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Analysis of gene expression profile of TPM3-ALK positive anaplastic large cell lymphoma reveals overlapping and unique patterns with that of NPM-ALK positive anaplastic large cell lymphoma

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Abstract

Anaplastic large cell lymphoma (ALCL) comprises a group of non-Hodgkin lymphomas characterized by the expression of the CD30/Ki-1 antigen. A subset of ALCL is characterized by chromosomal translocations involving the anaplastic lymphoma kinase (ALK) gene on chromosome 2. While the most common translocation is the t(2;5)(p23;q35) involving the nucleophosmin (NPM) gene on chromosome 5, up to 12 other translocations partners of the ALK gene have been identified. One of these is the t(1;2)(q25;p23) which results in the formation of the chimeric protein TPM3-ALK. While several of the signaling pathways induced by NPM-ALK have been elucidated, those involved in ALCLs harboring TPM3-ALK are largely unknown. In order to investigate the expression profiles of ALCLs carrying the NPM-ALK and TPM3-ALK fusions, we carried out cDNA microarray analysis of two ALCL tissue samples, one expressing the NPM-ALK fusion protein and the other the TPM3-ALK fusion protein. RNA was extracted from snap-frozen tissues, labeled with fluorescent dyes and analyzed using cDNAs microarray containing ~9200 genes and expressed sequence tags (ESTs). Quantitative fluorescence RT-PCR was performed to validate the cDNA microarray data on nine selected gene targets. Our results show a significant overlap of genes deregulated in the NPM-ALK and TPM-ALK positive lymphomas. These deregulated genes are involved in diverse cellular functions, such as cell cycle regulation, apoptosis, proliferation, and adhesion. Interestingly, a subset of the genes was distinct in their expression pattern in the two types of lymphomas. More importantly, many genes that were not previously associated with ALK positive lymphomas were identified. Our results demonstrate the overlapping and unique transcriptional patterns associated with the NPM-ALK and TPM3-ALK fusions in ALCL.

Keywords: NPM-ALK; TPM3-ALK; cDNA microarray; Quantitative RT-PCR; ALCL

1. Introduction

Anaplastic large cell lymphoma (ALCL) is a subtype of aggressive non-Hodgkin lymphoma typically presenting as systemic disease, with or without extranodal involvement [1]. The World Health Organization defines ALCL as a systemic T-cell lymphoma composed of large pleomorphic cells with abundant cytoplasm, horseshoe-shaped nuclei with expression of CD30 and cytotoxic granule-associated proteins [2]. The majority of ALCLs exhibit a T-cell or null phenotype and demonstrate expression of the anaplastic lymphoma kinase (ALK) protein [2]. Approximately 80–85% of the ALK positive ALCLs are associated with the t(2;5)(p23;q35) [3–6] which juxtaposes the nucleophosmin (NPM) gene at 5q35, a nucleolar protein involved in shuttling ribonucleoproteins from the cytoplasm to the nucleus, to the anaplastic lymphoma kinase (ALK) gene at 2p23, a tyrosine kinase receptor belonging to the insulin receptor superfamily [7,8]. Normal expression of ALK is stringently controlled and limited to

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the cytoplasm of the testis, ganglion cells of the intestine, and neural tissues [8]. The NPM-ALK fusion protein has been shown by immunohistochemistry to localize in the cytoplasm and the nucleus of the neoplastic cells, thereby providing a distinctive marker for t(2;5) positive ALCLs [4].

Since the first report of the t(2;5) in ALCL, at least 12 molecular variants implicating the ALK gene have been described [9–12] in not just ALCLs, but in a subset of soft tissue tumors, called inflammatory myofibroblastic tumors [13]. Fifteen to 20% of ALK positive ALCLs harbor variant fusion partners, including the t(1;2)(q25;p23) [12,14]. This translocation leads to the fusion of the N-terminus of the nonmuscular tropomyosin, TPM3, on chromosome 1 to the cytoplasmic portion of ALK [12]. Like other translocation partners of ALK, TPM3 can self-associate, leading to the activation of the TPM3-ALK fusion protein [15].

Several signaling pathways have been implicated in the pathogenesis of NPM-ALK positive ALCLs. NPM-ALK has been shown to activate several members of the signal transducer and activator of transcription (STAT) family, including STAT3 and STAT5 [16,17]. Others have demonstrated downstream involvement of pathways involving PI3K, AKT and PLC-γ [18,19]. Less is known about the downstream consequences of TPM3-ALK expression, but TPM3-ALK expressing cells have been shown to utilize the PI3-kinase/AKT pathway [20].

cDNA microarray analysis is a useful tool to examine gene expression patterns between different cell populations and is useful for elucidation of deregulated signaling pathways important in the pathogenesis of cancer [21,22]. In this study, we utilized cDNA microarrays composed of approximately 9200 unique gene sequences and expressed sequence tags (ESTs) to compare the expression profiles of an ALCL with the t(2;5) NPM-ALK translocation and an ALCL with the t(1;2) TPM3-ALK translocation. Our results indicate that similar transcriptional pathways are affected in NPM-ALK positive and TPM3-ALK positive ALCLs. In addition, distinctive expression patterns are associated with either chimeric ALK fusion. Finally, our results provide novel insights into the transcriptionally deregulated pathways pathogenesis involved in ALK positive lymphomas.

2. Materials and methods

2.1. Patient samples

All tissues were obtained from the surgical pathology files of the Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah. This study was approved by the Institutional Review Board (IRB #10710). The NPM-ALK positive ALCL sample was obtained from a cervical lymph node from a 12-year-old male. The lymphoma expressed CD3, CD30, and nuclear and cytoplasmic ALK by immunohistochemistry (data not shown). The presence of the t(2;5)(p23;q35) translocation was verified by RT-PCR analysis which has been previously published [23]. The second case represents a cervical lymph node biopsy from a 32-year-old male which was involved by ALCL. The lymphoma expressed CD2, CD30 and cytoplasmic ALK. RT-PCR analysis for t(2;5)(p23;q35) was negative and 5′-RACE revealed the presence of the t(1;2)(q25;p23) (TPM3-ALK), as previously described [24]. Flow cytometry and cytogenetic analyses were not performed. Both tumors were obtained from diagnostic material prior to treatment. The reactive lymph node was obtained from Primary Children's Medical Center, in Salt Lake City, Utah. The absence of the t(2;5)(p23;q35) was verified by RT-PCR analysis for NPM-ALK (data not shown). Whole tissue sections from snap-frozen material were used for subsequent cDNA microarray analyses.

2.2. Cell lines

Our reference cDNA sample consisted of a composite cell line mixture containing an equal number of cells from five cell lines derived from hematologic malignancies. The cell lines included Jurkat (ATCC# = TIB-152, an immature acute lymphoblastic leukemia cell line), SKW-3 (a mature peripheral T-cell chronic lymphocytic lymphoma cell line), NCEB (a mantle cell lymphoma cell line), Raji (ATCC# = CCL-86, a mature B-cell t(8;14) positive Burkitt lymphoma cell line), and L-428 (a Hodgkin lymphoma cell line). These cell lines were maintained as previously described [25].

2.3. RNA extraction, linear RNA amplification and cDNA microarray experiments

Total RNA was extracted using Trizol™ reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The concentration and purity of RNA was determined based on $O.D._{260/280}$ measurements. Total RNA quality was assessed by 2% agarose gel electrophoresis. Total RNA from the patient samples and cell lines was subjected to linear amplification as previously described [26]. Microarray analysis was performed in the Huntsman Cancer Institute Microarray Core Facility at the University of Utah. Molecular Dynamics/Amersham Pharmacia Biotech (Piscataway, New Jersey, USA) instrumentation was used to print and scan microarray slides using techniques previously described [27]. This facility maintains a sequence-verified cDNA clone collection supplied by Research Genetics (Huntsville, Alabama, USA). In addition to these clones, the slides were customized to include a curated list of genes previously shown to be expressed in subsets of lymphoid cells for a total of 9200 clones per slide. Each cDNA clone was spotted in duplicate. All cDNA microarray experiments were carried out in quadruplicate.