Journal of Pediatric Surgery (2012) 47, 1789–1796

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Journal of Pediatric Surgery

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Original Articles

Clinical feature of anaplastic lymphoma kinase–mutated neuroblastoma

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Received 7 September 2011; revised 26 April 2012; accepted 3 May 2012

Key words:
ALK;
Neuroblastoma;
Mutation;
Amplification;
Mass screening;
Relapse;
Prognosis

Abstract

Purpose: Anaplastic lymphoma kinase (*ALK*) has recently been identified as a gene conferring a predisposition for neuroblastoma. We have analyzed tyrosine kinase domain mutations and amplification/expression of the *ALK* gene and focused on clinical features of neuroblastoma cases with *ALK* aberrations.

Methods: The frequency of *ALK* mutations, copy number gain, and expression were analyzed in 538 neuroblastoma tumors derived from 361 cases, including 161 cases detected by mass screening. These cases were analyzed according to clinicopathologic features including the International Neuroblastoma Staging System and patient outcomes.

Results: Three cases (0.8%) had *ALK* amplification, and 16 cases (5.2%) had missense mutations at positions F1174, F1245, D1249, and R1275. Among them, 7 cases were diagnosed at more than 14 months of age, and 11 cases were infants, including 9 cases detected by mass screening and 1 multiple neuroblastoma with a germline mutation. Of the 11 infants, 3 cases relapsed, and 1 case died of disease. Among cases detected by screening, activated *ALK* cases showed significantly worse prognosis ($P = .002$). Of 7 older cases, 5 had *MYC* amplifications, and 5 died of disease. The expression levels of *ALK* were up-regulated in cases with unfavorable outcomes. In cases with activated ALK neuroblastoma, survival rates of patients detected by screening were significantly better than those in the clinically detected group ($P = .025$).

Conclusions: The results of the present study support the hypothesis that activated *ALK* tumors represent a specific subset of neuroblastomas. These tumors usually develop in infants and may have a high capacity for recurrence.

Neuroblastoma, derived from neuroblasts in the neural crest, is the most common malignant solid tumor in children. The incidence of neuroblastoma is about 1 case per 7000 babies born per year [1]. More than 90% of neuroblastomas are diagnosed within the first 5 years of age, and they exhibit 3 distinct patterns of clinical behavior:

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http://dx.doi.org/10.1016/j.jpedsurg.2012.05.007

life-threatening progression, spontaneous regression, or maturation to ganglioneuroma. Many patients who are diagnosed at more than 1 year of age have advanced neuroblastomas with metastasis and usually show poor outcome despite multimodal therapies. Because more than 80% of neuroblastomas produce catecholamines, their metabolites (vanillylmandelic acid [VMA] and homovanillic acid [HVA]) are detectable in the urine. Consequently, mass screening projects to detect earlier-stage neuroblastomas have been carried out in several countries [2-5]. In Japan, large-scale mass screening of infants increased the annual incidence of the disease more than 2-fold. In contrast, both the incidence of advanced neuroblastoma in older children and the cumulative mortality rate of neuroblastoma were reduced significantly [2]. These findings indicate that a large number of neuroblastomas occur in infants and spontaneously regress or mature silently without clinical detection, whereas some of them may progress into malignant phenotypes [2]. These screening projects also provided us with a large number of neuroblastoma samples for investigation of the biologic characteristics of neuroblastoma. Transforming some tumors from a favorable type to unfavorable one has been suggested [6], but the precise molecular mechanism of malignant transformation of these tumors remains to be elucidated.

Clinically, it is essential to distinguish progressive neuroblastomas from favorable tumors because appropriate multimodal therapies are necessary to improve the prognosis of patients with progressive tumors. In contrast, aggressive therapy should be avoided in patients with favorable tumors to minimize side effects, late complications, and medical expenses. To more clearly distinguish the molecular and biologic heterogeneity of neuroblastomas, numerous analyses have been performed, and several distinct differences have been found including *MYCN* amplification, DNA ploidy, chromosomal loss or gain, expression of *NTRK1*, telomerase activation, and others [7].

ALK, a receptor tyrosine kinase, was initially identified through the analysis of a specific translocation in a subtype of non-Hodgkin lymphoma [8]. Recently, *ALK* was identified as a gene conferring a predisposition to neuroblastoma [9-12]. It harbors mutations in the tyrosine kinase domain (TKD) leading to aberrant activation of the gene and the downstream pathway [9]. Germline mutations of *ALK* were shown to be responsible for some hereditary neuroblastomas, and *ALK* has also been found to be mutated in some sporadic neuroblastomas [10-12]. Two hotspot mutations (F1174 and R1275) were identified as activating mutations [13], and several other mutations in TKD have been identified in sporadic neuroblastomas [14]. In these tumors, activating mutations and *ALK* amplification are the underlying mechanisms for *ALK*-dependent tumorigenesis and might be associated with a specific clinical phenotype in this disease [13,14].

In this article, we searched for acquired and germline *ALK* mutations in more than 300 Japanese neuroblastomas, including cases detected by mass screening. We investigated the characteristics of the neuroblastoma cases with *ALK* mutations, especially focusing on the treatment and the clinical outcomes of neuroblastoma cases with aberrant *ALK* genes. This study is the first to examine clinical features and investigate both mutations and copy number alterations of *ALK* in both a large set of sporadic neuroblastoma cases and cases detected by mass screening.

1. Materials and methods

1.1. Samples

Over the past 2 decades, more than 500 neuroblastomas derived from more than 400 cases, including approximately 180 detected by mass screening, have been stored at our university hospital. In some cases, we were consulted for molecular analysis by other hospitals in Japan. In the present study, genomic DNA was extracted from 361 neuroblastoma samples including 161 detected by mass screening. The tumor stages at surgery and tumor histology were classified according to the International Neuroblastoma Staging System (INSS) [15] and the International Neuroblastoma Pathological Classification (INPC) [16], respectively. Between 1991 and 2010, all patients were diagnosed at our hospital or affiliated hospitals as having neuroblastoma. Most patients were treated according to Japanese neuroblastoma protocols for infants or advanced stage neuroblastoma (A1, new A1, or A3) [17]. The follow-up period for all patients was more than 1 year. This research was approved by the ethical committee of our university (ID no. Hiro-Rin-20). Written informed consent for this research was obtained from parents of all patients. None of the patients had prior therapy before surgery or biopsy to obtain tumor specimens. Venous blood (5-7 mL) was taken from patients before surgery. Tumor DNA and constitutive DNA from each patient were extracted and purified using standard methods.

1.2. Affymetrix platform

Array experiments were done according to standard protocols for Affymetrix GeneChip Mapping 100K arrays (Affymetrix, Inc, Santa Clara, CA) [18]. The 100K single nucleotide polymorphisms (SNPs) or SNP 6.0 arrays were scanned with the Affymetrix GeneChip Scanner 3000 using GeneChip Operating System 1.2 (Affymetrix). Genotype calls and intensity of the SNPs were processed by GeneChip DNA Analysis Software. Individual SNP copy numbers and chromosomal regions with gains or losses were evaluated with the Affymetrix GeneChip Chromosome Number Tool 2.0.

1.3. *ALK* mutation analysis

Polymerase chain reaction (PCR) primers were designed for exons 23, 24, and 25 of the *ALK* gene, according to previous reports [9-12], and each exon was amplified from