MYC and TP53 aberrant of CSF tumor cells in children with metastatic medulloblastoma

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Abstract

Background: Medulloblastoma (MB) is the most common primary malignant brain tumor in childhood. MYC and TP53 aberrant is the high risk factor for its prognosis, but its values in tumor cells of CSF has not been reported.

Methods: During October 1, 2012 to April 10, 2020, a total of 185 metastatic MB were enrolled in this study, CSF cytology and MYC and TP53 aberrant statuses were analyzed.

Results: We found that forty-two patients were positive presence of tumor cells in CSF, and 39 cases were positive with MYC or TP53 aberrant, which was present in about 66% of Group 3 MB. The PFS and OS rates of patients with positive gene aberrant were much lower than that of negative aberrant group (P<0.01), and MYC and TP53 aberrant of tumor cells in CSF was high independent risk factor for OS of metastatic patients (95% CI was 0.266-0.998, P<0.05).

Conclusion: Thus detection of MYC and TP53 aberrant of tumor cells in CSF could be an important method to complement detection of surgical specimen, and can improve the molecular diagnosis.

Background

Pediatric brain tumor is the most common solid malignancy in childhood. It is currently the second cause of cancer-related mortality and morbidity in this age group [1]. Medulloblastoma (MB) approximately accounts for 20% of all malignant pediatric brain tumors [2]. With multimodal therapies, including surgical resection, craniospinal irradiation (CSI) with posterior fossa boost, and adjuvant chemotherapy, the 5-years overall survival (OS) rates of the MB children without metastasis at the time of diagnosis have remained over 80% [2, 3]. However, MB cells are prone to metastasize rapidly to adjacent sites via cerebrospinal fluid (CSF) or hematogenous route [2, 4], and metastasis is observed in up to 30% of children at initial presentation[2]. Patients with metastasis are more likely to experience relapse and to have poorer prognosis.

Integrative genomics and mRNA expression profiling have demonstrated that MB comprises a heterogeneous group of tumors. It includes four molecular subgroups (WNT, SHH, Group 3 and Group 4), which have distinct prognosis and biological behavior [5]. The outcome remains poor in patients with large-cell anaplastic pathology, metastatic disease, MYC/MYCN amplification, MYC and TP53 mutation, and chromosome 17 defects [5-7], suggesting the importance of gene detection in clinical practice. However, owning to the small amount of tumor tissues, difficult-to-define tumor boundaries, and tendency to infiltrate the leptomeninges or ventricles, gene aberrant of frozen tissues is inadequate to certificate high-risk MB. Additionally, CSF surrounds and supports the central nervous system (CNS), including the ventricles and subarachnoid space, interacts with the CNS directly [8], thus tumor cells will be easily obtained from CSF for molecular diagnosis of MB through lumbar puncture (LP). Therefore, detection of gene aberrant of tumor cells in CSF may be a new method to improve the molecular diagnosis of MB.
The present study aimed to compare the MYC and TP53 aberrant of tumor cells in CSF and those of surgical specimen, and reveal the importance of gene aberrant of tumor cells in CSF to the prognosis of patients with metastatic MB.

**Methods**

**Patients and study design**

MB children with postoperative metastatic disease treated in the Department of Pediatrics, Beijing Shijitan Hospital, Capital Medical University were retrospectively analyzed. This research was carried out according to the principles set out in the Declaration of Helsinki 1964 and all subsequent revisions, informed consent was obtained, and the relevant institutional review board had approved the study.

Patients enrolled in this study had the following characteristics: (1) age was under 18 years old at the time of diagnosis; (2) the diagnosis of MB was confirmed by brain MRI and CT images before surgery and pathological examination of the surgical specimens; (3) tumors were surgically resected before chemotherapy, and genetic profile of surgical specimens were examined; (4) the diagnosis of metastatic MB was determined by brain and spinal cord MRI images; (5) LP was performed before each stage of chemotherapy, and the CSF was centrifuged to look for tumor cells.

**Treatment protocol**

In this study, gene aberrant includes MYC amplification and mutation, TP53 mutation of tumor cells in CSF or surgical specimen. Once a gene aberrant was found either in CSF or in surgical specimen, this patient was classified into positive group. Then these metastatic patients were divided into aberrant positive group and negative group (including tumor cell negative in CSF), according to the results of gene detection. The treatment protocol was based on a modified HIT 2000 SKK regimen [9], which included induction chemotherapy with intrathecal methotrexate (IT-MTX), CSI, and consolidation or maintenance chemotherapy (Fig. 1).

**Induction chemotherapy**

The SKK induction chemotherapy included four stages. The first stage uses cyclophosphamide (CTX, 800 mg/m², days 1-3) and vincristine (VCR, 1.5 mg/m², day 1), followed by methotrexate (MTX, 5.0 g/m², day 1, followed by leucovorin rescue after 6 h) and VCR (day 1), which repeat once; the fourth stage uses carboplatin (200 mg/m², days 1-3) and etoposide (VP16, 150 mg/m², days 1-3). All above stages were accompanied by IT-MTX (12.5 mg/dose and dexamethasone 5 mg) with 2-week intervals. Patients in mutation negative group received another cycle of SKK chemotherapy. (Fig. 1)

**Irradiation therapy**

After induction chemotherapy, then CSI was applied with 36 Gy to the craniospinal axis in daily fractions of 1.8 Gy.
Consolidation or maintenance therapy

Patients in mutation negative group received maintenance chemotherapy, which included 4-6 cycles of cisplatin (75 mg/m², day 1), lomustine (70 mg/m², day 1), and VCR (days 1, 8, 15) every 42 days (A protocol). Patients in mutation positive group received consolidation chemotherapy, which includes two cycles of A protocol, then alternatively uses CTX and VCR, and carboplatin and VP16 (B protocol), which was described as AABAABAA protocol [9-11].

DNA extraction and genotyping

After lumber puncture, an aliquot of CSF was centrifuged on a glass slide, which was sent to pathologists for the examination of tumor cells in CSF. The rest of CSF was centrifuged immediately and genomic DNA in the cell pellet was extracted using a QIAamp tissue kit [12]. PCR primers were designed to amplify the coding exons of MYC and TP53. Nested PCR was performed using hi-fidelity Taq DNA polymerase to amplify several small amount of DNA samples. PCR product was directly sequenced. Primer sequences, PCR conditions and the detailed sequencing results are provided in Appendix.

Statistical analysis

Statistical analyses were performed using SPSS (version 20.0 SPSS Inc., USA). OS was defined as the interval between the date of treatment and that of death by any cause or of the last visit. PFS was defined as the interval between the date of treatment and that of the detection of subsequent progression, relapse; death by any cause, or last contact. Survival rate was estimated using the Kaplan-Meier method, and exact log-rank tests were used to compare two survival curves in the two groups. Multivariate regression models with forward and backward stepwise selection were used to analyze the effects of related factors (age, sex, tumor histopathology, surgical resection range, tumor cells in the CSF, molecular subgroup, and MYC and TP53 gene mutations) with score test $P$ values $\leq 0.05$ on survival (variables with likelihood ratio test $P$ values $\geq 0.10$ were excluded).

Results

Metastatic MB Patients

This research was carried out according to the ethics committee's principles of Beijing Shijitan Hospital, and informed consent was obtained from children's parents or their legal guardians.

During the period of October 1, 2012 through April 10, 2020, a total of 185 children, those with metastatic MB treated with chemotherapy and irradiation therapy after surgical resection were enrolled in this study. The median age was 6.8 years old (mean: 7.2 years, range: 0.2–16.6 years). Here, 111 patients were gross total resection (GTR), 60 were near total resection (NTR), 13 were subtotal resection (STR), and one was unable to be removed and had to be performed biopsy owning to the widespread of tumor cells in skull. Moreover, 115 patients were classic MB (CMB), 45 patients were desmoplastic/nodular and MB with extensive nodularity (DNMB and MBEN), and 23 patients were large-cell and anaplastic MB
As to the molecular subgroup, 57 cases were SHH, 27 cases were Group 3, 97 cases were Group 4, one case was WNT, and two cases were not otherwise specified (NOS).

Importantly, 42 patients were positive presence of tumor cells in CSF, and 39 cases were positive with *MYC* or *TP53* aberrant. The characteristics of these gene aberrant patients were shown in Table 1.

**Table 1** Demographics and disease characteristics of patients with *MYC/TP53* aberrant
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Metastatic Patients (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>28</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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</tr>
<tr>
<td>Median (range)</td>
<td>4.9 (1.1–10.3)</td>
</tr>
<tr>
<td>Resection extent</td>
<td></td>
</tr>
<tr>
<td>GTR</td>
<td>27</td>
</tr>
<tr>
<td>NTR</td>
<td>10</td>
</tr>
<tr>
<td>STR</td>
<td>1</td>
</tr>
<tr>
<td>Only Biopsy</td>
<td>1</td>
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<tr>
<td>Histopathology subtype</td>
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<tr>
<td>CMB</td>
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</tr>
<tr>
<td>DNMB</td>
<td>9</td>
</tr>
<tr>
<td>LCAMB</td>
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<tr>
<td>Molecular subgroup and $MYC/TP53$</td>
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</tr>
<tr>
<td>SHH</td>
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<tr>
<td>$MYC$ positive aberrant</td>
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</tr>
<tr>
<td>$TP53$ positive mutation</td>
<td>4</td>
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<tr>
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<tr>
<td>Group 3</td>
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<tr>
<td>$TP53$ positive mutation</td>
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</tr>
<tr>
<td>Group 4</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>$TP53$ positive mutation</td>
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</tr>
<tr>
<td>WNT</td>
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</tr>
<tr>
<td>$TP53$ positive mutation</td>
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</tr>
<tr>
<td>Tumor cells in CSF</td>
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</tbody>
</table>
Note: MB, medulloblastoma; CMB, classic medulloblastoma; DNMB, desmoplastic/nodular and medulloblastoma with extensive nodularity; LCAMB, large-cell and anaplastic MB; SHH, sonic hedgehog; CSF, cerebrospinal fluid; GTR, Gross total resection; NTR, near total resection; STR, subtotal resection.

In addition, among those MYC or TP53 positive aberrant patients, 6 cases were newly found MYC/TP53 aberrant in tumor cells of CSF, while not detected in surgical specimen. Meanwhile, MYC or TP53 aberrant were positive in 33 surgical specimens, and 14 cases were positive MYC or TP53 aberrant both in surgical specimens and CSF, 19 cases were positive only in surgical specimens (see Table 2).

**Table 2** The status of MYC/TP53 aberrant of tumor cells in CSF or surgical specimen of patients with metastatic MB

<table>
<thead>
<tr>
<th></th>
<th>MYC (+)</th>
<th>TP53 (+)</th>
<th>MYC and TP53 (+)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen (+) and CSF (-)</td>
<td>13</td>
<td>5</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Specimen (+) and CSF (+)</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Specimen (-) and CSF (+)</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>10</td>
<td>2</td>
<td>39</td>
</tr>
</tbody>
</table>

Moreover, the MYC amplification was present in more than half of Group 3 MB (55.5%), while TP53 mutation was present in about 11.1%. The relationship between MYC/TP53 aberrant in CSF and surgical specimen was further described in Fig. 2.

**Survival of patients**

Of all these 185 patients, the median survival time was 2.3 years (range: 0.2–7.6 years), and the estimated 5-year PFS and OS rates were 48.1 ± 4.8% and 53.6 ± 5.3% (± standard error), respectively. However, the survival of patients with positive gene aberrant was very poor, and much lower than those of negative aberrant group (P<0.01). In fact, the median survival time of those patients with MYC/TP53 positive aberrant was only one year (range: 0.2–2.5 years), and all patients were nearly died in 2.5 years. The one- and 2-year PFS/OS rates of patients with positive gene aberrant were 15.4 ± 5.8% / 51.9 ± 8.3%, and 2.6± 2.5% / 11.5 ± 5.4%, respectively. On the other hand, the median survival time of patients with negative gene aberrant was 2.2 years (range: 0.3–7.6 years), and the one- and 2-year PFS/OS rates were
91.6 ± 2.3% / 95.9 ± 1.7%, and 77.0 ± 3.7% / 90.5 ± 2.6%, respectively (see Fig. 3A, 3B). Even the 5-year PFS/OS rates were 61.1 ± 5.6% / 68.3 ± 6.1%, respectively.

**Risk factors associated with survival**

Multivariate analysis using risk factors with Cox’s proportional hazard model showed that molecular group and presence of tumor cells in CSF were independent predictive factors for PFS in patients with metastasis (95% CI was 1.153-2.026 and 1.651-5.387, respectively, and P<0.05). Meanwhile, besides of molecular group and presence of tumor cells in CSF, positive gene aberrant was an independent risk factor associated with OS of metastatic patients (95% CI was 1.378-2.728, 2.145-8.165 and 0.266-0.998, respectively, and P<0.05).

**Adverse effects**

More than 70% of patients experienced gastrointestinal disorders, including anorexia, nausea and abdominal pain, etc. But all these disorders were controlled promptly using antiemetics or probiotics [13]. Various degrees of myelosuppression appeared in all patients. Six patients had to decrease their carboplatin and etoposide doses by 10% owing to myelosuppression, and 6 patients received red cell or platelet transfusions. No patients were withdrawn from the chemotherapy regimen due to adverse events.

**Discussion**

Moreover, detection of the presence of high-risk factors with all possible methods is beneficial to respond effectively to suppress the MB progression, and testing of MYC/TP53 gene aberrant is a useful and feasible method. In this study, it was intriguingly that another 6 cases were detected positive MYC/TP53 gene profile of tumor cells in CSF, while not found in surgical specimen. This was not reported before, and we thought it might be the time and spatial heterogeneity of MB cells [22], and detecting gene aberrant from multi-sites or multi-specimens is necessary. Moreover, we further found that MYC and TP53 gene aberrant was an independent risk factor associated with OS, MYC amplification was present in more than half of Group 3 MB, and TP53 mutation was present in about 11.1% of Group 3. Meanwhile, molecular group and presence of tumor cells in CSF were independent predictive factors for the PFS and OS of metastatic MB, which consistent with previous reports [6, 16]. In fact, patients with MYC or TP53 aberrant were very high-risk, and the median survival time was very poor. As in our study, the mortality and morbidity within two years were nearly 100%.

Besides its high mortality, the estimated 5-year PFS/OS rates of all metastatic patients were 48.1% and 53.6% respectively, which was similar to Waszak SM’s reports [23]. In the present study, the median survival time was 2.2 years, and the 2- and 5-year PFS/OS of patients with negative gene profile (negative gene aberrant group) were 77.0%/90.5% and 61.1%/68.3%, respectively. However, those patients with positive gene aberrant showed a poorer prognosis, and the median survival time was only 1.0 years, and almost all patients were died in 2 years. The 2-year PFS/OS of patients with positive MYC/TP53 gene...
aberrant patients only were 2.6%/11.5%, respectively. These results powerfully further proofed the importance of detecting MYC/TP53 gene aberrant.

Conclusion

MYC and TP53 aberrant correlated with a worse prognostic outcome in children MB. Molecular genetic detection of surgical specimen had been popularized, but the genetic results of CSF tumor cells has been not reported. Although there were some limitations of our study, such as the inherent bias, small sample size and a single-center observation, we still could conclude that detection of MYC and TP53 aberrant of tumor cells in CSF maybe an important method to complement the molecular results of surgical specimen, and can improve to adjust the chemotherapy regimen of metastatic or refractory MB disease in time.

Abbreviations

CTX: Cyclophosphamide; CSF: Cerebrospinal fluid; CI: Confidence interval; CSI: Craniospinal irradiation; CNS: CMB: Classic MB; Central nervous system; DNMB and MBEN: Desmoplastic/nodular and MB with extensive nodularity; VP16: Etoposide;

GTR: Gross total resection; IT-MTX: Intrathecal methotrexate; LCAMB: Large-cell and anaplastic MB; LP: Lumbar puncture; MB: MTX: Methotrexate; Medulloblastoma; NOS: Not otherwise specified; NTR: Near total resection; OS: Overall survival; STR: Subtotal resection; VCR: Vincristine;

Declarations

Acknowledgments

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Authors’ contributions

Shuxu Du and Jiang Xiao designed the concepts of this study, Miao Li and Yujie Li performed and interpreted statistical and bioinformatic analysis, Yuan Wang, Jin Zhang, Xiaojun Gong and Yanling Sun provided data, Shuxu Du, and Miao Li wrote and revised the manuscript, Jiang Xiao, Wanshui Wu and Liming Sun supervised this study. All authors read and approved the final manuscript.

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Availability of data and materials

All reagents and devices used in the present study are commercially available and are not related to human confidentiality. The datasets are included in the manuscript. Other encrypted clinical data are stored at the Beijing Shijitan Hospital and are available upon request to the corresponding author.

Ethics approval and consent to participate

Ethics approval was obtained from the Beijing Shijitan Hospital (2015 approval for Other Categories No. 12). The informed consent to participate was obtained from the legal guardian.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

References


**Figures**
Figure 1

Treatment protocols for children with metastatic MB Notes: IT-MTX, intrathecal methotrexate, CSI, craniospinal irradiation.

Figure 2

MYC and TP53 gene aberrant of CSF and surgical specimen in patients with metastatic MB.
Figure 3

Survival of patients with metastatic MB. (A) PFS rates of patients; (B) OS rates of patients.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Appendix.docx
- TableS1.docx