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Expression of ALK protein, mRNA and fusion transcripts in anaplastic large cell lymphoma

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ABSTRACT

Systemic anaplastic large cell lymphoma (ALCL) can be divided into two subgroups, anaplastic lymphoma kinase (ALK)-positive and ALK-negative, based on the expression of ALK protein. Expression of this protein is due to genetic alterations of ALK at 2p23. Overall, observations on ALK protein, ALK mRNA, ALK-associated genetic alterations and their relationships, to one another are not often reported in the literature. In this study, we investigated the expression of ALK protein, mRNA and fusion transcripts involving ALK and their relationships in ALCL and analyzed formalin-fixed, paraffin-embedded tissues. Forty-five human cases were analyzed with immunohistochemistry for the ALK protein and RT-PCR for ALK mRNA and seven kinds of ALK involved fusion transcripts. Our results showed that the expression of ALK protein, ALK mRNA and ALK fusion transcripts were significantly related to one another ($P<0.01$). Consistent with the expression of ALK protein, patients presenting with ALK mRNA or ALK involved fusion transcripts were significantly younger than those lacking ALK gene alteration ($P<0.01$). This study demonstrates expression of both ALK protein and ALK mRNA are positively correlated with expression of ALK-associated fusion transcripts. Combined detection of ALK protein, ALK mRNA and ALK fusion transcripts can complement each other to aid in the diagnosis of ALCL.

Introduction

Anaplastic large-cell lymphoma (ALCL) was first described in 1985 by Stein and his co-workers, who reported that a subset of non-Hodgkin lymphoma expressed the CD30/Ki-1 antigen with frequent cohesive proliferation and lymph node infiltration (Stein et al., 1985). It is now agreed that ALCL is a T/null cell neoplasm frequently characterized by the aberrant anaplastic lymphoma kinase (ALK) protein expression, which results from chromosome translocation involving the ALK gene. About 80% of genetic alterations involve t(2;5)(p23;q35) translocation between the ALK gene on chromosome 2p23 and the nucleophosmin (NPM) gene on chromosome 5q35 (Delsol et al., 2001). Moreover, several studies have shown that the remaining 20% of ALK positive ALCLs are associated with other translocations in the ALK gene at 2p23, Some of these translocations include t(1;2)(q21;p23) creating the TPM3-ALK protein, t(2;19)(p23;q13.1) creating the TPM4-ALK Protein, t(2;3)(p23;q21) creating the TFG-ALK protein, t(2;17)(p23;q23) creating the CLTC-ALK protein, inv2(p23;q35) creating the ATIC-ALK protein, and t(2;17)(p23;q25) creating the ALO17-ALK protein (Pulford et al., 2004). All translocations involve ALK possesses significant oncogenic potential resulting from the constitutive activation of the tyrosine kinase ALK. This kinase activation can induce growth factor-independent proliferation, cellular transformation, protection from apoptosis, and resistance to therapeutic drugs (Li and Morris, 2008).

According to the current World Health Organization (WHO) classification of lymphomas, ALCL can be subdivided into two biologic subtypes based on the presence or absence of aberrant expression of ALK (Delsol et al., 2001). Moreover, studies have demonstrated that ALK-positive ALCL exhibits different molecular, pathological and clinical features, and suggest that it is a distinct entity (Salaverria et al., 2008). The examinations of ALK always present a challenge for the clinical pathologist in ALCL diagnosis. In previous studies, extensive immunophenotypic and molecular studies had used to detect ALK protein and related fusion transcripts (Dunphy et al., 2000; Rust et al., 2005; Schumacher et al., 2004; Suzuki et al., 2000). However, the simultaneous observation of ALK protein, ALK mRNA and ALK-associated fusion transcripts have been less frequently investigated, especially in formalin-fixed and paraffin-embedded tumors, and especially for their relationships to one another and their significances in pathological diagnosis.

In this study, we explored in ALCL tissues (a) the expression of ALK protein by immunohistochemistry and mRNA, and (b) seven kinds of ALK-related fusion transcripts by RT-PCR following gene sequencing. These procedures were done in an effort to clarify their potential

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Table 1
Primers used to detect PGK, ALK, and ALK fusion transcripts

Prime	Sequence 5′-3′	PCR product size (bp)	Annealling temperature (°C)
PGK	F-GGGCTGCATCACCATCATAGG R-GAGAGCATCCACCCCAGGAAG	148	57
ALK	F-GATGGACCCACCCAAGAACTG R-CGGCAAAGCGGTGTTGATTAC	154	60
NPM-ALK	F-TCCCTTGGGGGCTTTGAAATAACAC R-CGGCAAAGCGGTGTTGATTAC	177	60
ALO17	F-TGAAGATGTGTGGGAACGTG	115	60
TPM3	F-AGAGACCCGTGCTGAGTTTGCTGA	147	60
TPM4	F-TGAGACCCGTGCTGAATTTGCAGA	147	60
TFG	F-CAGCAGCCACCATATACAGGA	128	60
ATIC	F-TGGAATGAACCCACATCAGAC	144	60
CLTC	F-GAAGGAGTACTTGACAAAGGTGGAT	174	60
ALK†	R-CGGAGCTTGCTCAGCTTGTA		

F: Forward primer; R: reverse primer.
† A common reverse primer was used in RT-PCR for detection of various ALK fusion genes.

relevance and the contribution of ALK protein, ALK mRNA, and ALK-associated fusion transcripts in the clinicopathologic diagnosis of ALCL.

Materials and methods

Patient population and tissue samples

Samples for a total of 45 cases of primary systemic ALCL (formalin-fixed and paraffin-embedded tissues) were retrieved from the institutional and consultation files from two departments of pathology, Cancer Hospital, Fudan University and the department of pathology, Xinhua Hospital, Shanghai Jiao Tong University, Shanghai, P.R. China. All patients were diagnosed between January 1999 and June 2006. Each case was independently reviewed by two pathologists, who made a diagnosis based on morphological and immunophenotypic criteria, as described in the WHO classification (Delsol et al., 2001). Twenty-seven patients were male and 18 were female (M: F = 1.6:1), with a mean age of 31 years (range 3 to 71 years). Of them, 42 cases had at least one lymph node involved, and 3 cases had only extranodal disease observed (one skull, one right elbow, one peritoneal cavity).

Immunohistochemistry

Immunohistochemical staining was performed using an immunoperoxidase technique, as described elsewhere (Rassidakis et al., 2001). In brief, paraffin sections were dewaxed with xylene and rehydrated in a graded ethanol series. After heat-induced antigen retrieval in 0.01 mol/L citrate buffer (pH6.0), the sections were incubated with ALK monoclonal antibody (ALK1, 1:30 dilution; Dako), CD30 monoclonal antibody (Ber-H2, 1:30 dilution; Dako), CD20 monoclonal antibody (L26, 1:100 dilution; Dako) and CD3 polyclonal antibody (1:50 dilution; Dako) in a humidified chamber at room temperature for 60 min and then at 4 °C overnight. Slides known to express ALK, CD30, CD20 and CD3 were used as the positive controls and slides processed with tris buffered saline (TBS) instead of primary antibodies were used as the negative controls. On the second day, the sections were washed with phosphate buffered saline (PBS) three times, incubated with the EnVision reagent (Dako) at room

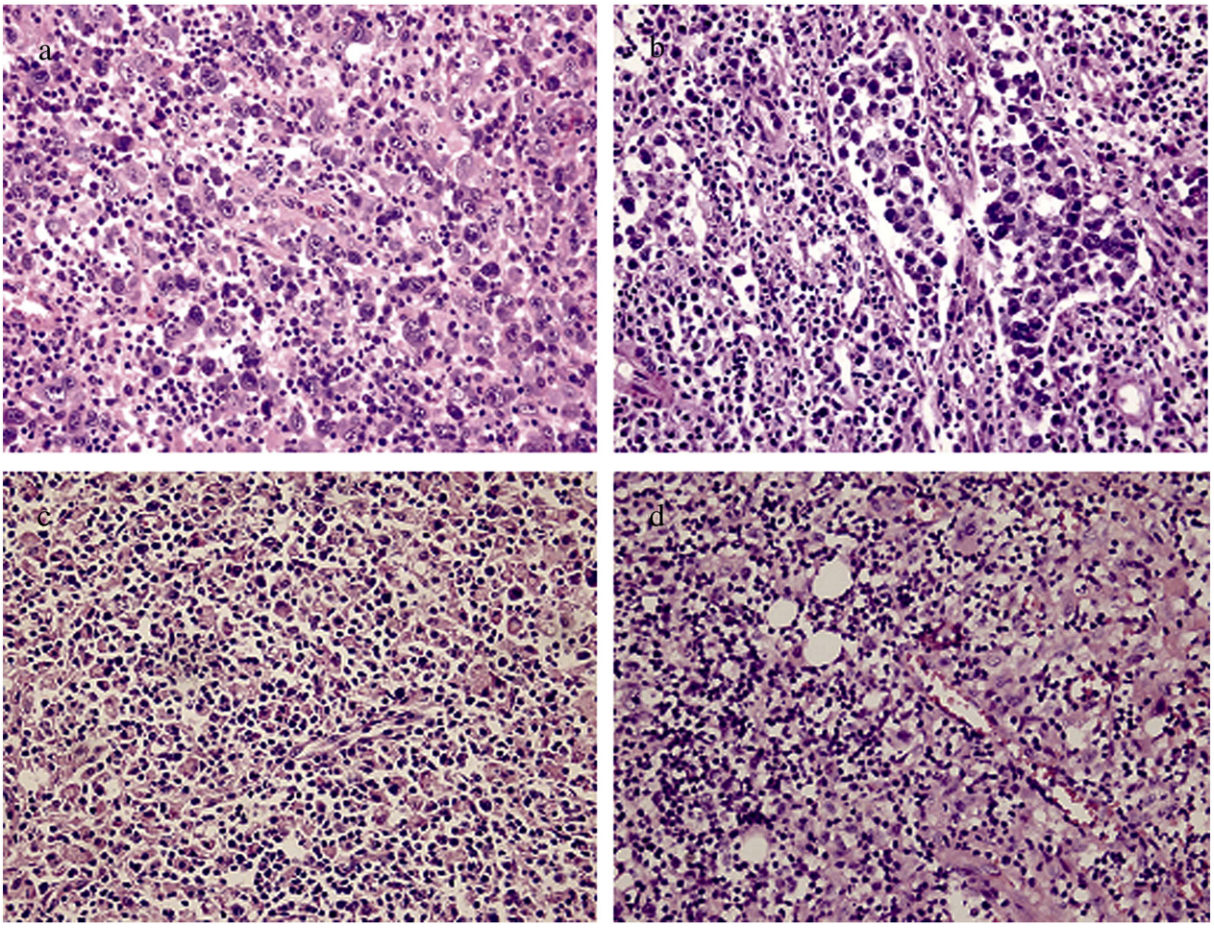

Fig. 1. Morphology of primary systemic ALCL: (a) a common histologic variant showing a diffuse and cohesive pattern (H&E, ×400); (b) a common histologic variant, showing sinusoidal involvement (H&E, ×400); (c) a lymphohistiocytic variant (H&E, ×400); (d) a small cell variant (H&E, ×400).