Circulating tumor DNA is present in the most aggressive meningiomas

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Recent discoveries of multiple driver mutations open promising perspectives for targeted therapies in meningioma. Nevertheless, iterative recurrences of most aggressive meningiomas as extended skull base meningiomas are not systematically operated and historically documented. This suggests the interest and the relevance of liquid biopsy in meningiomas. In a proof-of-concept study, we detected the NF2 mutation in 2 of 6 cell-free plasma DNAs and 1 of 1 cerebrospinal fluid (CSF) from high-grade recurrent cases, suggesting that identification of the driver mutation in blood and CSF is today feasible. Liquid biopsy could be an interesting tool to adapt the targeted therapy in meningiomas in the near future.

Since the discovery of NF2’s involvement, many advances have been performed in the description of meningioma mutational landscape. The therapeutic management of refractory meningioma to iterative surgeries and radiotherapy sessions remains an unmet medical need in neurooncology. Targeted therapies were suggested to be of interest in aggressive meningiomas, particularly for skull base ones given the large pattern of newly discovered driver mutations, which could be targeted by specific inhibitors. Recently, a case of AKT1-mutated skull base meningioma treated by AKT1 inhibitor was reported to be successful. PI3K–Akt–mTOR pathway targeting was also demonstrated to be relevant in meningiomas.

Although tumor tissue is likely available in recurrent high-grade meningiomas, iterative tumor recurrences are not systematically operated and often non-histologically documented. Recent studies demonstrated that the mutational pattern of meningioma recurrence could differ from the initial tumor to remote recurrence. Moreover, complex skull base meningiomas are often nonaccessible to surgery, treated without histology, and yet could benefit from targeted therapies. Therefore, in these different situations, liquid biopsy could be of interest in the patient diagnosis, prognosis, and to guide therapeutic management.

The detection of tumor mutation in cell-free plasma DNA (cfDNA) constitutes the most promising biomarker in cancer. In meningioma, in contrast to other brain tumors, the lack of brain–blood barrier makes possible the passage of circulating tumor DNA (ctDNA) in the blood. As an alternative solution tumor mutations could be searched in the CSF. In a proof-of-concept study, we analyzed cfDNA in blood from 15 patients and in CSF from 3 other patients, all previously identified with at least one pathogenic variant in their tumor, except one (M17, Table 1).

Eleven patients presented with benign meningioma (WHO grade I and II) and 7 with recurrent high-grade meningiomas were included. Written informed consent was required for each patient. The study was approved by the Aix-Marseille University IRB.

In all cases, the blood sample was taken before surgery. The CSF has been collected during surgery for thoracic benign meningioma (M10) and during ventriculoperitoneal shunt implantation in 2 cases (M11, benign and M17, aggressive). cfDNAs were extracted from 4 mL of serum and from 1.5 to 4 mL of CSF; all previously identified with at least one pathogenic variant in their tumor, except one (M17, Table 1).

Specimens were analyzed by ultradeep sequencing on a MiSeqDx (Illumina) using the Custom QIAseq targeted DNA Panel library preparation (Qiagen) targeting NF2, AKT1, SMO, KLF4, TRAF7, PIK3CA, SUFU, SMARCB1, SMARCE1, CDKN2A, CDKN2B, PTEN, and TERT. This library preparation
| Tumor Age (year) | Sex | WHO grade | Histological subtype | Tumoral location | Tumoral volume (cm\(^3\)) | Tumoral extension | Number of surgical removal | Mutated gene | Driver mutation(s) identified in tissue | Mutated allelic fraction \(^a\) in tissue (%) | Sample coverage \(^b\) | Coverage of depth at the position of the driver mutation \(^c\) | Allelic fraction positive reads/total reads (%) in cfDNA or CSF | Mutated allelic fraction detected \(^d\) in tumor biopsy |
|-----------------|-----|-----------|----------------------|-----------------|-----------------|----------------|----------------|--------------|---------------------------------|-----------------------------|----------------|-----------------------------------------|------------------------------------------------|----------------------------------|
| 7               | F   | Benign    | Meningothelial       | Tuberculum      | 7               | —              | 1              | TRAF7        | c.1606 G>A, p.(Gly536Ser)       | 36.7%                        | cfDNA No             | 1759×                                                  | 36.7%                                                             | No (28.7%)                         |
| 58              | F   | Benign    | Meningothelial       | Frontal         | 16              | 1              | TRAF7         | c.1225 A>C, p.(Gly410Asp)       | 46.9%                        | cfDNA No             | 2106×                                                  | 46.9%                                                             | No (11.4%)                         |
| 79              | F   | Benign    | Meningothelial       | Parasagittal    | 18              | 1              | TRAF7         | c.654 delA, p.(Glu218fs)        | 41.8%                        | cfDNA No             | 2178×                                                  | 41.8%                                                             | No (28.7%)                         |
| 57              | F   | Benign    | Meningothelial       | Conve'ne plaques | 57              | 1              | AKTI          | c.1657 G>T, p.(Glu553Lys)       | 31.7%                        | cfDNA No             | 2400×                                                  | 31.7%                                                             | No (28.7%)                         |
| 70              | M   | Benign    | Meningothelial       | Temporal        | 1              | 1              | TRAF7         | c.1657 G>T, p.(Glu553Lys)       | 31.7%                        | cfDNA No             | 2400×                                                  | 31.7%                                                             | No (28.7%)                         |
| 65              | F   | Recurrent high-grade meningiomas | Meningothelial | Thoracic | 3               | 2              | TRAF7         | c.37 del C, p.(Glu12fs)         | 47.3%                        | cfDNA No             | 2106×                                                  | 47.3%                                                             | No (28.7%)                         |
| 79              | F   | Recurrent high-grade meningiomas | Meningothelial | Thoracic | 3               | 2              | TRAF7         | c.37 del C, p.(Glu12fs)         | 47.3%                        | cfDNA No             | 1292×                                                  | 47.3%                                                             | No (28.7%)                         |
| 79              | F   | Recurrent high-grade meningiomas | Meningothelial | Thoracic | 3               | 2              | TRAF7         | c.37 del C, p.(Glu12fs)         | 47.3%                        | cfDNA No             | 1292×                                                  | 47.3%                                                             | No (28.7%)                         |
| 79              | F   | Recurrent high-grade meningiomas | Meningothelial | Thoracic | 3               | 2              | TRAF7         | c.37 del C, p.(Glu12fs)         | 47.3%                        | cfDNA No             | 1292×                                                  | 47.3%                                                             | No (28.7%)                         |
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| 79              | F   | Recurrent high-grade meningiomas | Meningothelial | Thoracic | 3               | 2              | TRAF7         | c.37 del C, p.(Glu12fs)         | 47.3%                        | cfDNA No             | 1292×                                                  | 47.3%                                                             | No (28.7%)                         |

\(^a\) The coding exons and exon–intron boundaries of 13 genes (\(\text{NF2}\) (NM_000268.3), \(\text{AKT1}\) (NM_001014431.1), \(\text{SMO}\) (NM_005631.4), \(\text{KLF4}\) (NM_004235.4), \(\text{TRAF7}\) (NM_032271.2), \(\text{PIK3CA}\), \(\text{SUFU}\), \(\text{SMARCB1}\) (NM_003073.3), \(\text{SMARCE1}\) (NM_003079.4), \(\text{CDKN2A}\) (NM_058195.3), \(\text{CDKN2B}\) (NM_004936.3), \(\text{PTEN}\) (NM_000314.4), and \(\text{TERT}\) (NM_198253.2) were sequenced using the Custom QIAseq targeted DNA Panel (Qiagen) on a MiSeqDx (Illumina) as previously described.

\(^b\) The mutated allelic fraction is the count of mutated alleles out of the total number of alleles (wild type + mutated).

\(^c\) Coverage of depth: number of times the nucleotide seat of the mutation in the tissue has been read by sequencing.

\(^d\) No mutation detected in the tumor biopsy.
It was the first study identifying ctDNA mutation driver from meningioma patients. A previous study analyzed cfDNA from 34 meningiomas. In this study, several variants have been identified, but no gene was a driver for meningioma. Moreover, no correlation was made with the tumor; therefore, the tumor origin of the identified variants cannot be asserted.

In the patient M17, even with a good quality of biopsy material, no NF2 genetic alteration was detected but the issue could be the area of tumor biopsy in this huge tumor. The lack of NF2 mutation is frequent in skull base meningiomas with meningothelial and secretory features. Two hypotheses may be done to explain the NF2 mutation detected in CSF: (1) a second genetic occurrence with an NF2 mutation leading to extremely aggressive meningioma or (2) a second meningioma occurrence. However, the second hypothesis is not in accordance with the MRI showing a clear tumor progression (Figure 1).

In benign meningiomas, mutation detection in circulating DNA remains negative suggesting a low level of ctDNA in blood and CSF. In the aggressive tumors, mutation drivers were detectable in cfDNA from 2 of 6 patients and in CSF from 1 of 1 patient. Recurrent high-grade meningiomas represent a low part of meningiomas, but their therapeutic management remains particularly challenging today. The role of the subcutaneous invasion in ctDNA detection remains unknown and requires further studies. It has been shown that patients with tumors limited to the central nervous system have significantly enriched ctDNA in CSF. Interestingly, we were able to detect in CSF an NF2 mutation not found in the tumor, a feature already reported in brain tumor, which may be due to the tumor heterogeneity. Further studies are required to assess the sensitivity and the interest of ctDNA.
analysis to address some limitations of tissue-based genetics. Circulating tumor DNA could be currently detected in the blood and the CSF of recurrent high-grade meningiomas. The presence of ctDNA in blood or CSF from meningioma patients could be an interesting tool in a near future in a selected population to determine the tumor mutational change in recurrent high-grade meningiomas and to adapt the targeted therapy.

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