Detection of Mitochondrial DNA Mutations in the Tumor and Cerebrospinal Fluid of Medulloblastoma Patients

Lee-Jun C. Wong, Maria Lueth, Xiao-Nan Li, Ching C. Lau, and Hannes Vogel

Abstract

Medulloblastoma is the most common malignant brain tumor in children. Although the prognosis has improved considerably, leptomeningeal spread of the tumor remains a significantly negative predictor of survival. Mitochondrial DNA (mtDNA) mutations have been detected in many types of human tumors but not in medulloblastomas. Using temporal temperature gradient gel electrophoresis, we have analyzed the entire mitochondrial genome in 15 cases of medulloblastoma and the corresponding cerebrospinal fluid (CSF) samples in 8 of 15 cases. Six of 15 cases (40%) showed at least one mtDNA mutation in each of the tumors. A total of 18 somatic mtDNA mutations was detected with one of the tumors having 11 mutations, of which 9 were novel. Seven of 8 CSF samples that were analyzed showed mtDNA mutations. One patient who showed persistent mtDNA mutation in the CSF collected at the end of therapy when there was no evidence of disease had a relapse 5 months later. In contrast, patients whose end-of-therapy CSF samples that showed either no detectable mtDNA mutation or different mutations from that of the tumor continue to be disease free. Our results demonstrate that mtDNA mutations are frequently found in medulloblastomas. The mtDNA alterations detected in CSF may be used as sensitive markers to monitor disease progression and predict relapse.

Introduction

Medulloblastoma is the most common pediatric malignant brain tumor involving the cerebellum. The pathogenesis of medulloblastoma is unknown in most cases, although certain germ-line and acquired genetic abnormalities are associated with a susceptibility to medulloblastoma in subsets of patients. Cytogenetic abnormalities include isochromosome 17q in ~50% of cases. Losses of genetic material have also been documented from chromosomes 1q (1) and 10q (2) in 20–40% of medulloblastomas. Mutations of the human homologue of the Drosophila patched gene (PTCH), as well as other members of the sonic hedgehog pathway have also been reported in 10–15% of sporadic medulloblastomas (3). In addition, the adenomatous polyposis coli gene and its corresponding WNT signaling pathway have been implicated in ~13% of sporadic medulloblastomas (4). MYC amplification has been associated with large cell medulloblastomas (5).

Mitochondrial abnormalities in medulloblastoma have not been specifically investigated beyond their morphological features. A study of the role of mitochondria in in vitro studies of apoptosis in medulloblastoma cell lines indicates that ceramide-induced apoptosis may require respiratory chain activity and can occur independently of the mitochondrial apoptosis pathway (6). Because the net growth of medulloblastomas represents a balance between cell division and apoptosis, the role of mitochondria in energy metabolism and apoptosis is likely to be of importance in understanding both the biology of medulloblastoma and as a potential target of therapy.

In an increasing list of diverse neoplasms, mtDNA alterations have been documented (7). These have proven to be of interest as tumor markers and as evidence that they may play a role in neoplastic cellular adaptation to altered energy requirements. The nuclear genome has also been investigated in cancer in relation to mitochondrial physiology upon observing that mutations in genes encoding structural subunits of complex II are present in autosomal dominant hereditary paragangioma (8). These mutations lead to a defect in oxygen sensing and the response to hypoxia, functionally resulting in loss of the tumor suppressor’s role for complex II (9). Nonsense and germ-line mutations involving complex II subunits have also been reported in familial and sporadic pheochromocytoma (10–12). These observations lead to the present study in which the complete mitochondrial genome sequences in 15 cases of medulloblastoma are compared with those in blood and CSF from these patients. The possibility of using mtDNA alterations in CSF to monitor disease recurrence in medulloblastoma patient was also explored.

Materials and Methods

Patients. Tumor tissues, blood, and CSF samples were collected and banked with informed consent through an Institutional Review Board- approved protocol at Baylor College of Medicine and affiliated hospitals. Tumor tissues were collected at the time of surgery and were snap frozen in liquid nitrogen within 30 min after resection and stored at ~80°C. Blood samples were collected at the time of surgery and before any additional therapy. DNA was extracted from blood samples immediately after collection. CSF samples were collected at various times during routine staging work-up and therapy according to the treatment protocol. CSF was spun at 1000 rpm for 10 min immediately after collection and the supernatant frozen and stored at ~80°C in 200-μl aliquots. Treatment of all patients involved in this study, except patients 116 and 118, was according to the same in-house protocol for medulloblastoma, which included craniospinal radiation and chemotherapy. Patient 116 expired before receiving any therapy because of rapid progression of disease after initial surgery. Patient 118 received only chemotherapy and no radiation therapy because of his young age. Tissues from 15 patients, 12 male and 3 female, were used in this study with the age at diagnosis ranging from 14 months to 14 years (mean of 6.25 years).

DNA Isolation. DNA was extracted from frozen tumor tissues with Trizol reagent (Invitrogen, Carlsbad, CA) following the manufacturer’s instruction. Briefly, after the aqueous phase containing RNA has been removed, DNA was precipitated with 100% ethanol, washed twice with 0.1 M sodium citrate at 10% ethanol, and once with 75% ethanol. After the DNA pellet was dried and redissolved in 8 mM NaOH, 0.1 M HEPES was added to adjust the pH to 8.4.

The abbreviations used are: mtDNA, mitochondrial DNA; CSF, cerebrospinal fluid; TTGE, temporal temperature gradient gel electrophoresis; ND4, NADH dehydrogenase subunit 4.
DNA was extracted from blood leukocytes using the Wizard kit (Promega, Madison, WI) and stored at 4°C. Total DNA was quantified using fluorescent Hoechst dye H33258 with Dyna Quant 200. DNA was diluted to 5 ng/µl to be used in PCR reactions. CSF was heated at 95°C for 5 min. Two µl of heated CSF were used for PCR without DNA extraction.

**Mutational Analysis of the Entire Mitochondrial Genome.** Thirty-two pairs of overlapping primers were used to amplify the entire mitochondrial genome (13). The DNA fragments vary in size from 306 to 805 bp with an average of 594 bp. The amplified fragments had ~70-bp overlap at each end. The position and the sequence of the PCR primers, and the PCR and TTGE conditions have recently been published (13). Briefly, the DNA template, after the initial denaturation at 94°C for 5 min, was amplified with 35 cycles of 45 s each at 94°C for denaturation, 55°C for reannealing, and 72°C for extension, and completed by 4 min of extension at 72°C. PCR products were denatured at 95°C for 30 s and slowly cooled to 45°C for a period of 45 min at a rate of 1.1°C/min. The reannealed homoduplexes and heteroduplexes were kept at 4°C until loading onto the gel. TTGE analysis was performed on a Bio-Rad D-Code apparatus (Bio-Rad Laboratories, Hercules, CA). Polyacrylamide (acrylamide:Bis = 37.5:1) gels were prepared in 1.2X TAE buffer containing 6 M urea. Five µl of denatured and reannealed PCR products were loaded onto the gel. Electrophoresis was carried out at 145 V for 4-5 h at a constant temperature increment of between 1-2°C/h (13, 14). The temperature range was determined by computer simulation (MacMelt software; Bio-Rad Laboratories). The gels were stained with 2 mg/liter ethidium bromide for 5 min and imaged with a digital CCD gel documentation system. On TTGE analysis, a single bandshift represents a homoplasmic DNA alteration and a multiple banding pattern represents a heteroplasmic mutation (14). PCR products from blood and tumor tissues of the same patient were analyzed side-by-side. Any DNA fragments showing different banding patterns between the blood and tumor samples were sequenced to identify the exact mutation(s).

**Sequencing Analysis.** The confirmation of mutation was performed by direct DNA sequencing of the purified PCR product using the original PCR primers and a BigDye terminator cycle sequencing kit (Perkin-Elmer Life Sciences, Boston, MA) on an ABI 377 (Applied Biosystems, Foster City, CA) automated sequencer. The results of DNA sequence analysis were compared with the published Cambridge sequence using Mac Vector 7.0 (Oxford Molecular Ltd., Oxford, United Kingdom) software. Sequence variations found in both tumor and blood mtDNA were scored as germ-line variations. Each was then checked against the Mitomap database. Those not recorded in the database were categorized as novel mtDNA variations, and those appeared in the database were reported as polymorphisms. Any DNA sequences that were different between tumor and matched blood mtDNA were scored as somatic mutations. mtDNA from CSF was sequenced and compared with those from the blood and tumor of the same individual.

**RESULTS**

**Somatic mtDNA Mutations in Medulloblastomas.** TTGE analysis revealed numerous homoplasmic and heteroplasmatic somatic mtDNA mutations in medulloblastomas. Fig. 1A shows three homoplasmic mtDNA alterations: G7337A, T7389C, and G7521A, within a DNA fragment. Change from homoplasy in blood to heteroplasy in tumor is illustrated in Fig. 1B. Fig. 1C depicts a change in the degree of heteroplasmy detectable by both TTGE and sequencing.

Overall, a total of 18 somatic mtDNA mutations were detected (Table 1A) in 6 of 15 medulloblastomas (40%). Among these mutations, 5 (29.4%) were insertions or deletions in np 303–315 polycytosine tract region. The remaining 13 somatic mutations were single-base substitutions (72.2%). Eleven (61%) mutations are in the D-loop region, 3 in tRNA (G7521A, T15904C, and A15937G) and 4 in mRNA (protein coding region; Table 1A). Two of the protein coding region mutations are silent mutations and the other two are nonsense mutations: Y496H in cytochrome c oxidase subunit I (COX I) and L96P in ND4. Among the 18 somatic mutations, 8 were changing from homoplasmic wild-type to homoplasmic variant. The T15904C in tumor 124 was a change from homoplasmatic variant in blood back to homoplasmic wild-type in tumor. Five changed from heteroplasmic in blood to homoplasmic in tumor, 1 changed from homoplasmic in blood to heteroplasmic in tumor, and 4 were heteroplasmic in both blood and tumor but with a quantitatively different proportion of the mutant mtDNA.

Three tumors have one somatic mtDNA mutation, 2 have two mutations, and 1 (tumor 124) has 11 mutations. Because of the numerous somatic mtDNA mutations in tumor 124, sample mishandling was suspected. To verify that blood and tumor DNA were indeed from the same patient, 4 polymorphic markers: the short tandem repeat in intron 3 of the phenylalanine hydroxylase gene (chromosome 12), the CTG repeats of the myotonin protein kinase gene (disease gene for myotonic dystrophy, chromosome 19), the CAG repeats of the androgen receptor gene (X chromosome), and of spinocerebellar ataxia type 3 (chromosome 4) were analyzed. The identity of the blood specimen was found to match the corresponding tumor tissue at >98% probability.

**Germ-Line Sequence Variations.** DNA fragments showing different banding patterns between blood and tumor tissues on TTGE analysis are indications of somatic mutations. During the sequence analysis to identify the somatic mutations (Table 1A), it was noted that there were numerous germ-line sequence variations when the sequence of normal tissue (blood) was compared with that of the published Cambridge sequence. A total of 72 distinct germ-line variations was identified from the sequenced fragments. These do not represent all of the sequence variations in the specimens analyzed because only the DNA regions that show somatic mutations by TTGE were sequenced. Thirteen of these variations are novel (Table 1B), and 59 (data not shown) of them have been previously recorded in the Mitomap database. Eleven of 13 novel variations occurred only once (Table 1B), whereas 24 of 59 reported polymorphisms occurred multiple times. All germ-line variations are silent nucleotide substitutions or alterations in noncoding D-loop region or tRNA genes.

**mtDNA Mutations in CSF.** CSF was available from 10 patients, including the 6 who harbored somatic mtDNA mutations in their tumors. We were able to amplify the regions containing the mutations in 7 of them, 5 of which were from cases with mtDNA mutations in the tumor. The CSF sample of patient 126 failed to amplify. CSF of patients 143 and 146 was not investigated because there was no mutation found in the tumor. Table 2 summarizes the age of diagnosis, staging, clinical status, and somatic mtDNA mutations in tumor and CSF. The average age of diagnosis of the patients with somatic mtDNA mutations is higher (8.5 year) than that (5.1 year) of those who do not have mtDNA mutations. The proportion of tumors having somatic mtDNA mutation is independent of the metastatic status of the patient, both at 40%. With patient 122, the same T152C somatic mutation found in tumor was also detected in CSF sample (Fig. 1C). The T152C mutation was the only mutation found in the tumor and was in heteroplasmic state in blood, tumor, and CSF, but the proportion of mutant was lowest in blood and highest in CSF (Fig. 1C). The CSF sample of this patient was taken 1 month after completion of all therapy at which time there was no evidence of disease based on magnetic resonance imaging studies, as well as CSF cytology. However, this patient subsequently developed recurrent disease 5 months later. These results suggest that mtDNA mutation in CSF can be detected long before the recurrence of disease. Presumably, cells resistant to therapy continued to divide and the mutant mtDNA progressively accumulated.

Three regions, SD, TP, and D-loop, of CSF mtDNA of patient 124 were amplified. This patient harbored a total of 11 somatic mtDNA mutations in the tumor, two of which, T15904C and A15937G, were novel (Table 2). Only the T15904C mutation in tRNA threonine was
found in the CSF sample (Fig. 1D). The mutant mtDNA appeared to be homoplastic. This patient initially presented with metastatic disease involving the spinal cord. The CSF sample was taken 6 months after all therapy was completed at which time there was no evidence of disease by magnetic resonance imaging and CSF cytology. The patient has remained disease free after 40 months of follow-up. Unlike in the case of 122 where the mtDNA mutations in the tumor and the CSF were identical and the patient eventually relapsed, in this case, at least 7 mtDNA mutations found in the tumor were not detected in the CSF. These results suggest that residual mtDNA from tumor cells can still be found long after therapy has been completed when no tumor cells can be detected by conventional methods such as imaging and cytology. Furthermore, because only one of several mtDNA mutations found in the tumor was detected in CSF and was homoplastic, this suggests that the original spinal metastases could represent an earlier clone of tumor cells that had migrated down the neuraxis and developed independently of the cerebellar tumor. This is supported by the other mutation found in the CSF of this patient in the 303–309 polyC tract region. It was C8/C7 heteroplasy in blood, homoplasy C7 in tumor, and C9/C8 heteroplasy in CSF. Although the possibility of normal cell contamination and the random effects of therapy cannot be ruled out, it is still hard to explain why the CSF and tumor share some of the same mutations, especially rare ones like T15904C. Furthermore, in the case of patient 149 where the CSF was collected before any therapy was given, mtDNA mutations in the 303–309 polyC tract region were also different between the tumor and the CSF. Interestingly, patient 149 was diagnosed with nonmetastatic disease and the fact that the pretherapy CSF sample contains mtDNA.
Table 1  Medulloblastoma

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<th>Amino acid change</th>
<th>Function</th>
<th>Previously reported in tumors</th>
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**Table 1** Medulloblastoma

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<th>Germ-line mutation</th>
<th>Frequency</th>
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**Gene/Region**

- D-loop
- ND4L
- ND4
- COXI
- SD
- T10810C

**Amino acid change**

- COXII Novel
- COXII Novel
- COXII Novel
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- COXII Novel
- COXII Novel

**Function**

- Conserv. seq block
gastric esoph, ov, brca
- Conserved sequence block
gastric esoph, ov, brca
- H-strand origin
gastric esoph, ov, brca
- Replication primer
gastric esoph, ov, brca
- H-strand origin
gastric esoph, ov, brca
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gastric esoph, ov, brca

**Previously reported in tumors**

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**Mutations**

- Somatic mtDNA alterations in medulloblastomas and other tumors (17, 19). Back change from variant in normal tissue to wild-type in tumor tissue has also been observed (Table 1A, T149; Ref. 17). These results demonstrate that tumorigenesis is a progressive process during which the mutant mitochondria may gain replicative advantage and somatic mutations accumulate (15, 20).

The frequent mutation in np303–309 polyC region probably reflects mitochondrial genomic instability in this hypervariable triple helix region. To investigate if this is the result of generalized microsatellite instability, we sequenced 10 mtDNA regions containing the poly mononucleotide tracts, and no mutation was detected in these regions. These results suggest that the instability at np 303–309 is probably not because of generalized microsatellite instability but because it locates at the relatively unstable triple strand displacement loop region. The somatic mtDNA mutations observed in tumors are not PCR artifacts for we have analyzed mtDNA in at least 50 muscle/blood specimens of individuals of various ages and somatic mtDNA mutations have not been found. Our observations substantiate the concept that genomic instability in tumors should include the mitochondrial genome.

**mtDNA Mutation Spectrum**

The spectrum of somatic mtDNA mutations in medulloblastoma was compared with that of neurofibromatosis type 1, glioblastoma, and breast cancer (Table 3). The insertions and deletions at np 303–309 polyC regions occurred most frequently, consistent with the hot spot for mitochondrial genomic instability in all tumors. If counting only the distinct somatic mtDNA mutations, 71% (10 of 14) were novel in medulloblastoma (Table 3). All of the somatic mtDNA mutations in neurofibromatosis type 1 and 79% of the somatic mtDNA mutations in breast cancer are in the D-loop region (17, 19), but only 50% of the distinct somatic mtDNA muta-
mutations of medulloblastoma are in the D-loop, which is similar to that of glioblastoma multiforme (56%; Ref. 18). The average number of somatic mtDNA mutations/tumor is around one to three. Kirches et al. (18) reported 19 somatic mtDNA mutations found in a single glioblastoma. One of our patients harbored 11 somatic mtDNA mutations.

**Significance of Somatic mtDNA Mutations.** Mutations in coding region may play a role in the pathogenesis of tumors. The G7521A mutation in tRNA aspartate changes a GT base pairing to AT base pairing at the amino acyl stem region. This alteration perhaps makes the stem region more stable and allows the tRNA to work more efficiently. Similarly, the T15904C mutation in tRNA threonine at the 3′ end of the loop region and the A15937G mutation at the first bp next to the loop may play a role in the pathogenesis of tumors. The G7337A mutation in tRNA ubiquitin (Ubi) may also affect tRNA structure and stability. Two missense mutations, Y496H in cytochrome oxidase III and L96P in NADH subunit 4, involved in the substitution of the hydrophobic aromatic tyrosine with a helix destabilizing secondary amino acid proline, are nonconserved changes that are expected to bring about significant structural/functional alteration. Mutations in noncoding region, although not directly involved in amino acid change, are located in transcription factor binding sites, replication primer sites, origin of replication, or conserved sequence blocks. Alterations in these positions may affect mtDNA replication, transcription, and overall expression.

**Table 2. mtDNA alterations in blood, tumor, and CSF of patients with medulloblastoma**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Gender</th>
<th>Age at DX</th>
<th>Staging</th>
<th>Clinical</th>
<th>Sequence in blood</th>
<th>Sequence in tumor</th>
<th>Sequence in CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>Female</td>
<td>8 years</td>
<td>M*</td>
<td>Dead</td>
<td>Cambridge seq.</td>
<td>Cambridge seq.</td>
<td>Not investigated</td>
</tr>
<tr>
<td>112</td>
<td>Male</td>
<td>5 years</td>
<td>M</td>
<td>NED</td>
<td>Cambridge seq.</td>
<td>Cambridge seq.</td>
<td>Not investigated</td>
</tr>
<tr>
<td>114</td>
<td>Male</td>
<td>14 years</td>
<td>NM</td>
<td>NED</td>
<td>Cambridge seq.</td>
<td>Cambridge seq.</td>
<td>Not investigated</td>
</tr>
<tr>
<td>116</td>
<td>Male</td>
<td>30 months</td>
<td>NM</td>
<td>Dead</td>
<td>Cambridge seq.</td>
<td>Cambridge seq.</td>
<td>Not investigated</td>
</tr>
<tr>
<td>118</td>
<td>Male</td>
<td>14 months</td>
<td>NM</td>
<td>Dead</td>
<td>Cambridge seq.</td>
<td>Cambridge seq.</td>
<td>Not investigated</td>
</tr>
<tr>
<td>120</td>
<td>Female</td>
<td>4 years</td>
<td>NM</td>
<td>NED</td>
<td>303–309 C9 (hm)</td>
<td>303–309 C9 (hm)</td>
<td>303–309 C9 (hm)</td>
</tr>
<tr>
<td>122</td>
<td>Male</td>
<td>6.5 years</td>
<td>M</td>
<td>AWD</td>
<td>T152</td>
<td>T152C&gt;T&gt;C(hm)</td>
<td>T152C&gt;T&gt;C(hm)</td>
</tr>
<tr>
<td>124</td>
<td>Male</td>
<td>6.5 years</td>
<td>M</td>
<td>NED</td>
<td>C151</td>
<td>C151T</td>
<td>No amplification</td>
</tr>
<tr>
<td>126</td>
<td>Male</td>
<td>12 years</td>
<td>NM</td>
<td>AWD</td>
<td>T11046C(hm)</td>
<td>T11046C(hm)</td>
<td>No amplification</td>
</tr>
<tr>
<td>135</td>
<td>Male</td>
<td>9 years</td>
<td>NM</td>
<td>NED</td>
<td>G247A(hm)</td>
<td>G247A(hm)</td>
<td>G247A(hm)</td>
</tr>
<tr>
<td>140</td>
<td>Male</td>
<td>3 years</td>
<td>NM</td>
<td>NED</td>
<td>Cambridge seq.</td>
<td>Cambridge seq.</td>
<td>Not investigated</td>
</tr>
<tr>
<td>143</td>
<td>Male</td>
<td>5 years</td>
<td>NM</td>
<td>NED</td>
<td>C295T(hm)</td>
<td>C295T(hm)</td>
<td>T295C&gt;T(hm)</td>
</tr>
<tr>
<td>146</td>
<td>Male</td>
<td>3 years</td>
<td>M</td>
<td>NED</td>
<td>Cambridge seq.</td>
<td>Cambridge seq.</td>
<td>Not investigated</td>
</tr>
<tr>
<td>149</td>
<td>Female</td>
<td>11 years</td>
<td>NM</td>
<td>NED</td>
<td>303–309 C9 (hm)</td>
<td>303–309 C9 (hm)</td>
<td>303–309 C9 (hm)</td>
</tr>
</tbody>
</table>

* M, Metastatic disease at the time of diagnosis; NM, nonmetastatic at the time of diagnosis; NED, no evidence of disease; AWD, alive with disease; Cambridge seq., cambridge sequence; hm, homoplasmic; ht, heteroplasmic.

**Somatic mtDNA Mutations in CSF as Potential Markers for Disease Prognosis.** Somatic mtDNA mutations were detectable in the CSF of patients with medulloblastoma with or without metastasis. There are three potential sources of mutated mtDNA in the CSF of these patients: the intracranial tumor; spinal metastases; and nontumor cells in the spinal compartment that have been mutated by the therapy. One would predict that the probability of detecting mtDNA mutations in the CSF would be higher in the metastatic cases than the nonmetastatic cases. Unfortunately, with the small sample size in this study and the fact that most of the CSF samples were collected at the end of therapy, we were unable to confirm this prediction. Interestingly, all of the CSF mutations in the nonmetastatic cases involved the np 303–309 polyC tract region, whereas none of the metastatic cases had CSF mutations in that region. Because all of the CSF samples except 2 (patients 146 and 149) were collected either right after radiation therapy (patient 135) or 1–9 months after the completion of all therapy, one would also predict that if the mutation in CSF is identical to that found in the original tumor, it could indicate the presence of resistant tumor cells and therefore might predict relapse of disease as seen in patient 122. Likewise, if the CSF mutation reverts back to that in normal tissue, it would signal the absence of tumor cells and therefore predict a disease free status as in the case of patient 124. Finally, for CSF mutation that is found in neither the normal nor...
tumor tissues as in the cases of 120 and 128, the origin of these mutated mtDNA is uncertain. Although it could arise from normal cells in the CSF that have been mutated by radiation and/or chemotherapy, it is hard to understand how these mtDNA would be able to persist in the CSF long after therapy has been completed, especially if the mutated cells are not proliferating as confirmed by the other surveillance studies. Would these mtDNA eventually disappear from the CSF over time? Such questions can be addressed by conducting a time series study sampling multiple CSF samples from the same patients at various times during long-term follow-up. Lastly, with CSF samples taken before any therapy, it would also be interesting to correlate clinical outcome with the similarity between the mutations in the CSF and tumor. Identical mutations in the CSF and tumor would imply higher turnover of the tumor cells, especially in the nonmetastatic cases. Because apoptosis is used as a marker for higher grade in medulloblastoma, identical mutations in CSF and tumor would suggest a higher grade tumor and therefore poorer prognosis. Conversely, divergent mutation patterns could be associated with better prognosis as seen in patient 149. Ultimately, a prospective study involving a larger patient population and serial CSF samples from each patient will be necessary to establish the utility of mtDNA mutations as biomarkers for prognostication and early detection of recurrent disease.

References

Detection of Mitochondrial DNA Mutations in the Tumor and Cerebrospinal Fluid of Medulloblastoma Patients

Lee-Jun C. Wong, Maria Lueth, Xiao-Nan Li, et al.


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