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Anaplastic lymphoma kinase gene expression in small round cell tumors of childhood—a comparative ımmunohistochemical study☆,☆☆

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ABSTRACT

The focus of this study was to investigate anaplastic lymphoma kinase (ALK) expression by immunohistochemistry using a highly specific antibody. Distribution and frequency of ALK expression may provide a clue for ALK inhibitor use in small round cell tumors of childhood. The study group involved 76 small round cell tumors of childhood, which composed of 11 rhabdomyosarcomas, 13 Wilms tumors, 7 Ewing sarcoma/primitive neuroectodermal tumors, 34 peripheral neuroblastic tumors, and 11 acute lymphoblastic lymphoma. Anaplastic lymphoma kinase protein expression in small round cell tumors of childhood is poorly described in the literature. The findings of our study highlight a potential and possible role of targeting ALK in pediatric solid tumors by using ALK immunohistochemistry. Anaplastic lymphoma kinase may also have an oncogenic role in rhabdomyosarcomas and peripheral neuroblastic tumors, and they may possibly be treated with ALK inhibitors. Anaplastic lymphoma kinase expression in Wilms tumors is not reported in the literature, previously. Our study evaluated ALK expression in Wilms tumor samples.

1. Introduction

The anaplastic lymphoma kinase (ALK) gene is a member of the insulin receptor family involved in the development of nervous system during embryogenesis [1]. Anaplastic lymphoma kinase is also known as ALK tyrosine receptor kinase, or CD246. Anaplastic lymphoma kinase gene was first shown to have a role in anaplastic large cell lymphomas, and studies performed later revealed that inhibition of the kinase by specific ALK targeting drugs results in tumor growth arrest and cell death in patients with non–small cell lung cancer (NSCLC) [2].

The receptor ALK was found to be mutated in many cases of lymphomas, inflammatory myofibroblastic tumors, esophageal cancers, breast cancers, colorectal cancers, and NSCLC and was targeted in biological therapies [3]. Anaplastic lymphoma kinase rearrangement–positive patients may be treated with crizotinib, a novel ALK inhibitor that was approved by the Food and Drug Administration in 2011 for the treatment for patients with locally advanced or metastatic NSCLC that is ALK positive. If additional pediatric solid tumor types would be identified by ALK fusion expression, such cases could possibly benefit from new ALK inhibitor therapies.

The focus of this study was to investigate ALK presence by immunohistochemistry (IHC) using a highly specific ALK antibody in order to define the frequency and distribution of ALK expression. Distribution and frequency of ALK expression may provide a clue for ALK inhibitor use in small round cell tumors of childhood. Among the small round cell tumors of childhood, we included rhabdomyosarcomas (RMSs), Wilms tumors, Ewing sarcoma/primitive neuroectodermal tumors (ES/PNETs), peripheral neuroblastic tumors (pNTs), acute lymphoblastic lymphoma (ALL). To our knowledge, immunohistochemical comparison studies based on ALK expression on such a heterogenous collection of pediatric tumors are quite rare.

2. Materials and methods

2.1. Tissue specimens

The study group consisted of 76 small round cell tumors of childhood sampled from patients treated at Ankara Children's Hematology and Oncology Research and Training Hospital. Ethical approval for the study was given by the ethical committee (2014-078) at the Ankara Children's Hematology and Oncology Research and Training Hospital, Ankara, Turkey.

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2.2. Immunohistochemistry

Tissue samples were processed for routine histologic examination with standard formalin fixation and paraffin embedding, and 5-μm thin sections were stained with hematoxylin-eosin. Samples were immunohistochemically stained for ALK expression. For IHC, 3-μm-thick sections were tested with Ventana Benchmark GX immunostainer (Ventana, Tucson, Arizona). Diaminobenzidine was used as chromogen. Immunohistochemistry was performed with modified tissue microarray blocks, constructed for each case. The individual cases were represented with 4 different 0.5-cm cores in the blocks.

Anti-ALK antibody (anti-CD246, clone ALK1; DAKO, Glostrup, Denmark) was used. We used sample of anaplastic large cell lymphomas as positive controls. For negative controls, the primary antibodies were omitted. A pathologist evaluated immunohistochemical staining of ALK in a blinded fashion. Immunoreactivity was scored as follows: 0, no staining; 1, faint cytoplasmic staining without any background staining; 2, moderate cytoplasmic staining; and 3, strong granular cytoplasmic staining in at least 10% of tumor cells [4].

3. Results

The study group involved 76 small round cell tumors of childhood, which composed of 11 RMSs, 13 Wilms tumors, 7 ES/PNETs, 34 pNTs, and 11 ALLs. These samples were obtained from patients treated at the Ankara Children's Hematology and Oncology Research and Training Hospital between 2006 and 2014. Staining characteristics of the tumors are detailed in Table 1.

3.1. Rhabdomyosarcomas

Nine (81%) of 11 RMSs showed positivity for ALK, all with cytoplasmic staining pattern. Four (36%) of 11 showed moderate to strong cytoplasmic, dot-like, and membranous staining. Among the histologic parameters, pure solid growth pattern, pleomorphic and spindle cell morphologies were significantly associated with ALK status (Fig. 1).

3.2. Wilms tumors

Five (38%) of 13 Wilms tumors showed positivity for ALK in the epithelial component, all with cytoplasmic staining pattern. One (7%) of 13 showed moderate to strong cytoplasmic, dot-like, and membranous staining (Fig. 2). Renal tubules also showed strong cytoplasmic and membranous staining.

3.3. Ewing sarcoma/primitive neuroectodermal tumors

Two (28%) of 7 ES/PNETs showed positivity for ALK, all with cytoplasmic staining pattern. None of the them showed moderate to strong ALK positivity.

Table 1
Results of IHC staining for ALK in small round cell tumors of childhood

	Pediatric solid tumors	Tumors with any cells positive for ALK, n (%)	Tumors with moderate-strong staining for ALK, n (%)
RMSs	11	9 (81)	4 (36)
Wilms tumors	13	5 (38)	1 (7)
ES/PNET	7	2 (28)	0 (0)
pNTs	34	27 (79)	18 (52)
ALL	11	0 (0)	0 (0)

Moderate-strong, 2-3+ positivity for ALK.

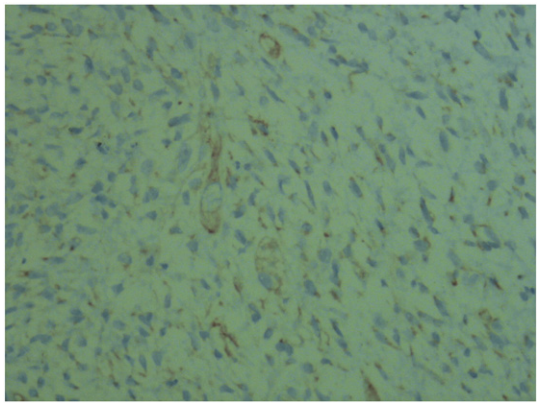

Fig. 1. Moderately ALK staining in ERMS. Original magnifications ×400.

3.4. Peripheral neuroblastic tumors

Twenty-seven (84%) of 34 pNTs showed positivity for ALK, all with cytoplasmic staining pattern. Eighteen (52%) of 34 showed moderate to strong cytoplasmic, dot-like, and membranous staining (Fig. 3A-C). Peripheral neuroblastic tumors are a generic term including neuroblastoma (NB), ganglioneuroblastoma (GNB), and ganglioneuroma (GN). The distribution ALK expression in the pNTs subtypes is detailed in Table 2. It was observed that higher frequency of ALK expression in pNTs correlated with advanced tumor types.

3.5. Acute lymphoblastic lymphoma

None of the 11 ALL showed ALK positivity.

4. Discussion

This study evaluates ALK expression in a group of pediatric solid tumors by IHC. Studies in this field and around ALK expression may contribute to identification of possible new targets for new agents. There is still a need to develop new treatments for patients with refractory diseases. Multiple malignancies such as ALCL, NSCLC, inflammatory myofibroblastic tumor, colorectal cancer, breast cancer, and NB are known to show ALK expressions. A study performed by Tennstedt et al [3] reported a comprehensive analysis of solid tumor types for ALK fusion and demonstrated that ALK was also expressed in thyroid cancers, seminomas, and lung and ovarian cancer.

Limited data exist regarding expression and genetic alterations and the potential effect of ALK inhibitors in RMSs. van Gaal et al [5] reported that ALK expression was increased in alveolar RMS (ARMS) as

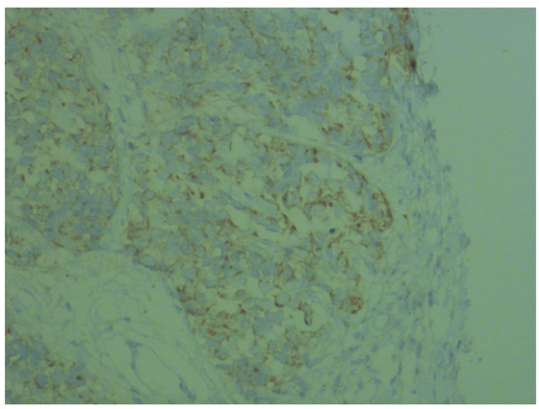

Fig. 2. Immunohistochemical staining of Wilms tumor specimen with ALK. Original magnifications ×200.