

Case Report

EXO1 homozygous deletion suppresses the hydroxyurea sensitivity in anaplastic meningioma with extracranial metastases

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Abstract: Background: Anaplastic meningioma is exceedingly rare, and its clinicopathological features are distinctive. Distant meningioma metastases have previously been reported to be very rare. The most common site of metastasis is the lung, which accounts for 61% of all meningioma metastases. The standard therapy for meningiomas is total resection and/or radiation therapy. Case Report: A 71-year-old man was admitted with headache and left hemiparesis. Magnetic resonance imaging (MRI) revealed mass lesions at the right frontal convexity and left occipital lobe. Following surgery, pathological examinations demonstrated an anaplastic meningioma. At 3 months after the operation and radiation therapy, lung tumor was removed. The pathological findings for the lung tumor resembled those of the brain tumor. A diagnosis of metastatic lung tumor from anaplastic meningioma was made. At 2 months after the lung surgery, MRI disclosed focal recurrence in the frontal convexity area and progression into the cavernous sinus. Abdominal CT detected a new metastatic lesion in the patient's liver. He underwent adjuvant reirradiation consisting of whole brain radiotherapy. In addition, he received chemotherapy using angiogenesis receptor (bevacizumab). Chemotherapy with hydroxyurea was initiated after 2 courses of the bevacizumab chemotherapy, because his hepatic lesion had become more aggressive. The intracranial mass was reduced by 50% at 8 weeks after initiation of the hydroxyurea chemotherapy. However, there was no significant effect on the liver and lung metastases. Analysis and Conclusion: Extracranial metastases have been estimated to occur in only 0.1% of all meningiomas and most often in association with anaplastic meningiomas. While total resection and/or radiation treatment provide the standard therapy for meningiomas, use of chemotherapy against meningiomas has been limited to distant metastases or based on out of surgical criteria. Several reports have demonstrated that hydroxyurea provides an effective chemotherapy for meningiomas including grade I cases. Hydroxyurea halts meningioma cell growth through arrest of the S-phase of the cell cycle, thus inducing apoptosis. Our analysis revealed *EXO1* homozygous deletion at exons 8 and 10 in the patient's extracranial metastatic meningioma. The findings suggested that *EXO1* homozygous deletion may be associated with suppression of hydroxyurea sensitivity in anaplastic meningiomas. Hydroxyurea should be a justified therapeutic adaptation in cases of anaplastic meningioma without *EXO1* homozygous deletion.

Keywords: Anaplastic meningioma, *EXO1*, hydroxyurea

Introduction

Meningiomas are the most common adult central nervous system (CNS) tumors. Their frequency accounts for 13-26% of all primary intracranial tumors [1]. The World Health Organization (WHO) classifies meningioma into benign (WHO grade I), atypical (WHO grade II) and

anaplastic (WHO grade III) types, which comprise 90%, 7% and 3% of all meningiomas [2]. Anaplastic meningiomas are characterized by elevated atypia, anaplasia, proliferation rate, mitotic index (20 or more mitoses) and necrosis areas [3]. They carry a particularly poor prognosis, with a median survival time of less than 2 years and a 5-year mortality rate of 68% [3, 4].

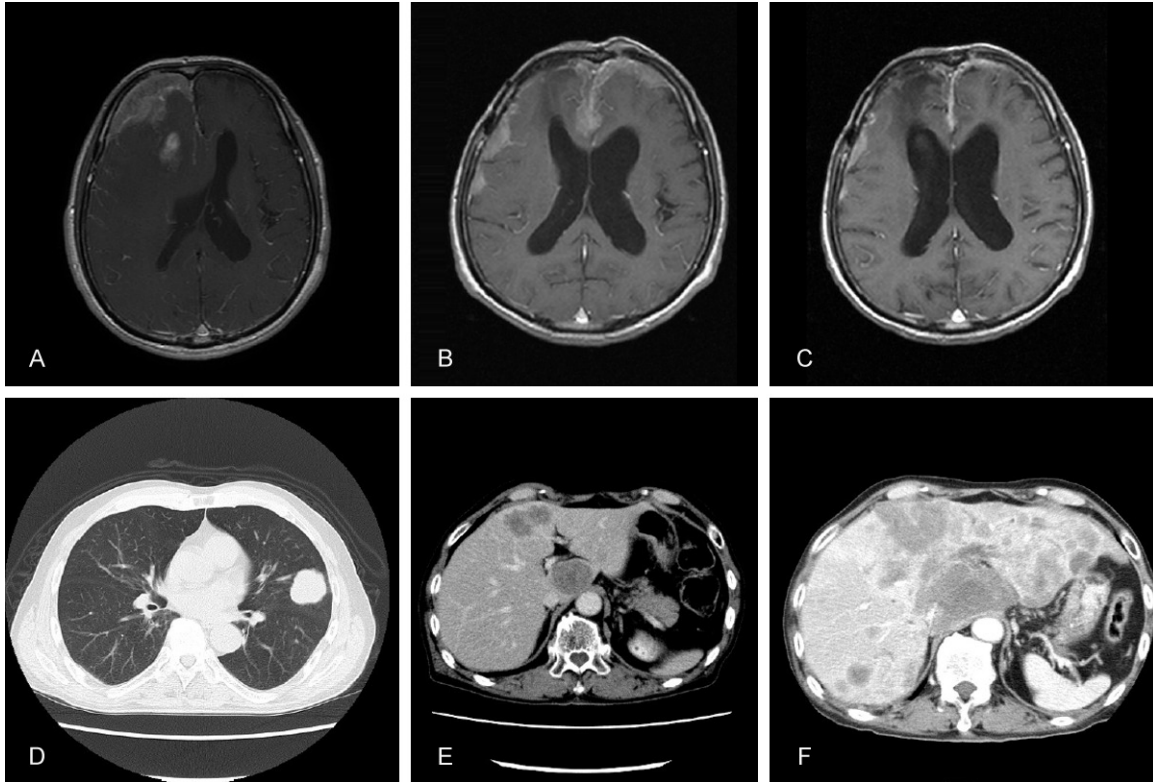


Figure 1. MRI T1-weighted images with Gd-enhancement. A: Pre-operative image. B: Post-operative, pre-chemotherapy image. C: Post-chemotherapy image. D: Chest CT image with enhancement at post removal of brain tumor. E: Abdominal CT image with enhancement at pre-chemotherapy. F: Abdominal CT image with enhancement at post-chemotherapy.

Anaplastic meningiomas display a high frequency of local recurrence, brain invasion, and metastases. Distant meningioma metastases have previously been reported to be very rare [5]. The most common site of metastasis is the lung, which accounts for 61% of all meningioma metastases [6, 7], followed by the liver, lymph nodes, and bones [6, 8].

The standard therapy for meningiomas is total resection and/or radiation therapy. Treatment of distant meningioma metastases is exceedingly difficult. We must choose the standard therapy for local control and general management including chemotherapy. In a phase II study, no patients were found to experience a complete or partial radiographic response to temozolomide therapy [9]. The majority of case series employing chemotherapy for anaplastic meningiomas have examined the effects of hydroxyurea, imatinib, somatostatin analogs, and angiogenesis inhibitors [10]. Hydroxyurea, an oral ribonucleotide reductase inhibitor, has become a standard therapy in the treatment of meningiomas refractory to surgery and radiation [10].

This report describes a case of anaplastic meningioma with focal recurrence and distant metastases to the lung and liver which was treated with hydroxyurea. The intracranial recurrence tumor was successfully brought under control. However, the distant metastatic lesions became more aggressive after 3 courses of hydroxyurea chemotherapy. We compare the genetic characteristics of anaplastic meningiomas between hydroxyurea-sensitive and anti-sensitive tumors.

Case report

A 71-year-old man had been in excellent health until he presented to another clinic with progressive headache. A computed tomography (CT) scan revealed an intracranial mass lesion, and he was then admitted to our hospital at one month after symptom appearance. The results of his physical examinations were essentially normal without mild disturbance of consciousness. A CT scan demonstrated mass lesions at the right frontal convexity and right parietal lobe. Magnetic resonance imaging (MRI) was performed, and the T1-weighted

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image disclosed a 60×40×30 mm low-intensity lesion in the right frontal convexity and a 5×5×5 mm low-intensity lesion in the left occipital lobe, with homogeneous contrast enhancement (**Figure 1**). Serum levels of tumor markers including CEA (carcinoembryonic antigen), CA19-9 (carbohydrate antigen 19-9), AFP (alpha-fetoprotein) and SCC (squamous cell carcinoma antigen) were negative. Chest X-ray and CT examinations revealed a 40×30×20 mm mass lesion in the left lung at the S3 area. The pre-operative diagnosis was metastatic brain tumors with lung cancer. On day 13 after admission, we performed surgery to remove the tumor at the right frontal convexity. Macroscopically, the tumor was reddish, soft, and vascular-rich. After the operation, there was no other neurological deficit, and the patient did not require care in his daily life. Pathological examinations demonstrated the tumor to be an anaplastic meningioma. Adjuvant therapy for the intracranial lesions including the operative lesion and left occipital lesion comprised targeted radiation therapy with a γ -knife.

At 3 months after the operation and radiation therapy, the lung tumor was removed. The pathological findings for the lung tumor resembled those of the brain tumor. A diagnosis of metastatic lung tumor from anaplastic meningioma was made.

At 2 months after the lung surgery, the patient displayed new symptoms, including appetite loss, polyopia and ptosis. MRI revealed focal recurrence in the frontal convexity area and progression into the cavernous sinus. Abdominal CT detected a new metastatic lesion in his liver. He underwent adjuvant reirradiation consisting of whole brain radiotherapy (30 Gy in 20 daily fractions of 1.5 Gy each). In addition, he received chemotherapy using angiogenesis receptor (bevacizumab). Chemotherapy with hydroxyurea (Hydrea; Bristol-Myers-Squibb, NY) was initiated after 2 courses of bevacizumab chemotherapy, because his hepatic lesion became more aggressive. As outlined in a previous report, the hydroxyurea was administered orally for 28 consecutive days (1,000 mg/m²/single daily dose) every 4 weeks (operationally defined as a cycle of therapy) [11].

The intracranial mass was reduced by 50% at 8 weeks of initiation of the chemotherapy with hydroxyurea. However, there was no significant effect on the liver and lung metastases (**Figure 1**).

Pathological findings

Intracranial lesion

The specimen exhibited increased cellular pleomorphism, nuclear atypia, presence of macronuclei and small cell cytology. There was a high mitotic index (20> mitoses) and area of necrosis as well as a meningomatous tissue pattern (**Figure 2**). Glial fibrillary acid protein (GFAP) and S-100 were immunohistochemically negative. Epithelial membrane antigen (EMA) was immunohistochemically positive. Vimentin was immunohistochemically positive in the focal area. The MIB-1 labeling index (LI) was 40%.

Intrathoracic lesion

Most findings resembled those of the intracranial lesion on HE staining.

Genetic alterations

Screening of 8 genes (*NF2* (*Neurofibromin 2, merlin*), *TRAF7* (*TNF Receptor-Associated Factor 7*), *KLF4* (*Kruppel-Like Factor 4*), *AKT1* (*V-Akt Murine Thymoma Viral Oncogene Homolog 1*), *SMO* (*Smoothened*), *KIT* (*V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog*), *ERBB2* (*Erb-B2 Receptor Tyrosine Kinase 2*), and *MET* (*MET Proto-Oncogene*)) for single-nucleotide variants (SNVs) and copy number variants (CNVs) was carried out by next generation sequencing of both the intracranial meningioma and the intrathoracic meningioma. Genomic DNA from paraffin sections was extracted as reported previously [12]. For the control of copy number alterations, genomic DNA was extracted from the blood of three healthy volunteers using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The GeneRead DNaseq Targeted Panel V2 (Qiagen) was used for library preparation with an amplicon sequencing panel targeting the entire exonic sequence of six genes (*NF2*, *AKT1*, *SMO*, *ERBB2*, *KIT*, and *MET*; GeneRead Mix-n-Match panel, 181905 MNGHS-00062X-296; Qiagen) and a custom panel targeting the whole exon of *TRAF7* and partial exon of *KLF4* (targeting *KLF4* K409Q) (GeneRead Custom panel, 181902 CNGHS-00120X-44; Qiagen). The libraries were sequenced using the Illumina MiSeq to produce 150-bp paired-end reads. The raw read data obtained from amplicon sequencing were processed by online analytical resources from the GeneRead DNaseq Variant Calling Service for analysis of mutations and copy number alterations.

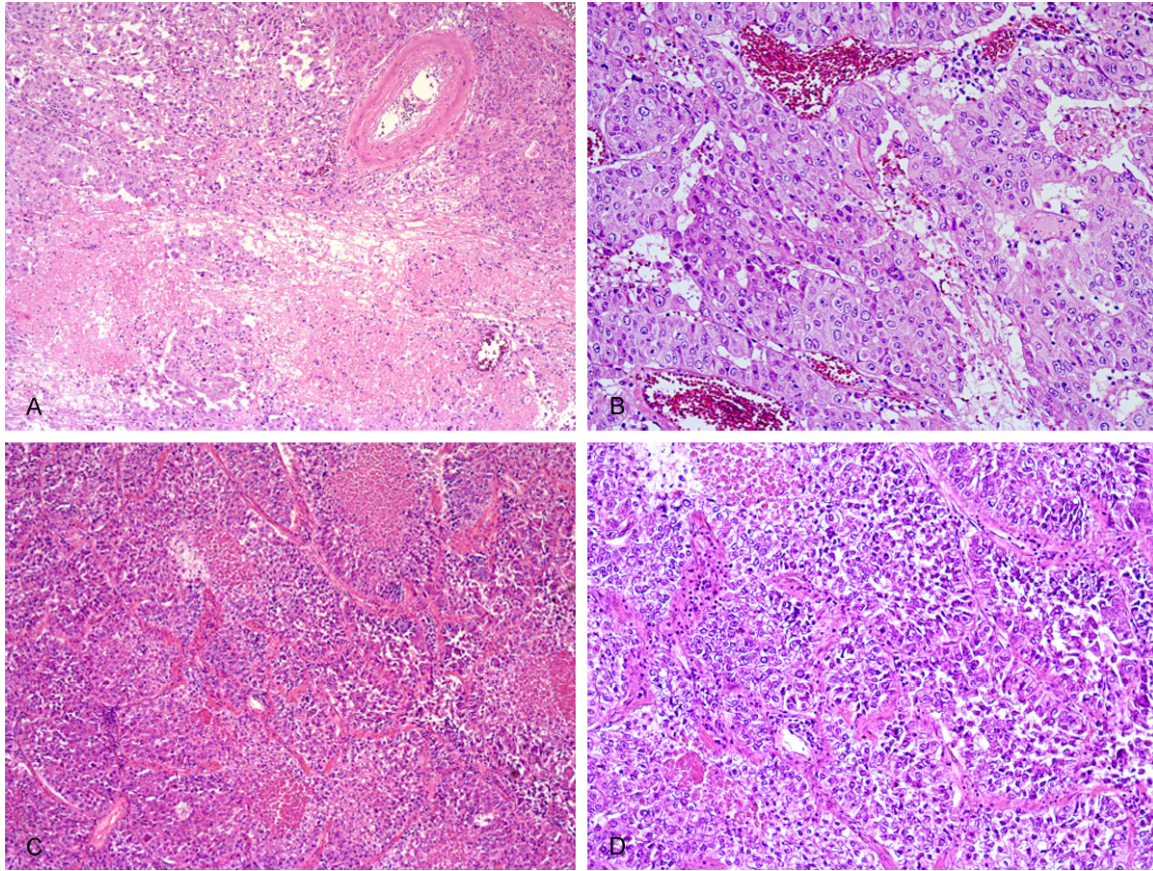


Figure 2. Histological features of the intracranial meningioma and extracranial metastatic meningioma. A: Intracranial tumor HE; original magnification, $\times 40$. B: Intracranial tumor HE; original magnification, $\times 100$. C: Extracranial metastatic tumor HE; original magnification, $\times 40$. D: Extracranial metastatic tumor HE; original magnification, $\times 100$.

Both samples displayed NF2 loss, KIT loss, MET loss and ERBB2 amplification (**Figure 3**). The genetic alterations were found to be similar in the intracranial and extracranial meningiomas.

Differential PCR for homozygous deletion of EXO1 genes

We carried out differential PCR for *EXO1* (*Exonuclease 1*) genes, using the cystic fibrosis transmembrane conductance regulator (*CFTR*) sequence as a reference. The primer sequences were 5'-AGA CGA CAA GCC AAT CTT CTT-3' (forward) and 5'-GCC ATG GCA TGT GTG ATA TT-3' (reverse) for *EXO1* exon 5 (product, 107 bp), 5'-TGA ATA TCA CGG TAC CAG AGG A-3' (forward) and 5'-TCT TCA TAG GCG TTC AGA GGA-3' (reverse) for *EXO1* exon 8 (PCR product, 121 bp), 5'-CCC CAC AAT TGA AGG AAA AT-3' (forward) and 5'-TTC TTG GTC TTT TCA CAA TGG A-3' (reverse) for *EXO1* exon 10 (PCR product, 105 bp), and 5'-TCA AAG CAT GCC AAC TAG AAG

A-3' (forward) and 5'-TGG GTA GTG TGA AGG GTT CAT-3' (reverse) for *CFTR* (PCR product, 79 bp).

Differential PCR was carried out using a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA) in a total volume of 10 μ l, consisting of PCR buffer (20 mM Tris-HCl, pH 8.0; 40 mM NaCl; 2 mM sodium phosphate; 0.1 mM EDTA; 1 mM DTT; stabilizers; and 50% glycerol), 3 mM $MgCl_2$, dNTPs (250 μ M each), sense and anti-sense primers (0.3 μ M each for *EXO1* exon 5 and exon 10, 0.2 μ M each for *EXO1* exon 8), *CFTR* sense and antisense primers (0.2 μ M each), 0.5 units of PLATINUM[®] *Taq* DNA polymerase (Invitrogen) and DNA (approx. 40 ng). Initial denaturing at 95°C for 5 min was followed by 32 cycles of denaturing at 95°C for 45 sec, annealing at 57°C for 45 sec, and extension at 72°C for 45 sec. A final extension step at 72°C for 5 min was added. The PCR products were subjected to electrophoresis on 8% acrylamide gels, and visualized with

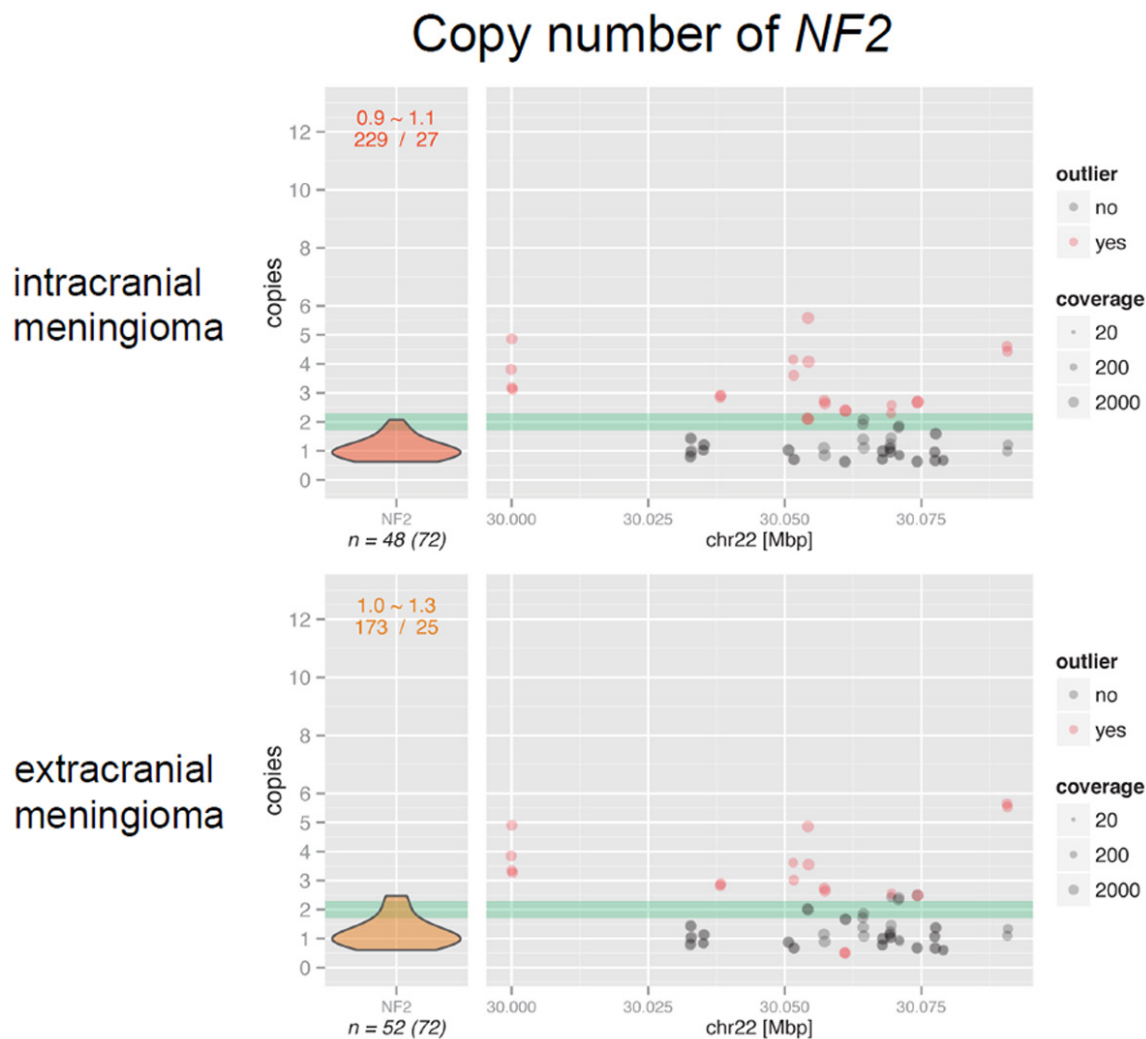


Figure 3. Left-side plot: Area-histogram (violin-plot) of the effective read depths used. The width of the shape is maximal where the density of all read counts is maximal, and it stretches up and down corresponding to the spread that is seen in the read counts. The main result is the copy number range estimate, labeled at the top of the left plot. Right-side plot: Read count ratios (sample/reference) for every individual primer as a marker at the primer binding position (x-axis). The marker size corresponds to the sum of coverage depths at this position (sample + reference), and a red color indicates that this point was excluded from the analysis because it represented an outlier. Upper: Copy number of *NF2* in the intracranial meningioma. Lower: Copy number of *NF2* in the extracranial metastatic meningioma.

GelRed™ Nucleic Acid Gel Stain (VWR International, Radnor, PA). Normal tissue DNA samples extracted from paraffin sections were included in each PCR reaction as negative controls. Quantitative analysis of the signal intensity was undertaken using Molecular Imager and Quantity One Analysis Software (Bio-Rad). Samples showing less than 20% of the *CFTR* signal were considered to exhibit homozygous deletion [13].

Differential PCR of the intrathoracic meningioma demonstrated homozygous deletion of the *EXO1* gene at exon 8 and exon 10, but such

findings were absent in the intracranial meningioma (Figure 4).

Discussion

Meningioma is the most frequent tumor of the CNS, and usually does not invade or metastasize. Most meningiomas are benign and correspond to WHO grade I. However, certain histological subtypes or meningiomas with specific combinations of morphological parameters are associated with less favorable clinical outcomes and correspond to WHO grade II (atypical) and III (anaplastic or malignant) [3].

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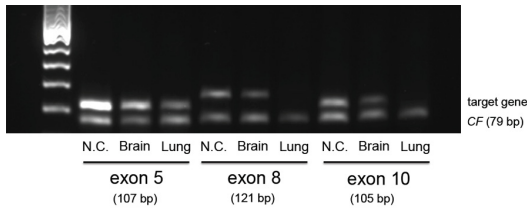


Figure 4. Homozygous deletion of the *EXO1* gene at exons 5, 8 and 10. NC, negative control; Brain, intracranial meningioma; Lung, extracranial metastatic meningioma.

Extracranial metastases have been estimated to occur in only 0.1% of all meningiomas (including hemangiopericytoma) and most often in association with anaplastic meningiomas [3, 6, 14, 15]. The most frequent pathway for metastasis to occur from an intracranial meningioma is via the paravertebral venous plexus [16]. The main sites of metastasis are thus the cervical lymph nodes, cervical soft tissue, parotid gland, thyroid gland, cervical bones and finally, the lung and pleura [17]. Surov A. et al. have summarized the locations and frequencies of distant metastases in meningiomas, showing liver metastases to be rare [18]. They reported that metastatic lesions were localized most commonly in the lung (37.2%), bones (16.5%), intraspinally (15.2%), and in the liver (9.2%) based on 164 distant metastatic meningiomas. According to Forest F. et al., 7 cases out of 40 distant metastatic meningiomas could be distinguished from hemangiopericytoma found among liver metastatic lesions [15]. Interestingly, 5 out of these 7 cases had multiple locations of metastasis involving the lung and bone. The liver may be affected if metastases pass through the right atrium into the inferior vena cava and further into the hepatic veins [18]. In our patient, there were two metastatic lesions: the first occurred in the lung, followed 4 months later by the appearance of a metastatic lesion in the liver.

While total resection and/or radiation treatment provide the standard therapy for meningiomas, use of chemotherapy against meningiomas has been limited to distant metastases or based on out of surgical criteria. In such cases, hydroxyurea, imatinib, somatostatin analogs, and angiogenesis inhibitors have been employed as adjuvant therapy [19-22]. Several reports have demonstrated that hy-

droxyurea provides an effective chemotherapy for meningiomas including grade I cases [23-28]. Hydroxyurea halts meningioma cell growth through arrest of the S-phase of the cell cycle, thus inducing apoptosis [29]. We employed a schedule identical to that which Chamberlain M.C. et al. reported previously [11, 30]. In our case, the focal recurrence lesion and intracranial metastases shrank into a partial response state. In contrast, the extracranial metastases (lung and liver) demonstrated rapid enlargement. As a consequence, the serum levels of hepatic function markers (AST, ALT and γ GTP) also fell within the reference interval.

The most important genetic alterations in sporadic meningiomas include mutation and loss of *NF2* (*Neurofibromin 2*, *Merlin*) [31]. *NF2* is involved in the Salvador/Warts/Hippo (SWH) pathway, and plays a role in the modulation of organ size by regulating cell proliferation and apoptosis, and in the regulation of genetic and protein stability. Alterations in the Hedgehog signaling pathway including *TRAF7*, *KLF4*, *AKT1* and *SMO* mutations have been identified in meningiomas [32]. Mutations of these genes were found to be mutually exclusive of *NF2* mutations [32]. *KIT* is one of the proto-oncogenes, which involving in diverse neoplastic processes [33]. Saini et al. demonstrated that *KIT* over-expression, instead of gene amplification, played an important role in meningiomas [34]. *ERBB2* is a transmembrane receptor protein with tyrosine kinase activity and is known to be involved in proliferation, differentiation, migration, adhesion and apoptosis [35]. Under-expression of *ERBB2* has been reported to be significantly associated with meningioma recurrence following surgical resection [36, 37]. *MET* is a receptor of tyrosine kinase, which serves to regulate the cell proliferation and migration signal pathway. *MET* over-expression has also been found to be associated with invasion and recurrence in meningiomas [38].

In the present study, we identified genetic alterations of *NF2*, as well as essential genes of the Hedgehog signaling pathway (*TRAF7*, *KLF4*, *AKT1* and *SMO*), *KIT*, *ERBB2* and *MET* in both our intracranial and extracranial meningioma samples. These materials each revealed *NF2* loss without *TRAF7*, *KLF4*, *AKT1* and *SMO* mutations. In addition, we were unable

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to detect KIT amplification, ERBB2 loss and MET amplification in both samples. The findings obtained suggested that both samples lacked any tumor progression, migration and metastatic molecular markers.

EXO1 belongs to the RAD2 family, and functions in DNA mismatch repair (MMR) to excise mismatch-containing DNA tracts directed by double-stranded breaks located at either 5' or 3' to the mismatch. This gene contains 3 important domains; one has interaction with *MSH3* (*MutS Homolog 3*), the second has interaction with *MLH1* (*MutL Homolog 1*), and the third has interaction with *MSH2* (*MutS Homolog 2*). In a recent study, ability for *EXO1* deletion was shown to suppress hydroxyurea hypersensitivity in vitro [39]. We therefore investigated *EXO1* homozygous deletion including these 3 important domains, i.e. exons 5, 8 and 10. We observed *EXO1* homozygous deletion at exons 8 and 10 in the patient's extracranial metastatic meningioma. The findings suggested that *EXO1* homozygous deletion may be associated with suppression of the hydroxyurea sensitivity in anaplastic meningiomas.

In conclusion, we have described an unequivocal case of anaplastic meningioma with distant metastases to the lung and liver. Hydroxyurea should be a justified therapeutic adaptation in case of anaplastic meningioma without *EXO1* homozygous deletion.

Disclosure of conflict of interest

None.

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