA therapy and with a history of CPA discontinuation before establishing the indication for surgical intervention. We used array-comparative genomic hybridization (to characterize copy number changes in those 30 meningiomas and subsequently performed next-generation sequencing with the National Institute of Cancer (INCa) solid tumor panel, which is a targeted panel of clinically actionable genes. We also examined grade, type, and clinical features.

Results. We identified *AKT1* mutations or *PIK3CA* mutations in 33.3% of CPA meningiomas. *AKT1* and *PIK3CA* mutations were mutually exclusive. Enrichment in oncogenic *PIK3CA* mutations in the CPA cohort was detected. CPA meningiomas showed chromosomal stability and were located mainly in the skull base. Ninety percent of CPA meningiomas were low-grade meningiomas and 63.4% were meningotheliomas. Half of our CPA cohort had microcystic components.

Conclusion. Our study shows that low-grade meningothelial meningiomas of the skull base are predominant in CPA meningiomas. We identified PIK3CA/AKT1 pathway as a hypothetical actor in onco-pharmacological interaction between meningiomas and CPA. This signaling pathway could be an interesting target for precision medicine trials in meningioma patients who have been subjected to CPA. Our results could invite the scientific community to review the current classification of meningiomas and to evolve toward more specific histomolecular classification.

Key Points

1. Meningiomas developed on cyproterone acetate are mutated frequently on PI3K pathway.

2. Meningiomas developed on cyproterone acetate showed chromosomal stability.

Importance of the Study

Our study aims to define the histomolecular characteristics of adult intracranial meningiomas arising on antiandrogen treatment. So far, there is little data available in the literature on this topic. Our cohort of 30 meningiomas developed on high-dose cyproterone acetate (CPA) constitutes the largest series reported in the literature. The identification of genetic and histologic abnormalities involved in meningiomas developed on CPA would enable better understanding of the meningiomagenesis. To reach our objectives, we used 2 different molecular technologies, array comparative genomic hybridization and next-generation sequencing. Our results represent a major step toward an integrated histomolecular classification. And our study suggests that PIK3CA/AKT1 enrichment in this particular population could place targeting therapy in a central position for the management of patients with meningioma.

Meningiomas are the most frequent brain tumors in adults.[1] The prevalence is higher in females with a sex ratio of 2:1.[2] From a histopathologic perspective, 80% of meningiomas are grade I according to the World Health Organization (WHO) classification.[1] Among those, several architectural patterns have been identified, the most frequent being meningothelial, fibroblastic, and transitional meningiomas.[3] The standard of care is surgical resection. Some meningiomas have a high risk of recurrence and are challenging for surveillance, raising the indication for adjuvant therapy.[4] Although pharmacotherapies may offer some benefits to selected patients in terms of survival, their efficacy has not been demonstrated in prospective studies.[5]

Several studies have suggested an association between female sex hormones and the pathophysiology of meningiomas.[6,7] The role of different sex hormones in the development of meningiomas has been evaluated using various approaches. Numerous reports have dealt with the presence or absence of hormonal receptors in the tumors[8]; almost all of those detected being high progesterone receptors; however, it remains difficult to determine whether their function is activating or inhibiting.[9]

Cyproterone acetate (CPA) has been associated with a risk of meningioma occurrence.[10,11] CPA is a synthetic steroid derived from 17-hydroxyprogesterone with antiandrogenic and progestogenic properties. Long-term use of high-dose CPA seems to be a major risk factor for developing meningioma. Many studies have shown the regression of multiple meningiomas after discontinuation of progesterone agonist treatment.[12,13] Although the association between CPA administration and multiple intracranial meningioma development is well documented, the underlying mechanisms remain unknown.

Even though molecular classification of meningiomas is still limited, it appears that there are 2 distinct pathways according to *NF2* mutation status.[14] Typical non-*NF2*-mutant meningiomas do not present major chromosomal copy number anomalies, whereas *NF2*-mutant meningiomas, or those with chromosome 22 monosomy, present a higher chromosomal instability.[15] Recent genetic analysis showed that *TRAF7*, *KLF4*, *AKT1*, *PIK3CA*, and *SMO genes* were frequently mutated in non-*NF2*-mutant meningiomas.[14] Although these mutations may be involved in tumor progression, their role in meningioma genesis has yet to be elucidated.[16] *PIK3CA* is mutated in 4.7% of all meningiomas and in 7% of non-*NF2*-mutant meningiomas.[17,18] In addition to gene mutations, recurrent chromosomal copy number changes have been reported in meningioma, differing according to the grade.[18] However, the histologic and genetic profiles of adult intracranial meningiomas developed on Androcur® use have barely been explored.

Here, we have assessed clinical cases of progression of meningiomas on progesterone agonist treatment by array-comparative genomic hybridization (array-CGH) and next-generation sequencing (NGS) and highlighted typical histologic and genetic features.

Materials and Methods

This is an observational study, descriptive and comparative, conducted at the University Hospital Center of Poitiers (UHCP), in collaboration with the Adolphe de Rothschild Foundation Hospital (RFH) in Paris. The study was validated by the Committee of Biologic Resources of UHCP (identification number BB0033-00068).

Patients and Specimens

We retrospectively selected patients operated for a meningioma with informed history of prolonged exposure to high-dose CPA therapy and documented meningioma evolution after CPA discontinuation. This selection was made regardless of grade, age, and sex to study a general CPA cohort. The tumor samples were obtained from patients who had undergone surgery between 2005 and 2017. None had received radiotherapy before the surgery. The specimens consisted of formalin-fixed, paraffin-embedded (FFPE) tissue sections, with at least 30% of tumor cell. All meningioma samples were reviewed by a neuropathologist (S.M.) and classified according to the 2016 WHO classification.[19] Tumor samples of meningiomas developed on CPA cohort were collected from the tumor banks (5 from the tumor bank of UHCP and 25 from RFH).

Our results were compared with a series of 150 adult meningiomas without informed history of Androcur® described by Abedalthagafi et al.[17] This cohort was used as a "control cohort" in our article.

DNA Extraction and Quantification

6 × 10 µm FFPE sections per sample were subject to a deparaffinization protocol using Tween® 20 detergent, before DNA extraction with the Maxwell® 16 FFPE Tissue LEV Purification kit. DNA was quantified by Quantus® Fluorometer (Promega).

Array Genomic Comparative Hybridization

The minimum DNA input required to perform the hybridization was 2 µg. Tumoral and reference DNAs (Promega) were labeled using the ULS Labeling Kit (Agilent Technologies) and hybridized onto SurePrint G3 Human CGH Microarray Chip 4 × 180K (Agilent® Technologies) for 24 hours at 67°C in a rotating oven at 20 rpm. After washing, the microarrays were scanned on the Agilent SureScan Microarray Scanner. Agilent CytoGenomics® was used to feature extract tiff. images generated by the scanner and to analyze the extracted data. Somatic copy number changes of 5 consecutive probes with mean $\log_2$ ratios 0.25 for gains and –0.25 for losses were called using the ADM2 algorithm. A second analysis strategy detecting aberrations with mean $\log_2$ ratios of 0.07 (absolute value) was adopted for samples with low tumoral cell percentage (<50%) to report low-level copy number aberrations. Genomic coordinates were reported according to the Hg19.

To evaluate the copy number alterations (CNAs) of each patient, the number of detected CNA was calculated. One chromosome could carry several CNAs. We defined 3 chromosomal profiles: flat profiles with no chromosomal aberrations (0 CNA), simple profiles with 1–2 CNAs, and complex profiles with 3 or more CNAs. In addition, we calculated the cytogenetic abnormality score (CAS), as defined by Aizer et al.[20] and previously used in the "control cohort" (Abedalthagafi et al.[17]).

Targeted NGS

An in-house actionable gene panel was designed as per the recommendations of the French National Institute of Cancer for the detection of theranostic mutations. The panel included a total of 212 primers, covering hotspot exons of 20 genes (Supplementary Table1). Double libraries were prepared using the Fluidigm Access Array system: after a pre-amplification step, multiplex polymerase chain reaction using target-specific primers was performed and samples were dual-barcoded during the target enrichment process. The amplicons were purified using Ampure XP beads and librairies were quantified and pooled with equimolar concentration. Sequencing was performed on a MiSeq platform using 300-cycle MiSeq Reagent Kit v2 (Illumina). Generated data (FASTQ) were processed according to an in-house developed bioinformatic pipeline. The workflow comprised 4 distinct steps: filtering data using fastQC and prinseq programs, aligning sequences according to the reference genome (hg19); using bowtie2 and BWA, pre-processing alignment files (.bam files); and using SAMtools, picardtools, GATK and FATBAM and FreeBayes. Finally, variants were annotated using ANNOVAR and homemade tools. All NGS analyses were performed in duplicate. For quality assessment, a positive control and 4 blanks were performed in the same run and analyzed with the same bioinformatic pipeline as the samples. All alterations were manually inspected by Integrated Genome Viewer (http://www.broadinstitute.org/igv). Only variants present in both duplicates with an allelic frequency ≥5% were considered. Clinical impact of variants was determined using Alamut® software and classified as per The Association of Molecular Pathology (AMP) guidelines..[21] Briefly, variants with strong or potential clinical significance (Tier I and II) were called "pathogenic," variants of unknown clinical significance were called "VOUS" (Tier III) and benign or likely benign variants were not reported.

Statistical Analysis

We used R3.3.2. software for the statistical analysis. The relationship between the variables was assessed with the chi-square independence test.

Results

Clinical and Histopathological Data

Our CPA cohort consisted of 29 female patients and only 1 male patient. The mean age was 50 years. The control cohort comprised 95 females and 55 males, with mean age of 58 years. The sex ratio was unbalanced in both cohorts with a strong prevalence of females in our cohort as they are the patients in whom Androcur® therapy is indicated, in a majority of cases. All women in the CPA cohort had received 25 mg/day Andocur® for the management of hirsutism. One male patient of our cohort had used Andocur® at a dose of 100 mg/day for the treatment of prostate cancer. Of the 30 patients who had been exposed to Andocur®, 26 (86.7%) had prolonged exposure >10 years and 21 (70%) showed clinical improvement and/or tumor regression on discontinuation of the treatment (Tables 1 and 2) but remained symptomatic. Twenty-six (86.7%) meningiomas were localized in the skull base and only 4 (13.3%) in the convexities (Figure 1B), whereas in the control cohort, 73 (48.7%) were localized in the skull base (Table 2). A positive relationship was found between the skull base localization of meningiomas and use of Andocur® (P = .0003; Table 2).

Twenty-seven (90%) meningiomas of the CPA cohort were grade I, and 3 (10%) were grade II (classified grade II based on focal invasion of the cerebral parenchyma n = 2 or due to the presence of a major chordoid component n = 1). There was no grade III meningioma in our series. The control cohort presented 104 (69.3%) grade I, 41 (27.3%) grade II, and 5 grade III (3.4%) meningiomas. Low-grade meningioma and exposure to Androcur® tended to be associated, although not significantly (P = .06; Figure 1 and Table 2).

Our CPA cohort included 19 (63.4%) meningothelial meningiomas, 6 (20%) transitional meningiomas, 3 (10%)

Table 1. Clinical Information on Patients in the Cyproterone Acetate (CPA) Cohort. CPA Cohort Consisted of 29 Female Patients and Only 1 Male Patient

	Age (years)	Sex	CPA indication	CPA daily dose (mg/d)	CPA exposure (years)	Symptoms appeared under CPA	Radio-clinical evolution after CPA discontinuation
Patient 1	63	M	Prostate cancer	100	<10	Left visual loss, memory disorders	Improvement
Patient 2	33	F	Hirsutism	25	>10	Right vestibular syndrom, right hypoacusis	Stable
Patient 3	49	F	Hirsutism	25	>10	Left visual loss	Improvement
Patient 4	48	F	Hirsutism	25	>10	Right visual loss	Improvement
Patient 5	52	F	Hirsutism	25	>10	Left visual loss	Improvement
Patient 6	29	F	Hirsutism	25	<10	Right V1, V2 and V3 deficit, right vestibular syndrom	Stable
Patient 7	52	F	Hirsutism	25	>10	Right visual loss	Improvement
Patient 8	29	F	Hirsutism	25	>10	Right visual loss	Improvement
Patient 9	64	F	Hirsutism	25	>10	Left visual loss	Stable
Patient 10	49	F	Hirsutism	25	>10	Right visual loss	Improvement
Patient 11	50	F	Hirsutism	25	>10	Left visual loss	Improvement
Patient 12	53	F	Hirsutism	25	>10	Left visual loss	Stable
Patient 13	38	F	Hirsutism	25	>10	Bilateral visual loss	Stable
Patient 14	62	F	Hirsutism	25	<10	Right visual loss	Improvement
Patient 15	68	F	Hirsutism	25	>10	Right visual loss	Improvement
Patient 16	47	F	Hirsutism	25	>10	Left exophtalmia, left visual loss	Improvement
Patient 17	49	F	Hirsutism	25	>10	Right visual loss	Improvement
Patient 18	51	F	Hirsutism	25	>10	Frontal swelling	Improvement
Patient 19	33	F	Hirsutism	25	>10	Frontal swelling	Improvement
Patient 20	47	F	Hirsutism	25	>10	Right visual loss	Stable
Patient 21	43	F	Hirsutism	25	>10	Right visual loss	Stable
Patient 22	37	F	Hirsutism	25	>10	Left visual loss	Stable
Patient 23	68	F	Hirsutism	25	>10	Left visual loss	Improvement
Patient 24	50	F	Hirsutism	25	>10	Left visual loss	Improvement
Patient 25	67	F	Hirsutism	25	>10	Left visual loss	Improvement
Patient 26	56	F	Hirsutism	25	<10	Frontal syndrom, phasic disorders	Improvement
Patient 27	47	F	Hirsutism	25	>10	Left visual loss	Improvement
Patient 28	49	F	Hirsutism	25	>10	Left visual loss	Stable
Patient 29	50	F	Hirsutism	25	>10	Right visual loss	Improvement
Patient 30	58	F	Hirsutism	25	>10	Bilateral ophtalmoplegia	Improvement

microcystic, 1 (3.3%) metaplastic, and 1 (3.3%) chordoid (Figure 1 and Table 2). Fifteen (50%) meningiomas developed on Androcur® treatment had microcystic components. In addition, there were 38 (42.7%) meningothelial meningiomas in the control cohort, 45 (50.6%) transitional meningiomas, 6 (6.7%) were fibroblastic meningiomas, and none were of the microcystic, metaplastic, or chordoid subtypes (Figure 3). A positive association was shown between meningothelial and microcystic histology and Androcur® use (*P* = .0002), whereas a negative correlation seemed to exist between transitional subtype and having been exposed to Androcur® (*P* = .0002; Figure 1 and Table 2).

Meningiomas Developed on Cyproterone Acetate Are Mutated Frequently on PIK3CA/AKT1 Pathway

Five (16.7%) meningiomas of the CPA cohort were *AKT1* mutated; all 5 had the same E17K nonsense pathogenic mutation in exon 3. These 5 *AKT1* mutant meningiomas were all located in the skull base. One *AKT1*-mutated meningioma carried an additional mutation in the *MET* gene, G1108S. This mutation was classified as VOUS according to AMP recommendations. The control cohort presented fewer meningiomas with E17K mutation in *AKT1* (6%) than

Table 2. Demographic and Pathological Data of the Cyproterone acetate (CPA) Cohort Compared With the Control Cohort

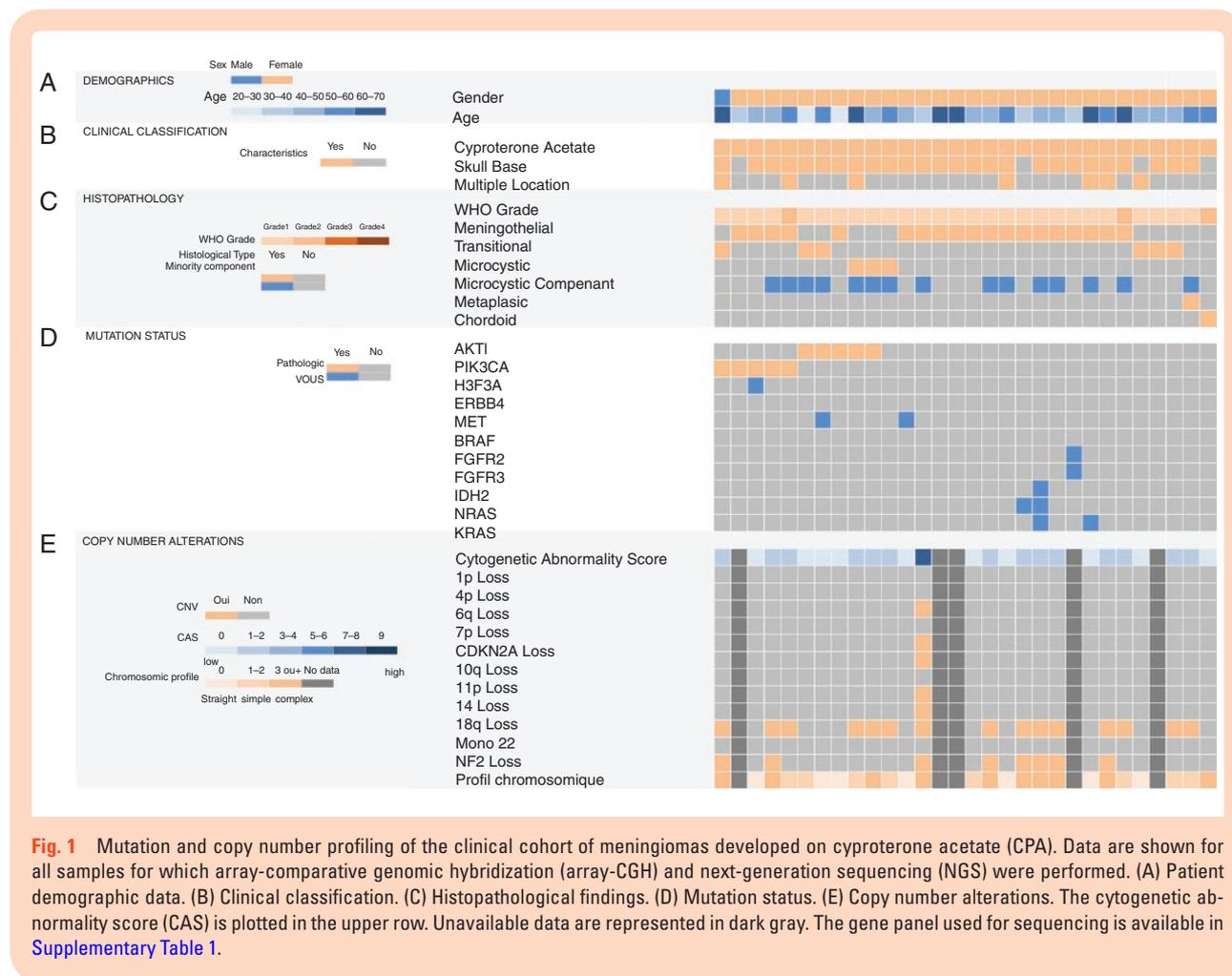|	CPA cohort	Control cohort	Chi2	Degree of freedom	*P*
Demographics data					
Total cases	30	150			
Age, mean (range), y	50 (29–68)	58 (22–90)			
Sex (F:M)	29:1	95:55			
Localization					
Skull base	26 (86.7%)	73 (48.7%)	13.10	1	<.001
Convexity	4 (13.3%)	77 (51.3%)			
AKT1 status					
Mutated	5 (16.7%)	9 (6%)	2.62	1	.10
Wild-type	25 (83.3%)	141 (94%)			
PIK3CA status					
Mutated	5 (16.7%)	7 (4.7%)	4.02	1	.045
Wild-type	25 (83.3%)	143 (95.3%)			
PIK3CA/AKT1 pathway status					
Mutated	10 (33.3%)	16 (10.7%)	8.64	1	.003
Wild-type	20 (66.7%)	134 (89.3%)			
CAS					
0	10 (40%)	55 (36.7%)	10.35	5	.06
1–2	14 (56%)	56 (37.3%)			
3–4	0	15 (10%)			
5–6	0	17 (11.3%)			
7–8	0	6 (4%)			
9	1 (4%)	1 (0.7%)			
WHO grade					
Grade I	27 (90%)	104 (69.3%)	5.54	2	.06
Grade II	3 (10%)	41 (27.3%)			
Grade III	0	5 (3.4%)			
Histopathology					
Chordoid	1 (3.3%)	0	23.74	5	<.001
Meningothelial	19 (63.4%)	38 (42.7%)			
Metaplastic	1 (3.3%)	0			
Microcystic	3 (10%)	0			
Transitional	6 (20%)	45 (50.6%)			
Fibroblastic	0	6 (6.7%)			

our cohort, but the difference was not statistically significant (*P* = .10; Figure 1 and Table 2).

Five (16.7%) meningiomas of the CPA cohort had a mutation in the *PIK3CA* gene. All *PIK3CA* mutations were missense mutations known to be pathogenic: E542G and E542K in exon 9; H1047L, H1047R, and G1049R in exon 20. One of these meningiomas carried 2 concomitant *PIK3CA* mutations, E542K and H1047R at different allele frequencies (58% and 7% respectively). The H1047L *PIK3CA* mutant meningioma also had 1 VOUS: A22V in *H3F3A* gene. Four of these 5 *PIK3CA* mutated meningiomas were located in the skull base. In Abedalthagafi et al. cohort, only 4.7% of the meningiomas carried oncogenic *PIK3CA* variants, 4 in exons 9 and 20 (E545K and 3 cases had H1047R mutations) and the rest

in exons 1, 5, and 8, respectively (E110del, N345K, E453K mutations). *PIK3CA* mutational state was associated with Androcur® exposure with a significant *P*-value (*P* = .045; Figure 1 and Table 2).

AKT1 and *PIK3CA* mutations in our CPA cohort were mutually exclusive. All in all, 33.3% meningiomas harbored at least 1 pathogenic mutation of the PIK3CA/AKT1 pathway. Compared with the control cohort, whereby only 10.7% had oncogenic mutations in the PIK3CA/AKT1 pathway, a positive association was established between *AKT1* or *PIK3CA* mutational status and Androcur® exposure (*P* = .003; Figures 1 and 2 and Table 2).

Other point mutations were identified in the CPA cohort. One meningioma had a *KRAS* mutation R149K. Two meningiomas carried mutations in the *NRAS* gene: C118Y

Fig. 1 Mutation and copy number profiling of the clinical cohort of meningiomas developed on cyproterone acetate (CPA). Data are shown for all samples for which array-comparative genomic hybridization (array-CGH) and next-generation sequencing (NGS) were performed. (A) Patient demographic data. (B) Clinical classification. (C) Histopathological findings. (D) Mutation status. (E) Copy number alterations. The cytogenetic abnormality score (CAS) is plotted in the upper row. Unavailable data are represented in dark gray. The gene panel used for sequencing is available in Supplementary Table 1.

and D119N. The meningioma with the *KRAS* mutation (R149K), *NRAS* mutation (D119N) also carried a mutation in the *IDH2* gene, R149W. *FGFR2* was mutated in 1 meningioma A284V and further mutated in the *FGFR3* gene, F384L. All of these mutations were categorized as VOUS.

Among the 5 *AKT1* mutated meningiomas of the CPA cohort, 2 were transitional meningiomas, 2 had microcystic subtype, and the remaining 1 was a meningothelial meningioma. All were WHO grade I. Four of the 5 *PIK3CA*-mutant meningiomas in the CPA series presented a meningothelial pattern and 1 was a transitional meningioma. The *PIK3CA*-mutated meningiomas were classified grade I, except for the meningioma that carried double *PIK3CA* mutations, classified as grade II meningioma because of a focal invasion of the parenchyma (Figure 1).

Chromosome Instability of Meningiomas Developed on Cyproterone Acetate

In 5 meningiomas, array-CGH could not be carried out due to insufficient DNA quantity. Among the 25 meningiomas analyzed, 24 (96%) had a low CAS score and none presented focal amplification. They included the 5 *AKT1*-mutated, the 5 *PIK3CA*-mutated, and the 3 microcystic meningiomas. The only meningioma within the CPA cohort

with a high CAS score was a meningothelial meningioma grade I, located in the skull base, *AKT1,* and *PIK3CA* wild-type. A majority of low CAS meningiomas (*n* = 126, 84%) were likewise found in the cohort without Androcur® exposure. No significant correlation between the CAS and Androcur® exposure was revealed, though a trend was noted (*P* = .06; Figure 1 and Table 2).

Seven (28%) meningiomas of the CPA cohort had a flat genomic profile on array-CGH, 8 (32%) had a simple profile, and 10 (40%) presented a complex profile (Figure 1 and Supplementary Table 2). The most frequently detected cytogenetic aberrations involved chromosomes 19 and 22. Chromosome 19 CNA was found in 16 (53.3%) meningiomas, whereas CNA of chromosome 22 was revealed in 9 (30%) meningiomas. Eight (26.67%) meningiomas presented 22q loss, a chromosomic region containing *NF2* gene (band 22q12). Two meningiomas with *NF2* gene deletion were *PIK3CA* mutant. The only meningioma with a high CAS score also presented a complex genomic profile with a gain of 3q and 14q regions, comprising the genes *PIK3CA* (band 3q26) and *AKT1* (band 14q32; Table 3). One complex case with CAS zero presented an atypical chromosomal profile with multiple whole-chromosome gains: chromosomes 4, 5, 8, 10, 11, 12, 13, 14, 17, 19, 20, and 21 (Table 3). Monosomy of chr22 was also highlighted in the control cohort without Androcur®
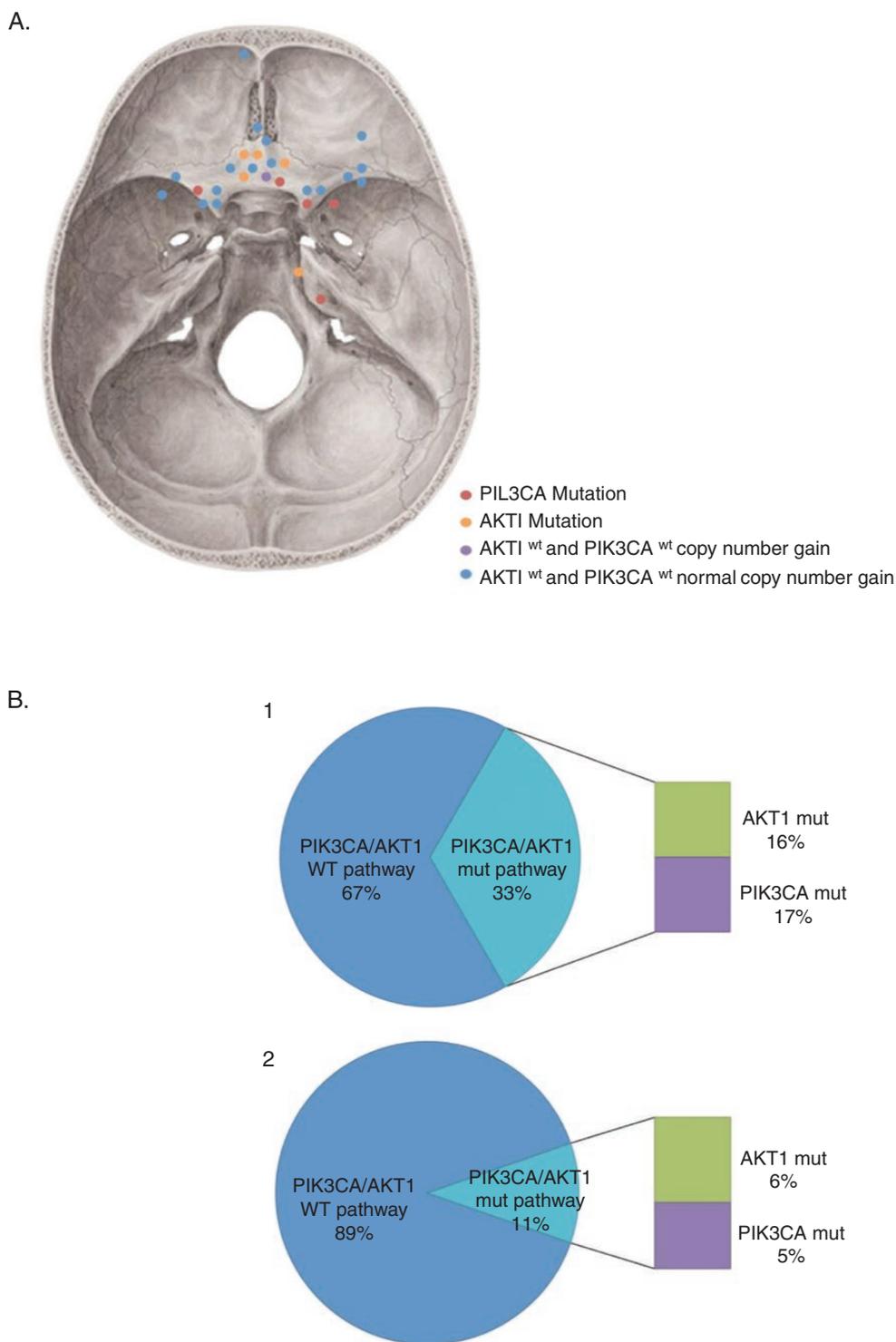
A.

- ● PIL3CA Mutation
- ● AKTI Mutation
- ● AKTI ^{wt} and PIK3CA ^{wt} copy number gain
- ● AKTI ^{wt} and PIK3CA ^{wt} normal copy number gain

B.

1

PIK3CA/AKT1
WT pathway
67%

PIK3CA/AKT1
mut pathway
33%

AKT1 mut
16%

PIK3CA mut
17%

2

PIK3CA/AKT1
WT pathway
89%

PIK3CA/AKT1
mut pathway
11%

AKT1 mut
6%

PIK3CA mut
5%

Fig. 2 Diagrams summarizing the mutation analysis of the *PIK3CA/AKT1* pathway in meningiomas developed on cyproterone acetate (CPA). (A) Schematic diagram depicting the approximate location of CPA meningiomas in the skull base. *PIK3CA* mutant, *AKT1* mutant, *AKT1* wild-type and *PIK3CA* wild-type with copy number gain and *PIK3CA* wild-type and *AKT1* wild-type with normal copy number are distinguished by different colored spots. (B) Diagram showing the distribution of pathogenic mutations of *AKT1* and *PIK3CA* genes in the CPA cohort (1) compared to the control cohort (2).
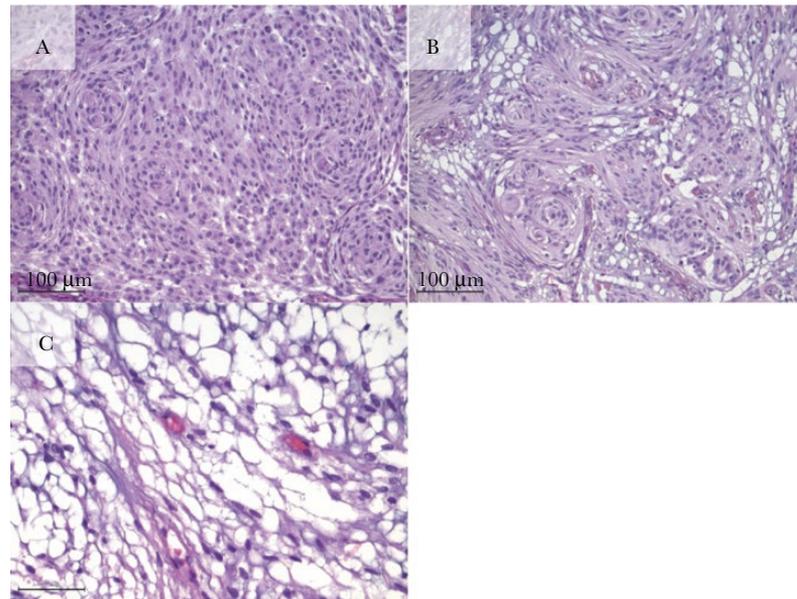
Fig. 3 Hematoxylin and eosin stain sections of the 3 main subtypes of meningiomas developed on cyproterone acetate (CPA): (A) meningothelial, characterized by sheets, whorls or syncytia of neoplastic cells which have round or oval centrally located nuclei and dispersed chromatin, smooth nuclear profiles and small indistinct nucleoli. (B) Transitional defined by mixed histology, typically containing meningothelial, and fibrous components and (C) microcystic with extracellular spaces, scattered throughout the meningioma substrate. (Scale length: 100 μm).

exposure, but the frequency of chr19 CNA appeared less frequent. Complete information on detected CNA is available in Supplementary Table 2.

Discussion

From a molecular standpoint, our results suggest that meningiomas arising on Androcur® are significantly enriched in pathogenic *PIK3CA* mutations. The gene *PIK3CA* encodes the catalytic subunit of the phosphatidylinositol 3-kinase enzyme involved in cellular proliferation through the PIK3CA/AKT1/mTOR signaling. Pathogenic mutations in *PIK3CA* lead to overactivation of the *PIK3* pathway.[22] The most frequently identified mutation in our cohort was H1047R in exon 20, in accordance with *PIK3CA*-mutated meningiomas described in the literature.[23] The detected mutations in the CPA series (E542K and E542G in exon 9, H1047L, H1047R, and G1049R in exon 20) were hotspot mutations, underlining a role in meningioma development.[24] Among them, E542G mutation has previously been reported in other tumors but not yet documented in meningiomas.[25] *PIK3CA* and *AKT1* mutations are known to be mutually exclusive[17,18] as was the case in our CPA cohort. We identified 2 *PIK3CA* mutations in the same meningioma tumor, which is in accordance with the intratumoral heterogeneity described in the literature.[18] Our results indicate a higher rate of PIK3CA/AKT1 pathway mutations in meningiomas developing on Androcur® treatment than in sporadic meningiomas. Peyre et al.[26] recently studied a series of progestin-associated meningiomas and also

reported a higher frequency of *PIK3CA* mutations in comparison with a control population. Incidence of *PIK3CA* mutation was higher compared with our study (35% vs 16.7%) but they did not observe any *AKT1* mutation enrichment. It is worth noting that Peyre et al. included patients with different progestin treatment (eg, egestrol acetate and chlormadinone acetate), whereas in our study, only patients with history of high-dose CPA treatment were included. Finally, only exons 9 and 20, which are known to contain most of the relevant oncogenic mutations, were analyzed in our study, whereas Peyre et al. analyzed all the exons of the gene.

Prior studies have shown that *PIK3CA* and *AKT1* mutations were more frequent in skull base meningiomas[17,27] and the results of our CPA cohort point toward preferential skull base localization for meningiomas arising on Androcur® use. Although correlating mutational status to tumor location, we can hypothesize that a mutation in the *PIK3CA/AKT1* signaling constitutes a favorable environment to onco-pharmacologic interaction between meningioma and Androcur®. In further investigation, it would be interesting to compare a CPA cohort, similar to ours, to a matched control for skull base location.

From a cytogenetic perspective, *PIK3CA*- or *AKT1*-mutated meningiomas have a stable chromosome profile.[16,17] That much said, our data did not disclose a statistically significant association regarding the cytogenetic abnormality score and whether or not Androcur® treatment had occurred. We can, therefore, extrapolate this stable genomic profile, characteristic of meningiomas harboring a *PIK3CA* or *AKT*1 mutation, to meningiomas

Table 3.   Two Meningiomas Developed on Cyproterone Acetate (CPA) With Atypical and Complex Chromosome Profile

	Loss	Cytobands	Genes with copy number loss	Gain	Cytobands	Genes with copy number gain
Patient 13	2q	q36,1-q37,3		1p	p31,1-p11,2	NOTCH2
	6q	q16,3-q22,1		1q	Whole	
	6q	q22,33-q25,2		2p	p23,3-p11,1	
	8	whole		3q	q26,1-q29	PIK3CA
	9p	p24,02-p21,1	CDKN2A, CDKN2B	6q	q22,32-q22,33	
	10p	p13-p12,31		7	Whole	EGFR
	10p	p12,1-p11,1		9q	Whole	KLF4
	10q	q21,1		10q	q11,22-q11,23	
	10q	q23,1-q23,31		11q	q14,3-q25	
	10q	q25,2-q26,2		12p	Whole	
	13q	q13,3-q31,1		13q	q12,11-q13,3	
	14q	q23,1-q32,33		13q	q31,1-q34	
	17q	q11,2		14q	q11,2-q23,1	AKT1
	18p	Whole		15q	q22,2-q26,3	
	18q	q22,1-q23		17p	p13,1-p11,1	
	19q	q12-q13,11		19p	p13,3-p13,2	
	19q	q13,2-q13,43		19p	p13,11-p12	
	20p	p12,3-p12,2		20q	q11,23-q13,33	
	20p	p11,23-p11,1		22q	q13,1-q13,33	
	20q	q11,21-q11,22				
	21q	q11,2-q22,13				
	22q	q11,21-q13,1	NF2, SMARCB1, CHEK2			
Patient 30	—	—	—	4	Whole	
	—	—	—	5	Whole	PIK3R1, hTERT
	—	—	—	8	Whole	
	—	—	—	10	Whole	SUFU
	—	—	—	11	Whole	
	—	—	—	12	Whole	
	—	—	—	13	Whole	
	—	—	—	14	Whole	AKT1
	—	—	—	17	Whole	RPS6KB1, POLR2A, BRIP1, SMARCE1
	—	—	—	19	Whole	
	—	—	—	20	Whole	
	—	—	—	21	Whole	

arising on Androcur® treatment. Interestingly, 2 wild-type meningiomas *AKT1* and *PIK3CA* in our cohort presented copy number gains involving these genes: 1 meningioma had *AKT1* gain and the other had both *AKT1* and *PIK3CA gains*. These 2 tumors might have an activation of the *PIK3CA/AKT1* signaling path via different mechanisms than classic point mutations, further supporting the hypothesis that this molecular pathway could be a major actor of meningiomas developed on Androcur®.

Because *PIK3CA* and *AKT1* are therapeutic targets,[28,29] the molecular characterization of meningiomas developing on Androcur® impels a reevaluation of the diagnostic and therapeutic strategy of these tumors. The surgical management of patients affected by meningiomas at the skull base can be quite problematic, depending on proximal neurovascular structures. Tumoral growth and surgical resection could lead to significant morbidity.[30] Several clinical trials are ongoing for molecules targeting

PIK3CA/AKT1 signaling in several cancer types (eg, NCT01226316, NCT03006172, NCT02822482) and a recent case report described the response of an *AKT1*-mutant meningioma to AKT1 inhibition.[31] Targeting oncogenes would hinder cellular proliferation in patients not showing a suitable clinical and/or radiological response after Androcur® discontinuation. Acknowledging molecular profile of meningioma may help in deferring the surgical indication in cases of contra-indication to surgery or high-risk procedures, which are often the case in skull base meningiomas; or patients in whom stopping Androcur® treatment is unsought in light of the clinical context. The outcome of our report highlights the importance of *PIK3CA/AKT1* inhibition and suggests a need for further phase II and III prospective studies.

On a histopathologic level, it appeared that meningiomas arising on Androcur® exposure were essentially low-grade meningothelial meningiomas. Once again, if we associate this outcome with tumor location, it appears that meningiomas developed on Androcur® treatment were mainly meningothelial meningiomas of the skull base. Lee et al. showed that most of the meningiomas occurring in the skull base have meningothelial histology.[3] Our results are consequently consistent with data from the literature. Our CPA cohort contained only 3 malignant meningiomas, and all the remaining ones were benign. We did not find a statistically significant association between the use of Androcur® and WHO grade. These findings are in line with epidemiological studies that report a vast majority of grade I among meningiomas.[1,2]

Previous genomic studies revealed a direct link between quantitative chromosomal abnormalities and malignancy of meningiomas.[24,25,32,33] All meningiomas in our CPA cohort had low CAS, except for one. This observation is consistent with the fact that almost all were grade I meningiomas. However, our study was not designed to determine whether CAS is associated with molecular grade of tumor or with drug exposure. Moreover, several authors had reported that *PIK3CA*- or *AKT1*-mutated meningiomas were grade I[16,17] as was the case in our study. Three meningiomas of the CPA series were classified grade II according to histoprognostic criteria of the 2016 WHO classification. One had a dominant chordoid component and the 2 others presented on the initial histopathologic reports a focal invasion of the underlying parenchyma that was not seen on slide review. Although the malignancy of a chordoid meningioma is indisputable, focal invasion of the cerebral parenchyma is more controversial. Although it is well-known that parenchymal infiltration in a grade I meningioma confers relapse and mortality rates similar to that of a grade II meningioma, the previous WHO classification did not consider invasion of the cerebral parenchyma as a grading criteria.[34] To avoid confusion, the current 2016 WHO classification henceforth recognizes the invasion of the underlying parenchyma as an atypical character and a sufficient argument to classify as grade II.[1,19] Genetically speaking, the 2 meningiomas with focal invasion of the underlying parenchyma had low CAS, simple chromosomal profile, and 1 was mutated for *PIK3CA*, manifesting chromosomal stability as per Abedalthagafi et al.'s conclusions. From a molecular angle, they would have a slow tumoral progression comparable with that of a grade I meningioma. Further genomic tumor explorations are needed to identify additional molecular markers predictive of meningioma progression and to ultimately integrate the molecular findings into histological classifications, as with gliomas.[35]

Half of the meningiomas in the CPA cohort showed microcystic components: 3 had major components and 12 had minor microcystic components of variable importance. Knowing that microcystic meningiomas commonly represent only 1.6% of meningiomas,[36] our findings imply an association between microcystic component and exposure to Androcur®. Nevertheless, meningiomas are polymorphous tumors and minor components have not been thoroughly described. The presence of microcystic histology might be an indirect marker of underlying genetic mechanisms that could play a role in the onco-pharmacologic meningioma-Androcur® interaction. Variation in tumor size post-discontinuation of the treatment could thereby be attributed to the variation in microcystic components ratio. Several hypotheses have been put forward on the pathogenesis of intra- and extra-cellular microcysts,[37,38] but further implication has yet to be demonstrated first in sporadic meningiomas and later in meningiomas occurring on Androcur® exposure.

Posterior meninges develop from the mesoderm embryonic tissue, whereas meninges of the anterior floor and anterior cranial vault originate from the neural crest.[3,39] Genomic studies have demonstrated that genetic aberrations mimicked this structure. Alterations of *NF2* gene preferentially concerned meninges of the posterior cranial fossa and cerebral convexity, whereas alterations of *TRAF7, SMO, KLF4, AKT1*, and *PIK3CA* genes involved meninges of the anterior cranial fossa.[17,40] Moreover, meningiomas of the anterior and middle cranial cavities are *NF2* wild-type and almost all are benign with chromosomal stability. On the other hand, meningiomas arising from cerebral and cerebellar convexities harbor mutations in *NF2* gene and/or chromosome 22 deletion and are more likely to be atypical tumors with chromosomal instability.[40] In our study, we found that 2 *PIK3CA* mutant meningiomas had *NF2* gene deletion, which is uncommon but has been reported.[18] To the best of our knowledge, there have not been yet any previous publications on the molecular status of meningiomas arising on Androcur® treatment. Moreover, the strength of our study lies in its sample size: with 30 meningiomas developed on CPA intake, it constitutes the largest cohort described in the literature. As for the limitations, our gene panel only contained actionable genes with prospective therapeutic targets and the other genes described in the literature for meningioma, such as *NF2,* were not characterized. This strategy has the advantage of detecting fewer mutations with uncertain significance.

In addition, because the molecular characterization of tumor sample requires tumor material, our recruitment was built on operated meningiomas that did not sufficiently respond to withdrawal of Androcur® treatment. This might create a selection bias regarding underlying oncogenic mechanisms. Nonetheless, despite the need for surgical intervention, 70% of the patients showed clinical and/or radiological improvement after discontinuation of the treatment. It is, therefore, conceivable that the histomolecular

characteristics observed in operated meningiomas are the same as in nonoperated meningiomas.

Finally, the sex ratio between our cohort and the control cohort was quite different and a subsequent study with age and sex-matched control would be of great interest to ascertain our results.

In conclusion, the advent of new genetic technologies over the past years has generated major progress in understanding the molecular and cytogenetic events involved in meningiomagenesis. Given the current state of evidence, several oncogenic mechanisms exist, each with a different molecular pathway. The refinement of our knowledge on molecular genetics of meningiomas could have a direct impact on the diagnosis and management of these tumors. Our publication is the first to analyze the histomolecular status of meningiomas developed on Androcur® treatment. The discovery of divergences between sporadic meningiomas and those arising on Androcur® intake facilitates understanding of the underlying oncogenesis.

Supplementary Material

Supplementary data are available at *Neuro-Oncology Advances* online.

Keywords

AKT1 | cyproterone acetate | meningioma | molecular pathology | PIK3CA.

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