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Frequent MN1 Gene Mutations in Malignant Peripheral Nerve Sheath Tumor

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Abstract. *Background/aim: Malignant peripheral nerve sheath tumor (MPNST) is a rare soft-tissue tumor, and its diagnosis is usually made histopathologically. The effectiveness of chemotherapy and radiotherapy has not been established. We elucidated prognostic factors, diagnostic markers, and therapeutic targets. Materials and Methods: Cases of MPNST were studied using next-generation sequencing. A total of 24 tumor samples, 11 from von Recklinghausen's disease-associated MPNST (vRH-MPNST), 11 from sporadic non-vRH MPNST, and two neurofibroma (NF) cases were retrieved, on which next-generation sequencing and survival analysis were performed. Results: We identified NF1 gene mutations, including three mutations in two NFs, and 10 mutations in eight MPNSTs (five vRH-MPNSTs and three sporadic MPNSTs). Meningioma 1 (MN1) gene alteration was detected in six cases of vRH-MPNST. It is considered that MN1 gene alteration is related to the tumorigenesis of vRH-MPNST. Conclusion: MN1 gene mutation was detected in more than half of our cases, it may have potential for use as a therapeutic target in vRH-MPNST.*

Malignant peripheral nerve sheath tumor (MPNST) is a rare soft-tissue tumor, accounting for 5% of all malignant soft-tissue tumors. Up to 50% of MPNST occur, in patients with von Recklinghausen's disease (neurofibromatosis type 1, NF1), 10% are radiation-induced, and the rest are sporadic (1). The prognosis is usually unfavorable, especially in patients with von Recklinghausen's disease. Moreover, the 5- and 10-year survival rates in patients without distant metastasis and the 10-year survival rate in patients with distant metastasis are reported to be 50%, 30%, and 8% respectively (2-4). The conventional treatment for MPNST is surgical resection, and the effectiveness of chemotherapy and radiotherapy for it has not been established.

Although diagnosis is based on morphological and immunohistochemical features, MPNST frequently displays non-specific histological findings and immunophenotype (5-7). MPNST exhibits the expression of S-100 protein, SRY-box transcription factor 10, and glial fibrillary acidic protein at rates of only <50%, 30%, and 20-30% (1, 8, 9), respectively, and there are no specific immunohistochemical markers. Recently, the usefulness of assessing trimethylation at lysine 27 of histone H3 (H3K27me3) has been reported in MPNST but complete loss of staining for H3K27me3 does not occur in all MPNST cases; the prevalence of H3K27me3 loss ranges from 30% to 70% (5, 6, 8, 10). Moreover, the differential diagnosis of MPNST is wide and includes various spindle-cell sarcomas and pleomorphic sarcomas, which have also been reported to exhibit complete loss of H3K27me3 (6, 11, 12). The diagnosis of MPNST is performed mainly by exclusion diagnosis for the above reasons.

Recently, next-generation sequencing (NGS) has revealed numerous novel gene aberrations in various tumors. In this study, we analyzed cases of MPNST associated with von Recklinghausen's disease (vRH-MPNST), sporadic non-vRH-MPNST, and neurofibroma (NF), using NGS to reveal prognostic factors, diagnostic markers, and therapeutic targets in MPNST.

Materials and Methods

Patients. This study was conducted in accordance with the principles embodied in the Declaration of Helsinki. This study was also approved by the Ethics Committee of Kyushu University (No.

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29-429, 29-625) and conducted in accordance with the Ethical Guidelines for Epidemiological Research enacted by the Japanese Government. Informed consent was obtained from the patients or their guardians.

Frozen tumor tissue samples were selected in the form of 11 samples from non-vRH-MPNST, 11 samples from vRH-MPNST, and two samples from NF cases that had been diagnosed at the Department of Anatomic Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, between 1988 and 2008 (Table I). These samples had been obtained from surgically resected tumors. Two vRH-MPNST samples (tumors 7A and 7B) were obtained from the same patient, one in the primary operation and the other in a subsequent operation. Survival data of patients with MPNST were available for all cases, with follow-up ranging from 4 to 156 months (median=17 months, mean=44.3 months).

Amplicon sequencing. Genomic DNA was prepared from tumor samples using Tissue DNA Purification Kit (Promega, Madison, WI, USA). All exons of 409 cancer-related genes were sequenced using the Ion AmpliSeq Comprehensive Cancer Panel (CCP) (Thermo Fisher Scientific, Cleveland, OH, USA) with an Ion Proton DNA sequencer (Thermo Fisher Scientific), in accordance with the manufacturer's protocol.

Sequencing data analysis. Obtained short-read sequences were mapped to human genome assembly hg19 using Torrent Suite Software (Thermo Fisher Scientific). Short variants were called using Ion Reporter Software (Thermo Fisher Scientific), and annotated by Ion Reporter and SnpEff (13). From the called variants, those with variant allelic frequency of less than 1% were removed as sequencing errors. Next, variants identical to single-nucleotide polymorphisms (SNPs) with a minor allelic frequency of more than 5% in The University of California Santa Cruz Genome Browser (14) or in the Human Genetic Variation Database (15) and those identified in five or more out of the 24 samples were removed as common SNPs. DNA copy numbers were estimated using Ion Reporter Software with AmpliSeq CCP single-sample analysis workflow. As a copy number baseline, CCP sequencing data of blood samples from five healthy males were used.

Survival analysis. Survival analysis was performed using the R statistics software package survival (16, 17).

Public mutation data. Mutation data of neurofibromin 1 *(NF1)* and meningioma 1 *(MN1)* genes in various cancer types were downloaded from cBio Cancer Genomics Portal (18, 19).

Results

NF1 gene mutations in MPNST and NF. The data of *NF1* gene alteration measured by NGS are shown in Table II. In samples from two patients with NF, we identified three mutations: One missense mutation (K1640fs) and two nonsense mutations (V497fs and R1362*). Both of the samples from patients with NF harbored mutations resulting in the introduction of a premature stop codon in the *NF1* gene. In nine samples from 22 patients with MPNST, we identified 10 mutations including frameshift mutations (Y1659fs, N292fs, S47fs), missense mutations (D1623G, M1, M645V), and nonsense mutations (R1241*, R1513*, R1748*, R2237*) (Figure 1A). In addition, copy-number variation analysis with data measured by NGS showed heterozygous loss in the *NF1* gene in eight samples from patients with MPNST. Of 10 MPNST samples harboring mutation, four samples revealed heterozygous deletion of *NF1*. Considering the mutation and copy-number variation analyses, 14 out of 22 samples from patients with MPNST might have had loss of *NF1* gene expression.

MN1 gene mutations are associated with the pathogenesis of MPNST through von Recklinghausen's disease. We explored the genetic difference between non-vRH-MPNST and vRH-MPNST. Among the 24 samples assessed by NGS, there were 11 samples derived from non-vRH-MPNST and 11 from vRH-MPNST. As a result of analysis using genetic data from NGS, we identified the *MN1* gene that was mutated only in vRH-MPNST (Table III). *MN1* gene mutation was observed in six out of 11 samples. All four *MN1* mutations that we identified were missense mutations located at Q533P, Q540R, Q542E, and V595M (Figure 1B). To confirm the possibility of *MN1* gene mutations, we analyzed the genetic difference between our data and data in cBioPortal (http://www.cbioportal.org/). cBioPortal provides genetic data from 147 studies on several cancer types that do not include MPNST. The data in cBioPortal showed that the mutations in the *MN1* gene were located across the entire genome. The mutations were concentrated at the region of 530 to 550 amino acids within the *MN1* gene, and all mutations were frameshifts. The *MN1* gene mutations at 530-550 amino acids were observed in adrenocortical carcinoma (6/90 samples), melanoma (4/491 samples), and lung adenocarcinoma (2/576 samples) (Figure 1C), according to data in cBioPortal (http://www.cbioportal.org/). Although the types of mutations differed, *MN1* gene mutations in MPNST and several cancer types were concentrated at a similar region, indicating that this region in the *MN1* gene might be a mutation hotspot. These findings suggest that *MN1* mutations are involved in MPNST as well as other neoplasms described above.

Mutations of B-cell lymphoma 2 (BCL2) apoptosis regulator are found in both vRH- and non-vRH-MPNST. The data of *BCL2* gene alteration measured by NGS are summarized in Table IV. Two mutations, namely, one frameshift mutation (p40fs) and one missense mutation (A43G), were detected in vRH-MPNST. One case of sporadic MPNST harbored a missense mutation (A43G). Interestingly, tumor 7A and tumor 7B had *MN1* and *BCL2* gene mutations, respectively. This suggests that *MN1* and *BCL2* gene mutations may be mutually exclusive genetic events in vRH-MPNST.

We compared the survival data for patients with vRH-MPNST and those with non-vRH-MPNST. Our analysis of

Table I. *Clinical findings*.

Case no.	Tumor no.	Age, years	Gender	Location
vRH-MPNST				
1	1	46	M	Forearm
2	2	68	M	Brachial muscle
3	3	22	M	Muscle of back
4	4	34	M	Sacral region
5	5	30	F	Radial nerve
6	6	69	M	Subcutis of thigh
7	7A	70	M	Thoracic vertebra-thoracic cavity
	7B	70	M	Thoracic vertebra 1/2
8	8	25	F	Retroperitoneum
9	9	20	F	Thigh
10	10	72	M	Thigh
Non vRH-MPNST				
11	11	17	M	Pelvis
12	12	77	F	Posterior mediastinum
13	13	61	F	Subcutis of thigh
14	14	47	M	Knee
15	15	73	M	Femoral muscle
16	16	68	M	Ankle joint
17	17	47	M	Retroperitoneum
18	18	78	M	Triceps brachii muscle
19	19	66	F	Subcutis of thigh
20	20	25	F	Axilla
21	21	21	M	Back
NF				
22	22	38	F	Back
23	23	58	M	Face

M: Male; F: female; vRH-MPNST: von Recklinghausen's disease-associated malignant peripheral nerve sheath tumor; NF: neurofibroma.

Table II. *Neurofibromin 1 (NF1) gene mutations in malignant peripheral nerve sheath tumor (MPNST) and neurofibroma (NF).*

Case no.	Tumor no.	*NF1* gene mutation	Location of *NF1* gene mutation
vRH-MPNST			
3	3	Frame-shift	N292fs
4	4	Nonsense mutation	R2237*
6	6	Missense mutation	D1623G
7	7A	Missense mutation	M1
	7B	Missense mutation	M1
9	9	Frame-shift	Y1659fs
Non vRH-MPNST			
11	11	Nonsense mutation	R1513*, R1748*
19	19	Frame-shift	S47fs
21	21	Nonsense mutation	R1241*
		Missense mutation	M645V
NF			
22	22	Nonsense mutation	V497fs, R1362*
23	23	Missense mutation	K1640fs

vRH-MPNST: von Recklinghausen's disease-associated malignant peripheral nerve sheath tumor; NF: neurofibroma.

the survival rate in vRH-MPNST showed that such cases had a poorer prognosis than those with non-vRH-MPNST (Figure 2A).

Next, to explore the mutations associated with prognosis in MPNST, we compared the genetic status and survival data. We found that *MN1* gene and *BCL2* gene mutations were associated with poorer prognosis (Figure 2B and C, respectively).

Discussion

MPNST is usually diagnosed on the basis of histopathological findings because no specific diagnostic immunohistochemical and genetic markers are currently available. Although H3K27me3 was previously reported to be a helpful marker to diagnose MPNST, it is not sufficiently sensitive to use as a diagnostic marker for almost all MPNST cases. Furthermore, MPNST may be a heterogeneous tumor category because the diagnosis of MPNST is basically through exclusion. Herein, we reported that *MN1* gene mutation was present in most vRH-MPNST cases. To the best of our knowledge, this is the first report of gene aberration in *MN1* occurring in vRH-MPNST. It is desirable to clarify the role of the *MN1* gene in vRH-MPNST and to reclassify MPNST by accumulating more cases.

Generally, MPNST is a malignant neoplasm with peripheral nerve sheath differentiation arising from a peripheral nerve bundle, and occurs in patients with von Recklinghausen's disease (20). MPNST frequently includes a complex karyotype with translocation, duplication, and DNA copy number alteration (21, 22). A certain proportion of patients with vRH-MPNST carry a germline alteration of the *NF1* gene with complete inactivation of one *NF1* allele (23). At the same time, somatic mutation of the *NF1* gene is found in about 40% of both non-vRH-MPNST and vRH-MPNST; most aberrations lead to inactivation of gene function (23). In this study, *NF1* gene mutation or heterozygous deletion was detected in 14 out of 22 MPNST cases, as previously reported (23). The presence of vRH-MPNST was significantly associated with prognosis but *NF1* gene alteration was not a prognostic factor in MPNST. This suggests that *NF1* gene alteration may be associated with the early phase of tumorigenesis in non-vRH-MPNST because

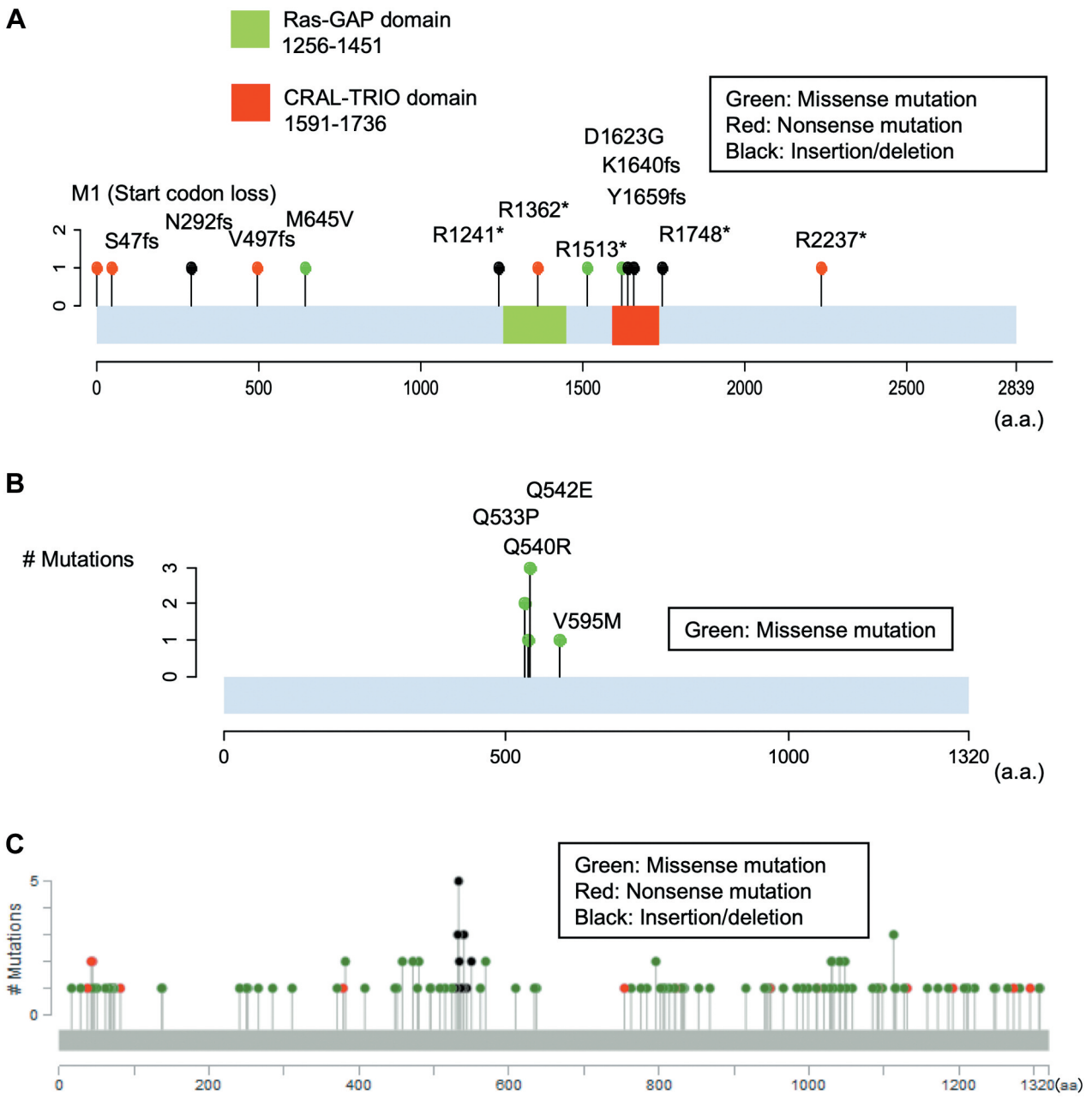
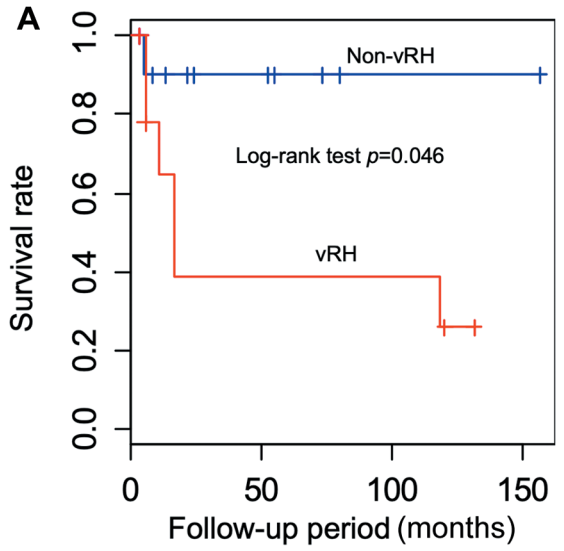
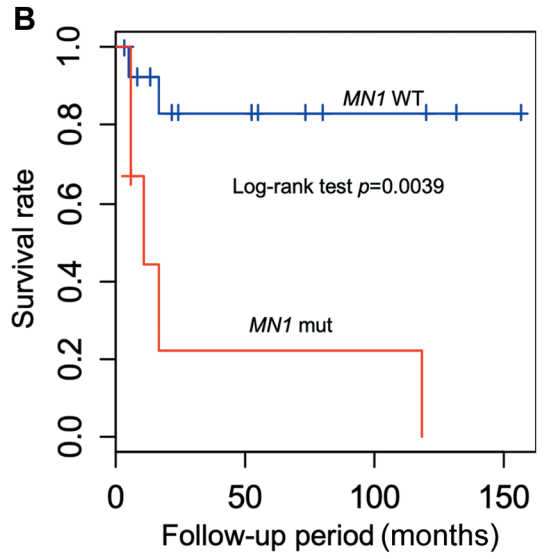


Figure 1. *A: Neurofibromin 1 (NF1) gene mutation identified in patients with malignant peripheral nerve sheath tumor (MPNST) and neurofibroma (NF). We identified three mutations: one missense mutation and two nonsense mutations in two patients with NF. In nine samples from patients with MPNST, 10 mutations were found, including frame-shift, missense mutations and nonsense mutations. B: Meningioma 1 (MN1) gene mutations detected in six samples derived from von Recklinghausen-MPNST. All four mutations were missense mutations located at Q533P, Q540R, Q542E and V595M. C: MN1 gene mutations were concentrated at 530 to 550 amino acids (aa) in adrenocortical carcinoma, melanoma and lung adenocarcinoma.*

this gene alteration is also detected in von Recklinghausen's disease-associated neurofibroma.

The *MN1* gene encodes a protein associated with the transcriptional coactivator or corepressor stimulating retinoid acid receptor (RAR)/retinoid X receptor (RXR) or vitamin D receptor-mediated transcription (24, 25). The *MN1* gene is located at chromosome band 22q12 and was found to be disrupted by balanced translocation (4;22)(p16;q11) in meningioma. In addition, a fusion protein, *MN1–translocation ETS leukemia (TEL)*, was reported in patients





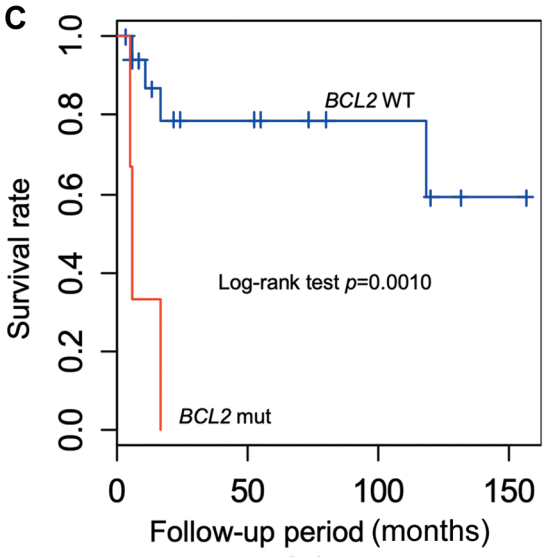


Figure 2. *A: Comparing the survival data of patients with malignant peripheral nerve sheath tumor (MPNST) with and without von Recklinghausen's disease (vRH). B: Meningioma 1 (MN1) gene mutation was associated with poorer prognosis. C: B-Cell lymphoma 2 (BCL2) gene mutation was associated with poorer prognosis. WT: Wild-type; mut: mutant.*

Table III. *Meningioma 1 (MN1) gene mutations in malignant peripheral nerve sheath tumor (MPNST).*

Case no.	Tumor no.	Location of mutation
2	2	Q533P
3	3	Q540R
4	4	Q542E
5	5	Q542E
7	7A	Q533P
10	10	V595M

with t(12;22)(p13;q12) in myeloid leukemia or myelodysplastic syndrome (26). As to the role of MN1–TEL1 fusion protein in hematopoietic cells, it was reported that *it* has transforming activity in NIH3T3 cells, which are sensitive to transformation by erythroblastic transformation specific factors, and is likely to act as a dysregulated transcription factor (27). Moreover, *MN1–TEL1* functions as an oncogene *in vivo* by promoting myeloid and lymphoid progenitors and blocking their differentiation (28). On the other hand, information about the function of the *MN1* gene are limited, although MN1 overexpression has been reported to predict worse prognosis regardless of the presence of a fusion gene in acute myeloid leukemia (29). It was reported that the *MN1* gene promotes leukemogenesis by cooperating

Table IV. *B-Cell lymphoma 2 (BCL2) gene mutations in malignant peripheral nerve sheath tumor (MPNST).*

Case no.	Tumor no.	*BCL2* gene mutation	Location of mutation
vRH-MPNST			
1	1	Frame-shift	P40fs
7	7B	Missense mutation	A43G
Non vRH-MPNST			
11	11	Missense mutation	A43G

vRH-MPNST: von Recklinghausen's disease-associated malignant peripheral nerve sheath tumor.

with some oncogenic fusion genes, such as nucleoporin 98 (NUP98)–homeobox D13 (*HOXD13*), mixed lineage leukemia (*MLL*)–ALL1 fused gene from chromosome 9 (*AF9*), and MLL–eleven-nineteen leukemia (*ENL*), as well as mutated Runt-related transcription factor 1 (*RUNX1*), and by becoming a target of insertional mutagenesis in a hematopoietic stem cell gene therapy trial (30). *MN1* gene aberration has also been detected in adrenocortical carcinoma, malignant melanoma, and lung adenocarcinoma, according to data in cBioPortal (http://www.cbioportal.org/). The present study demonstrated *MN1* gene alteration in six cases of vRH-MPNST. The mutations were concentrated in the same region, although the mutant sites varied in each case. *MN1* gene aberration may be related to the tumorigenesis of vRH-MPNST (31).

Effective therapy for MPNST has not been established yet, which is one of the reasons for the poor prognosis of MPNST. Our study demonstrated from the gene mutations in tumor no. 7 that there might be mutual exclusivity between *MN1* and *BCL2* gene mutations. This suggests that MPNST exhibits molecular heterogeneity within a single tumor of the same case, which may make the treatment of patients with MPNST difficult. In previous work, *MN1* was reported as a potential therapeutic target; moreover, MN1-silencing RNA significantly inhibited the colony-forming potential of *MLL*-rearranged primary leukemia cells, in which *MN1* was required for leukemogenicity (30). *MN1* may thus be a potent therapeutic target for MPNST.

In previous studies, from the relationships between MPNST and NF1 syndrome, tumor size, malignancy grade, and site of involvement were reported as prognostic factors (7, 32, 33). Histopathologically, rhabdomyoblastic differentiation (malignant triton tumor) is also associated with a poor prognosis (34). In the present study, we found that the prognosis of vRH-MPNST was worse than that of non-vRH MPNST, which supported previous findings. *MN1* gene mutation can be considered as a candidate prognostic factor but we determined that such mutations may be an independent prognostic factor because they were associated with the pathogenesis of vRH-MPNST and might be a confounding factor.

BCL2 has been reported as an oncogene regulating the cell cycle as well as suppressing apoptosis, and was identified by analysis of follicular lymphoma (35). In a number of previous studies, the tumorigenesis of non-vRH- or vRH-MPNST was found to be involved in apoptosis and cell-cycle regulation. Furthermore, BCL2 inhibitor, having the ability to suppress C-X-C motif chemokine 12 (CXCL12), might be therapeutically useful in MPNST (36). *BCL2* gene mutation was found to be a significant prognostic factor in our study. *BCL2* mutation might reflect that regulation of the cell cycle or CXCL12/CXCR4 signal transduction is related to tumor development, which might be exploited as a therapeutic target.

Several studies have reported cell-cycle regulators to be involved in the tumorigenesis of MPNST. If the development of MPNST is a multistep process, it is considered that *MN1* aberration may be one step towards tumorigenesis based on alterations of a cell-cycle regulator.

Conclusion

It is considered that the *MN1* gene might be associated with the tumorigenesis of vRH-MPNST. *MN1* gene mutation was detected in more than half of our vRH-MPNST cases, and might have potential as a therapeutic target.

Conflicts of Interest

The Authors declare that they have no competing interests.

Authors' Contributions

Izumi Kinoshita performed the research and wrote the article. Yuichi Yamada, Kenichi Kohashi, Hidetaka Yamamoto, Takeshi Iwasaki, Shin Ishihara, Yu Toda, Yousuke Susuki, Kengo Kawaguchi, Toshio Ichiki, Yuki Sato contributed to the research design and slide review. Masutaka Furue and Yasuharu Nakashima contributed to the sample collection and research design. Yoshinao Oda designed the research and gave final approval of the article. All Authors critically reviewed and approved the article.

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