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Preoperative analysis of 11q loss using circulating tumor-released DNA in serum: A novel diagnostic tool for therapy stratification of neuroblastoma

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ABSTRACT

Allelic deletion of the long arm of chromosome 11 (11q loss) is closely associated with the prognosis of neuroblastoma (NB). Here we examined 11q loss using tumor-released DNA fragments in the sera of 24 cases. The allelic intensity score of a panel of polymorphic markers in 11q23 in serum DNA was significantly different between the 11q loss-positive group and the11q loss-negative group. The 11q loss-positive and -negative groups did not overlap when a cut-off value of 0.5 was chosen for the allelic intensity score. Our serum-based 11q loss analysis could predict the allelic status of 11q in tumors.

1. Introduction

Neuroblastoma (NB) is the most common extracranial solid tumor of early childhood. Genomic changes in the tumor correlate with the behavior and outcome of NB patients. Amplification of the *MYCN* gene (MNA), located on 2p24, was detected in about 22% of the patients with NB, and is considered the strongest prognostic factor [1–3]. The non-MNA NBs fall into two clinically distinct subgroups: a low-risk subgroup with overall survival rates of more than 95% without any intensive therapy, and a high-risk subgroup with overall survival rates of less than 40% even though they are given dose-intensive, multimodal therapy. In the high-risk non-MNA group, development of NB depends on factors other than MNA, such as genetic expression profiles [4–6], aberrant hypermethylation of tumor suppressor gene [7–11], and chromosomal loss of heterozygosity (LOH) [12–14].

Non-MNA NB patients frequently have allelic deletion of the long arm of chromosome 11 (11q loss) [15,16], and have poor outcomes [12,17]. Accordingly, routine assessment of 11q loss status as well as MNA is required for therapy stratification of NB in the INRG staging system [18]. However, patients with localized NB and non-MNA, who were categorized as having low-risk NB, sometimes were given reduced therapy and were advised to accept a wait-and-see strategy [19,20]. Therefore, an early and non-invasive detection system for 11q loss that does not require any surgical procedure is needed to help select the appropriate therapy for these patients. We previously developed a test that uses tumor-released DNA that is

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present in the serum of NB patients [21,22] to decide which therapeutic strategy to use for treating NB before any invasive intervention. Preoperative combined assessments of MNA and 11q loss using serum DNA will make it possible to safely perform risk-adapted therapy. Here we describe a system for evaluating the allelic status of chromosome 11 using the sera of NB patients.

2. Patients and methods

2.1. Subjects

Twenty-four children diagnosed with NB at the Hospital of Kyoto Prefectural University of Medicine and Kyoto City Hospital were enrolled onto this study with the informed consent of their parents. The only inclusion criteria was the availability of outcome data and DNA samples from three tissues: serum obtained at the onset of NB, the tumor, and non-tumor tissue. At the time of diagnosis, 12 patients were younger than 1 year, and 12 were between 1 and 5 years of age. Seven of the patients had MNA, and 17 patients did not have MNA. According to the International Neuroblastoma Staging System [23], the 24 children included 9 in stage 1, 1 in stage 2A, 1 in stage 4S, 1 in stage 3, and 12 in stage 4. The serum and tumor samples were linked to clinical and biological information and the laboratory investigators were blinded to these data (Table 1).

2.2. Tumor and serum DNA preparation

DNA was extracted with a QIAamp DNA Mini kit (Qiagen) as per the manufacturer's protocol. Patients' sera were obtained before any therapy and surgery, and stored at −20 °C. Serum DNA was extracted as described previously [21,22]. For serum DNA isolation, serum was centrifuged at 15,000 rpm for 10 min to remove leukocytes, and then we used 100 μL of serum supernatant, which contained 1 μg of salmon testes DNA (Sigma) as a carrier DNA.

2.3. Allelic status of 11q by microsatellite analysis

Previous studies have revealed the smallest region of deletion overlap (SRO), which is a region of deletion that is shared by all cases of NB with 11q loss, and polymorphic markers involved in that region [12,15,16]. Thus, we chose three highly informative polymorphic markers from the SRO of 11q for microsatellite analysis (Supplementary Table S1). Sense primers were labeled with FAM, VIC, or NED fluorescent dyes respectively, and antisense primers were modified with a reverse tail to promote single nucleotide overhangs (Applied Biosystems, Foster City, CA, USA). To discriminate whole chromosome loss, we also chose three informative polymorphic markers that were located in the short arm of chromosome 11, and performed the microsatellite analysis simultaneously. When possible, the markers were combined in multiplex fluorescence screening panels. Samples that gave equivocal results were rescreened in a conventional uniplex polymerase chain reaction (PCR).

Electrophoresis was performed with an ABI PRISM 310 genetic analyzer (Applied Biosystems) and analyzed with Genescan software (Applied Biosystems). Allelic deletion of 11q23 was considered to have occurred at an individual marker when a comparison of the allelic intensity score

Table 1
Characteristics and results of 11q loss of the patients.

No.	INSS stage	Age of onset (mo.)	*MYCN* status	Mass screening	Outcome	11q loss in serum (representative allelic intensity score of STS marker on 11q23)	11q loss (MLPA)
1	4	13	Non-amp.		Died of disease	+(0.15)	+
2	4	48	Non-amp.		Died of disease	−(1.04)	−
3	4	8	Amp		Died of disease	−(1.08)	−
4	3	11	Amp.	+	Died of disease	−(0.99)	−
5	2	11	Non-amp.	+	Die of disease	+(0.30)	+
6	1	10	Amp.		Alive	−(1.14)	−
7	4	13	Amp.		Die of disease	−(1.00)	−
8	4	58	Gain		Alive with disease	−(0.65)	−
9	4	36	Non-amp.		Die of disease	−(1.17)	−
10	4S	2	Non-amp.		Alive	−(1.04)	−
11	1	4	Non-amp.		Alive	−(0.65)	−
12	4	60	Non-amp.		Alive with disease	+(0.38)	+
13	1	18	Non-amp.	+	Alive	−(1.01)	−
14	1	36	Non-amp.	+	Alive	−(1.09)	−
15	1	9	Non-amp.	+	Alive	−(1.00)	−
16	1	11	Non-amp.	+	Alive	−(0.67)	−
17	1	8	Non-amp.	+	Alive	−(1.05)	−
18	1	10	Non-amp.	+	Alive	−(0.67)	−
19	4	49	Amp.		Died of disease	−(0.93)	−
20	4	36	Non-amp.		Alive	−(0.99)	−
21	1	11	Non-amp.	+	Alive	−(1.00)	−
22	4	1	Non-amp.		Alive	−(0.97)	−
23	4	60	Non-amp.		Died of disease	+(0.20)	+
24	4	12	Amp.		Alive	+(0.10)	+