Clinicopathologic Effect of *DNMT3A* Mutation in Adult T-Cell Acute Lymphoblastic Leukemia

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Abstract

DNA extraction, amplification with sequencing analysis using the 310 ABI genetic analyzer for detection of a mutation (R882H) in 64 patients with T-cell acute lymphoblastic leukemia (T-ALL) at diagnosis. *DNMT3A* is frequently mutated among T-ALL patients and has been associated with a poor prognosis. These findings could help in risk stratification and treatment choice for patients with T-ALL.

Background: The present study aimed to determine the frequencies and clinicopathologic effect of a *DNMT3A* [DNA (cytosine-5)-methyltransferase 3A] mutation in patients with adult T-cell acute lymphoblastic leukemia (T-ALL). **Patients and Methods:** A total of 64 patients with T-ALL who had been admitted to Mansoura University Oncology Center were included in the present study. For all patients, DNA extraction and amplification with sequencing analysis using the 310 ABI genetic analyzer for detection of a mutation (R882H). **Results:** The *DNMT3A* mutation (R882H) was found in 12 of the 64 patients (18.8%). The *DNMT3A* mutation was frequently detected in the older age group and was associated with high leukocytic counts, a high bone marrow blast cell percentage, and the frequent presence of extramedullary disease. However, it was not associated with the hemoglobin level, red blood cell count, or platelet count. The patients with mutant T-ALL had a low tendency to achieve remission after induction. These patients had significantly shorter overall survival and shorter disease-free survival compared with those with wild-type T-ALL ($P = .037$ and $P = .006$, respectively). **Conclusion:** *DNMT3A* is frequently mutated in T-ALL and is associated with distinct clinicopathologic entities and a poor prognosis. These findings could help in risk stratification and treatment decisions for patients with T-ALL.

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Introduction

Epigenetic regulation of abnormal gene expression has been identified as a prominent feature of hematologic malignancy. Recurrent somatic alterations of key proteins involving DNA methylation, post-translational histone modification, and chromatin molding have been highlighted in the initiation and maintenance of malignancy. The rational use of targeted epigenetic therapy requires understanding the mechanisms of malignant transformation driven by aberrant epigenetic regulators.[1]

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive and heterogeneous disease with a poor prognosis and outcome. The diagnosis of ALL is based on immunophenotyping, which allows lineage specification and identification of prognostic markers.[2] Early T-cell precursor (ETP)-ALL is a high-risk subgroup of T-lineage ALL characterized by an associated distinctive immunophenotype [$CD1a^{(-)}$, $CD8^{(-)}$, $CD5^{(weak)}$] with stem cell or myeloid markers.[3,4]

Despite improved insights into T-ALL disease biology and its genetic landscape, the clinical prognosis has remained largely similar during the past decades. Treatment still consists of high-dose multiagent chemotherapy, potentially followed by hematopoietic stem cell transplantation. Even with aggressive treatment courses, which incur considerable side effects, the clinical outcome has remained extremely poor for a significant subset of patients with T-ALL because of therapy resistance or hematologic relapse.[5]

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The molecular mechanisms by which residual T-ALL tumor cells survive, resist chemotherapy, and act as a reservoir for leukemic progression and hematologic relapse remain poorly understood. Thus, enhanced molecular understanding of T-ALL disease biology will ultimately facilitate a targeted therapy-driven approach that can reduce the chemotherapy-associated toxicities and improve the survival of patients with refractory T-ALL.[6] A number of cytogenetic and molecular alterations have been discovered that have important value in the stratification of such patients toward targeted therapy.[7] Among these alterations is BCR-ABL.[4]

The Cancer Genome Atlas Research Network has reported that the incidence of DNA methylation-related genes is 24% in primary acute myeloid leukemia (AML) with nonsynonymous mutations.[8] This aberrant DNA methylation is a hallmark feature of AML and most likely mechanistic of carcinogenesis.[9] In addition, different AML subtypes exhibit different and specific patterns of DNA methylation with distinct difference in the regulation of gene expression.[10]

DNA (cytosine-5)-methyltransferase 3A (*DNMT3A*) belongs to a family of DNA methyl transferases, including *DNMT3A* and *DNMT3B*, that catalyze the addition of methyl groups to cytosine residues of CpG nucleotides.[11] Somatic mutations in *DNMT3A* were first observed by Yamashita et al[12] in 4% to 22% of AML patients. Most *DNMT3A* mutations are missense mutations that occur at residue R882 near the carboxyl terminus of the *DNMT3A* protein. However, a number of less common nonsense, frame shift, and splice site mutations have been found throughout the *DNMT3A* coding sequence.[13] Thus, mutations in *DNMT3A* are often classified as R882 versus non-R882 mutations. To date, no clinical difference has been described separating these 2 classes, and the reason for the high prevalence of R882 mutations remains unclear.[14]

Although the pattern and prognostic implications in myeloid malignancy have been clearly reported, the frequency and prognostic impact have not been fully elucidated in T-ALL. Therefore, we aimed to estimate the frequency of DNMT3A mutations and their prognostic impact in Egyptian patients with T-ALL.

Patients and Methods

Samples from 64 adult patients (33 men and 31 women) with newly diagnosed T-ALL at Mansoura University Oncology Center were studied. All the patients provided informed consent. The study period was January 2012 to January 2015. The age range was 17 to 65 years. Diagnostic bone marrow (BM) and/or peripheral blood samples were taken from all 64 patients. The diagnosis was based on morphologic examinations of the peripheral blood and BM smears and immunophenotyping by flow cytometry using an antibody panel against the following antigens: CD19, CD20, CD22, CD79a, TDT, CD34, CyCD3, CD2, CD1a, CD5, CD34, and CD7. The monoclonal antibodies were conjugated with the fluorochrome fluorescein isothiocyanate, phycoerythrin, allophycocyanin, and peridinin chlorophyll protein complex. All antibodies were purchased from BD Biosciences. The samples were analyzed using a BD FACSCalibur flow cytometer. Autofluorescence, viability, and isotype controls were included. Flow cytometric data acquisition and analysis were conducted using BD Cellquest Pro software.

The patients were treated according to the standard ALL regimen, including daunorubicin (60 mg/m^2 on days 1, 8, 15, and 22) and cytarabine (75 mg/m^2 on days 1-5) as induction therapy, followed by high-dose cytarabine (75 mg/m^2) as consolidation therapy. No patient included in our study received allogenic BM transplantation at first complete remission. The patients were followed up for 24 months or until death. The local medical ethics committee approved the study.

Sequencing Analysis of DNMT3A Mutation

DNA Extraction and Amplification. Genomic DNA was extracted from peripheral blood or BM samples using the GeneJET Genomic DNA purification kit (Thermo Fisher Scientific, Waltham, MA). The genomic region of the *DNMT3A* containing the mutational hotspot was amplified using the following primers: forward, TCCTGCTGTGTGGTTAGACG; reverse, ATGATGTCCAACCCTTTTCG. Polymerase chain reactions (PCRs) were performed in a total volume of 25 µL containing 12.5 µL of Maxima hot start PCR master mix 2X (400 µM each of Maxima hot Taq DNA polymerase, hot start PCR buffer, and deoxynucleotide and 4 mM Mg^{+2}; Thermo Scientific), 0.1 µL of each primer (100 pmol), and 1 µL of extracted DNA (20-50 ng) and completed to 25 µL with molecular grade water. The PCR was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) using the following steps: initial denaturation at 95°C for 4 minutes, 35 cycles at 95°C for 60 seconds, 56°C for 60 seconds, and 72°C for 60 seconds, and a final extension at 72°C for 7 minutes.

The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Cycle sequencing of the purified PCR products was performed using the Big Dye Terminator, version 3.1, Cycle Sequencing Kit. The reaction mix (10 µL) contains 2 µL of Big Dye Terminator Ready Reaction Mix (Applied Biosystems), 2 µL of 2X diluting buffer (Applied Biosystems), 5.7 PCR products, and 0.3 µL of *DNMT3A*-forward primer (10 pmol; AccuOligo; Bioneer Corp., Daejeon, Korea). Cycle sequencing was done using the GeneAmp PCR System 9700 (Applied Biosystems) by performing 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes.

Purification of Extension Products and Sequencing. Purification of the extension products was performed using the ethanol precipitation method. In brief, 50 µL of ethanol (100%) and 2 µL of 3M sodium acetate (pH 4.6) were added to each tube, mixed, and incubated in the dark at room temperature for 15 minutes, followed by centrifugation for 20 minutes at 14,000 rpm and careful aspiration of the supernatant. Next, 250 uL of 70% ethanol was added to the tubes, which were briefly vortexed, followed by centrifugation at 14,000 rpm for 5 minutes and careful aspiration of the supernatant. The tubes were placed with the lids open in a heat block at 90°C for 1 minute (to remove any remaining ethanol). Each pellet was suspended in 10 µL of Hi-Di Formamide, and the samples were sequenced on a 310 Genetic Analyzer (Applied Biosystems) and analyzed using the sequencing analysis, version 5.4, software (Applied Biosystems).