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Aberrant demethylation and expression of *MAGEB2* in a subset of malignant peripheral nerve sheath tumors from neurofibromatosis type 1

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ABSTRACT

Background: Malignant peripheral nerve sheath tumors (MPNSTs) occur in several percent of neurofibromatosis type 1 (NF-1) patients. When a CpG island (CGI) in the 5' region of a gene is methylated, transcription of that gene may be suppressed. Although cancer-testis antigens, including MAGEB2, are potential therapeutic targets for cancer in medical practice, information on MAGEB2 in MPNST is scarce.

Objective: The purpose is to clarify the methylation status and expression of *MAGEB2* in MPNSTs derived from patients with NF-1.

Methods: Quantitative real-time methylation-specific PCR (RT-MSP) and quantitative real-time reverse transcription-PCR (RT-PCR) were performed to measure methylation and mRNA expression, respectively, in MPNST cell lines and in MPNST and neurofibroma samples from patients with NF-1. Immunohistochemical analysis was also performed to assess MAGEB2 protein expression.

Results: RT-MSP and RT-PCR data showed low methylation levels and detectable mRNA expression of *MAGEB2*, respectively, in one MPNST cell line, but high methylation level and absence of expression in each other cell line and in normal cells. Based on RT-MSP data, 3 of 18 MPNST clinical samples exhibited low methylation levels; in contrast, all cutaneous and plexiform neurofibroma samples and normal cells exhibited high methylation levels. Methylation levels were not significantly associated with any clinical parameters. Immunohistochemical analysis revealed expression of *MAGEB2* protein in MPNST clinical samples with the low methylation level.

Conclusions: MAGEB2 can be aberrantly demethylated and expressed in MPNSTs. Conversely, the gene may not be demethylated in any types of neurofibroma, suggesting that the demethylation does not occur before malignant transformation.

1. Introduction

Neurofibromatosis type 1 (NF-1), also known as von Recklinghausen disease, is an autosomal dominant phacomatosis with neurocutaneous involvement caused by mutation of *NF1*, which encodes neurofibromin [1]. NF-1 patients present with various signs and symptoms including café-au-lait macules, neurofibromas, iris Lisch nodules, bone malformations, and malignant peripheral nerve sheath tumors (MPNSTs). MPNSTs occur in several percent of NF-1 patients and are often associated with poor prognosis [2]. Treatment options for MPNSTs are limited; consequently, molecular targets expressed specifically in tumor cells should be identified to develop more effective treatments.

DNA methylation is a common DNA modification involving the covalent binding of a methyl group to a DNA nucleotide; most commonly the cytosine of a CpG dinucleotide (genomic sites where a 5′ cytosine is adjacent to a 3′ guanine) is methylated [3]. CpG islands (CGIs) are dense clusters of CpGs and are often located in the 5′ regions of genes; CGI methylation can cause suppression of transcription [3]. For most genes in normal cells, the majority of CGIs within 5′ regions are unmethylated, and these genes can be expressed; however, for a minority of gene, CGIs within 5′ regions are methylated, and these genes (e.g., *ANKRD30A* [4],*PAX2* [5], and *SNCG* [6] on autosomes and *MAGEs* [7] on the X chromosome) are

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silenced in normal cells. Cancer-testis antigens (CTAs), encoded by cancer-testis genes (CTGs), are strong candidates as diagnostic markers and therapeutic targets for some types of cancers, since their expression, usually regulated by DNA methylation in 5′ CGIs, is normally restricted to only a few tissues such as testis tissue and aberrantly elevated in some types of cancers [7,8].

Melanoma antigen family B2 (MAGEB2) is a CTA that has recently been implicated in carcinogenesis and identified a potential cancer biomarker. van Duin et al. reported that MAGEB2 protein is of value for diagnosing multiple myeloma because it was expressed in 47% of newly diagnosed patients and 28% of relapsed patients [9]. Pattini et al. showed that *MAGEB2* is activated by promoter demethylation in head and neck squamous cell carcinoma and that it has growth promoting effects in a minimally transformed oral keratinocyte cell line [10]. On the other hand, the information on *MAGEB2* in MPNST is scarce. The present study was conducted to assess the methylation status of the *MAGEB2* 5′ CGI and *MAGEB2* expression in MPNSTs derived from NF-1 patients.

2. Materials and methods

2.1. Cell lines, clinical samples, and extraction of nucleic acid

The MPNST cell lines sNF02.2 and sNF96.2 were purchased from the American type culture collection (Manassas, VA). MPNST cell lines (HS-PSS, HS-Sch-2, NMS-2, and YST-1) and normal cultured fibroblasts (NB1-RGB cells) were provided by the Riken BioResources Center (Tsukuba, Japan). Normal cultured Schwann cells (HSC1700 cells) were provided by ScienCell (Carlsbad, CA). Eighteen paraffin-embedded MPNST samples were obtained from NF-1 patients (age range, 15–70 years) (Table 1a and Supplementary data Table S1). Eleven fresh-frozen cutaneous neurofibroma samples and 18 fresh-frozen plexiform neurofibroma samples were obtained from NF-1 patients without MPNST (age ranges are 30–64 years and 5–61 years, respectively) (Tables 1b and 1c). Eighteen paraffin-embedded plexiform neurofibroma samples were obtained from NF-1 patients without MPNST (age range, 12–79 years) (Table 1c). For each clinical specimen, written informed consent was obtained from reachable donors or their next of kin in cases of minor donors; alternatively, the ethics committee of The Jikei University School of Medicine waived the need for consent for unreachable donors including minor donors. Each diagnosis of NF-1 was based on diagnostic criteria for NF-1 published by the National Institutes of Health [11]. MPNST and neurofibroma were diagnosed histopathologically by at least two experienced board-certified pathologists. To extract DNA from paraffin-embedded samples, each sample was sliced into multiple sections (4–10 μm thick), deparaffinized, and then dissected with a fine needle. Genomic DNA was extracted via a standard phenol/chloroform extraction and ethanol precipitation procedure or with a QIAamp DNA mini kit (Qiagen, Valencia, CA). ISOGEN (Nippon Gene, Tokyo, Japan) was used to isolate total RNA from each specimen.

Table 1b
Characteristics of patients donating cutaneous neurofibroma samples.

Sample no.	Age (yrs)	Sex	Site	Methylation level (%)
#19	34	M	Trunk	95.7
#20	39	F	Trunk	91.8
#21	63	F	Trunk	100.0
#22	64	M	Upper limb	94.5
#23	60	F	Trunk	91.3
#24	63	F	Trunk	87.2
#25	53	F	Trunk	89.8
#26	30	M	Trunk	100.0
#27	72	F	Trunk	79.9
#28	31	M	Upper limb	97.6
#29	55	F	Trunk	95.5

M: male; F: female.

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2.2. Treatment with 5-aza-2′-deoxycytidine

For 5-aza-2'-deoxycytidine (5-aza-dC) treatment, MPNST cells were seeded at a density of 1.0–2.5 × 10^5 cells per 10 cm dish on day 0 and then exposed to medium containing 1 μM 5-aza-dC (Sigma–Aldrich, St Louis, MO) on days 1 and 3; as a result the cells were exposed during 96 h. Cells were harvested on day 5. For each cell line; 5-aza-dC-treated cultures showed mild growth suppression on day 5 compared with untreated cells.

Table 1a
Characteristics of patients donating MPNST samples.

Sample no.	Age (yrs)	Sex	Prognosis[a]	Site	Expression	Methylation[b]
#1	15	M	Dead, 13 months	Trunk	–	89.2
#2	66	M	Alive, 26 months	Trunk	+	8.5
#3	24	F	Dead, 28 months	Head/Neck	+	36.0
#4	41	F	Alive, 88 months	Head/Neck	–	86.0
#5	63	M	Alive, 62 months	Trunk	+	4.9
#6	57	M	Alive, 78 months	Upper limb	–	90.3
#7	66	M	Alive, 46 months	Trunk	–	93.5
#8	56	M	Alive, 45 months	Trunk	–	93.1
#9	42	F	Dead, 25 months	Lower limb	nd	100.0
#10	57	F	Alive, 146 months	Lower limb	nd	89.8
#11	34	M	Dead, 4 months	Trunk	–	89.7
#12	33	M	Dead, 11 months	Trunk	–	97.5
#13	50	M	Alive, 19 months	Upper limb	nd	73.8
#14	44	M	unknown	Head/Neck	nd	100.0
#15	66	M	Alive, 73 months	Trunk	nd	100.0
#16	17	M	Dead, 13 months	Head/Neck	nd	98.1
#17	70	F	Alive, 17 months	Trunk	nd	100.0
#18	24	M	Alive, 11 months	Trunk	nd	90.6

M: male; F: female; nd: not done.
[a] If the patient had died, the interval from diagnosis to death is stated. If the patient was alive at the end of the study, the period from diagnosis to the last day when the patient was confirmed to be alive is stated. [b]Methylation level (%).