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Identification Of Tet2 Gene Level Of Expression In Acute Leukemias

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Abstract

Objective: *TET2 gene (4q24) is one of the most important regulatory factors of the methylation process in human genome. The homologous protein is directly involved in conversion of 5-methylcytosine into 5-hydroximethylcytosine (1). Loss of function mutations in TET2 gene are considered to be driver mutations (initiating mutations) for leukemogenesis. In some cases these can be found in early clonal cells of Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL) or Myelodisplastic Syndromes (MDS) (2). The aim of this study is to determinate the frequency of TET2 mutations in Acute Leukemia (AL) patients.*

Materials and methods: *This retrospective study enrolled 24 patients newly diagnosed with AML (19 patients) and ALL (5 patients). By using Real-Time PCR we obtained a relative cuantification of TET2 gene level of expression taking ABL gene as reference.*

Outcomes: *Low levels of TET2 gene expression were found in 6 patients (25%), 3 of them being diagnosed with AML and 3 with ALL. No cytogenetic anomalies were noticed. However, all these patients had blastocytosis and higher white blood cells count than patients with normal levels of expression. There was one case with mutations in both TET2 gene and NPM1 gene and one case that associated a defective TET2 gene and an internal tandem duplication of FLT3-ITD.*

Conclusions: *In our study, TET2 gene loss of function mutations occured on a specific background which involved some characteristic mutations for AL and some particular features of cell morphology. We aim to continue this study by investigating the long term effects of this epigenetic mutation in AL patients.*

Keywords: epigenetic factors, TET2 gene, demethylation, acute leukemias.

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Introduction

Acute leukemia is a hematological malignancy characterized by an accelerated and uncontrolled production of abnormal blood cells in the bone marrow that subsequently discharge in the peripheral blood.

In hematopoietic stem cells, the disruption of epigenetic regulation of chromatin leads to modifications in certain loci that can be critical for leukemogenesis onset. An important epigenetic event is cytosine methylation at C5 position which starts in early embryonic development and continues throughout lifetime. It is the result of an intense activity of methylation and demethylation enzymes encoded by DNMT3, IDH and TET family genes (3). Recurrent mutations in these antagonistic genes were found in Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL) or Myelodysplastic Syndrome (MDS) (4).

TET2 (Ten-Eleven Translocation 2) gene (4q24) is one of the regulatory factors of the methylation process in human

genome. TET2 gene from hematopoietic stem cells contributes to self-renewal, commitment to the differentiation line and maturation of granulocytes, monocytes and lymphocytes. Mutations of TET2 gene in early precursors unbalance the hematopoiesis by stimulating the granulocytopoiesis to the detriment of lymphopoiesis and erytropoiesis.

Loss of function mutations in TET2 gene are considered to be initiating mutations in leukemogenesis. These events are not singular as in most of cases some associations with subsequent somatic mutations like FLT3, RUNX, NPM1 or CEBPA are mentioned (5).

The homologous TET2 protein is directly involved in conversion of 5-methylcytosine (5-mC) into 5-hydroximethylcytosine (5-hmC) in CpG genomic regions. A decreased function of TET2 protein is therefore associated with hypermethylated CpG loci. This hypermethylation process may occur in CpG loci that belong to regulatory genetic elements like gene enhancers, leading to a decrease in gene activity. Therefore, TET2 gene is considered a tumor supression gene (6).

In AML, the reported TET2 gene dysfunctions are the consequence of deletion, missense or nonsense mutations. Another incriminated mechanism is the absence of certain cofactors for the catalythic site of the TET2 enzyme while the gene keeps its optimal level of expression (7).

In ALL the main cause of a dysfunctional TET2 gene is the hypermethylation of some CpG loci from its own promoter region. However, the influence of TET2 gene underexpression in the prognosis of hematological malignancies is controversial (8).

Through multivariable analyses models some studies reported no implication in AML and ALL prognosis, whereas others suggested an unfavourable effect in cytogeneticaly normal (CN) AML patients (4,9). According to ELN classification, AML patients having CN AML are cathegorised into a favourable risk group (with mutations in CEBPA or NPM1 genes but no FLT3-ITD mutation) and a less favourable intermediate risk 1 group (the rest of CN AML patients). In two large observational studies (4,5) patients with CN AML and mutated TET2 gene had lower response rates and a higher risk of relapse as well as shorter event free survival (EFS) and disease free survival (DFS) compared to patients with CN AML and wild TET2 gene. It was also mentioned a possible association between parameters like old age, higher white blood cell count (WBC), higher peripheral blast count, higher levels of serum lactate dehydrogenase and TET2 mutations.

In this study, we investigated the prevalence of TET2 gene loss of function mutations in patients with AML and ALL and potential associations with molecular background and clinical characteristics.

Patients

We conducted a retrospective study on a group of 24 patients diagnosed with AML (19) and ALL (5) in Fundeni Clinical Institute between January 2022 and May 2023. The AML or ALL diagnoses were based on morphology, cytogenetics, flow-citometry and molecular biology identification procedures.

This study respects the ethical principles of the Helsinki Declaration and no experiments on human subjects are included.

We established the selection criteria mainly from the perspective of molecular changes, by referring to the most reported associations of TET2 gene mutations with trigger and cooperative mutations for the onset of AL. Therefore, we included patients with FLT3-ITD, NPM1 mutations and also patients with AML or ALL but no detectable mutations when performed our conventional Multiplex PCR panel. We assumed that some patients from this subgroup could have other specific mutations that are known to be significantly associated with loss of function mutations in TET2 gene.

The patients demographics and their genetic and clinical characteristics are summarized in table below:

According to FAB classification, the AML group included 3 cases with AML 0, 2 cases with AML 4, 2 cases with AML 5, followed by 1 case with AML 1. Meanwhile, all the patients from ALL group were diagnosed with common B-cell lymphoblastic leukemia, except a pro-B ALL case.

The previous cytogenetic analysis report indicated that 75% of patients had no changes in karyotype. Trisomy 8 was found in 3 of the remaining cases (12.5%), while the rest of patients had monosomies that do not fit into the notion of a clone.

The FISH technique recorded 17p deletion, 20q deletion and 5q deletion in 3 AML patients and a BCR-ABL rearrangement subsequent to (9,22) translocation in a ALL case. The other 4 cases of ALL were BCR-ABL negative.

Both 17p deletion and 5q deletion belong to the adverse risk category of ELN classification with a high probability of relapsing and poor overall survival.

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Characteristic	Patients
Median age (years) Range	52.6 1-73
Gender F M	 14 (58%) 10 (42%)
White blood cells Median Range	 87 x 10^9/ L 1.670 x 10^9/L – 336.770 x 10^9/L
Red blood cells Median Range	 8.52 g/dL 5.1 g/dL – 13.8 g/dL
Peripheral blasts % Median Range	 63.41 21-93
Bone marrow blasts % Median Range	 83.16 50-96
Cytogenetic tests Abnormalities Cytogenetic normal (CN)/ Negative FISH	 6 (25%) 18 (75%)
Flow-citometry	15 available results B-cell ALL antigen expression profile in 5 pacients AML expression profile in 10 pacients
Molecular identification	19 AML cases 5 ALL cases

Table 1: Demographic, clinical and genetic characteristics

The previous identification of specific mutations in both types of AL was possible through Multiplex PCR panels followed by Split-Out PCR. Our AML group of patients consisted in 9 patients with FLT3-ITD tandem duplication (37.50%), 2 patients with mutations in NPM1 gene (8.33%) and 1 patient with bcr 3 isoform of PMR- RARA (4.16 %). In ALL group of 5 patients, in 1 case the presence of both p190 and p210 transcript of BCR-ABL was identified, while another patient only had the p190 transcript. The rest of the selected patients from both groups did not have mutations compatible with our identification panel

Methods

The detection protocol for TET2 gene level of expression was performed only on the first collected set of blood samples from each patient. The purpose was to test the gene before the patients started to receive chemotherapy as there are evidences that therapeutical approach can modify TET2 gene initial level of expression. TET2 gene level of expression was tested by two steps Real-Time PCR (qPCR). The procedure involved mRNA isolation from blood samples, followed by reverstranscription to cDNA and Real Time amplification.

The amplification reactions and interpretation of results were performed on a Light Cycler 480 II (Roche). We chose the relative quantification method, using ABL1 as reference gene. For a quality control of reactions we added a non-DNA sample (blank), containing only reagents. A sample from a healthy subject was additionally included to serve as a comparison for TET2 levels of patients.

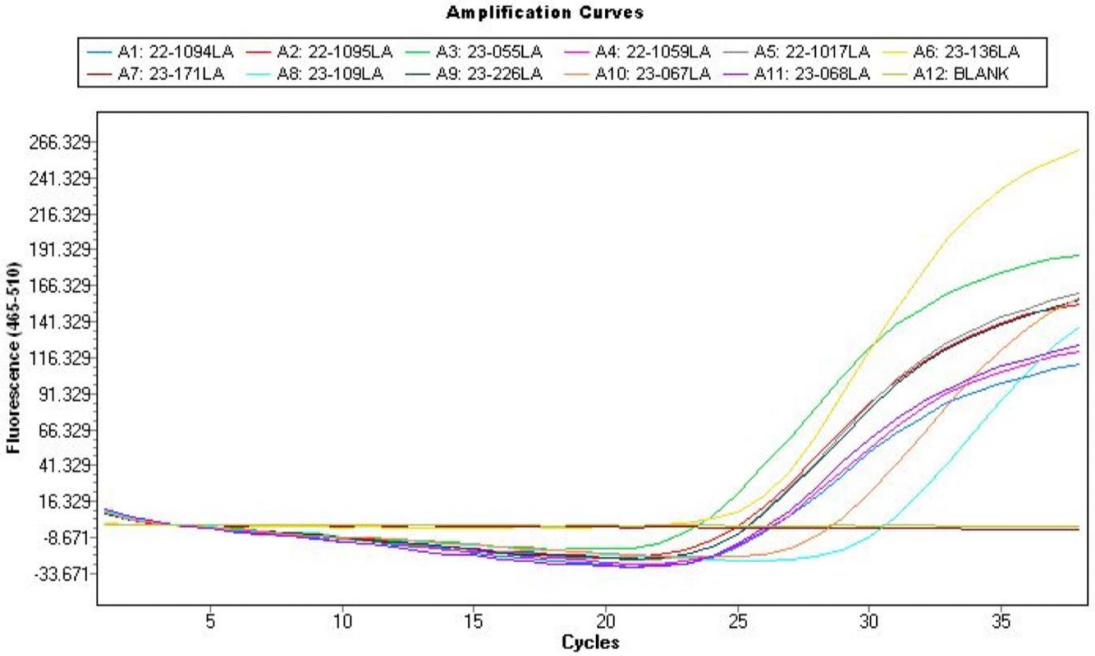

Fig.1 Amplification curve of TET2 gene in 12 samples

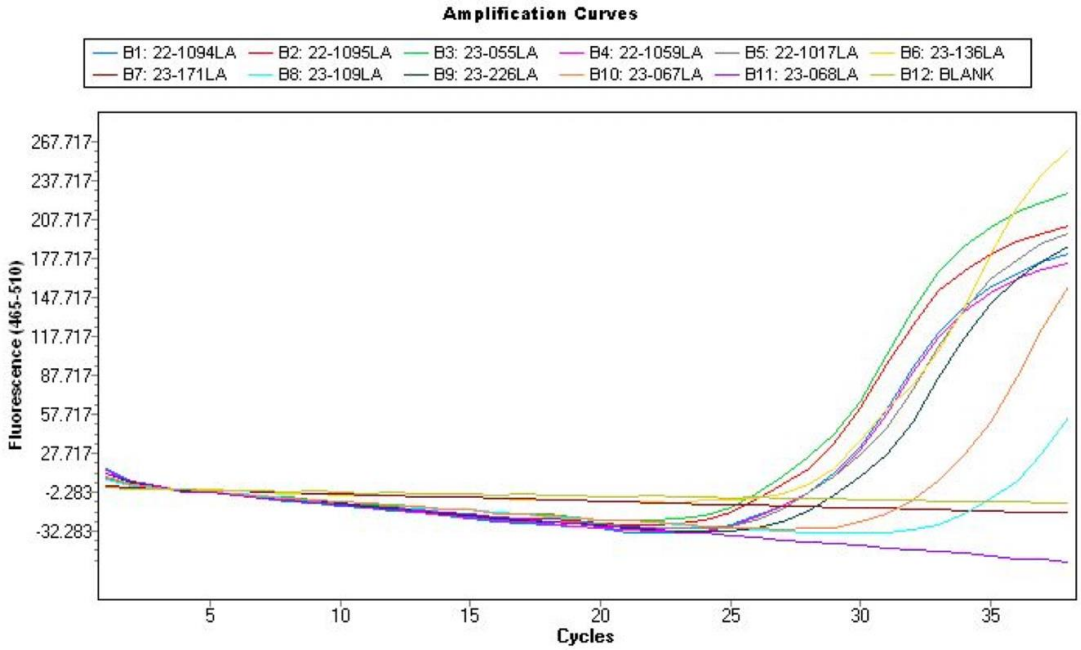

Fig.2 Amplification curve of ABL1 gene in 12 samples

Following the qPCR, we designed a ROC (Receiver Operating Characteristic) curve using the online analysis program ROC Curve Calculator John Hopkins Medicine (10). The purpose of this curve was to differentiate between low and normal levels of expression for TET2 gene by comparing ΔCt values of AML and ALL groups. Considering all the values, ΔCt=0.1935 was assigned as the cut-off for normal level of TET2 expression with a sensibility of 81.32%.

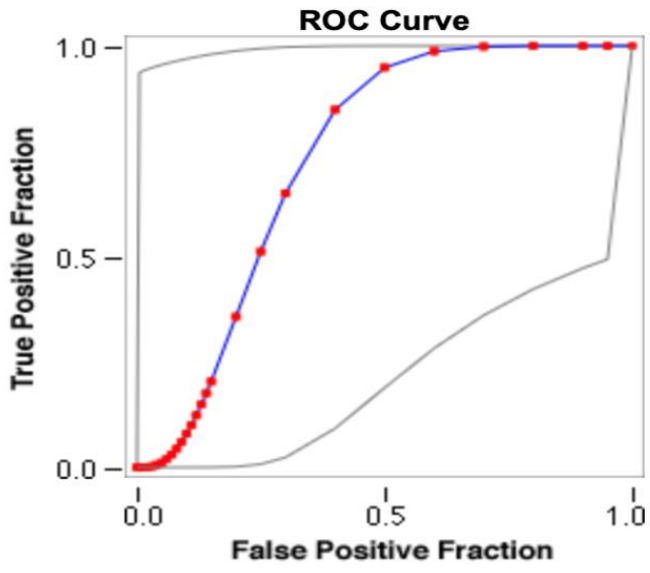

Fig.3 ROC curve of ΔCt values

Results

We identified 6 cases (25%) with low levels of TET2 gene expression as ΔCt values were lower than 0.1935. The distribution was identical in AML and ALL groups, each group containing 3 cases with dysfunctional TET2 gene. Among AML cases, a down-regulated TET 2 gene was found in those patients with no detectable mutations in our Multiplex PCR panel and no cytogenetic modifications. Remarkably, one case with low levels of TET2 gene had a FLT3-ITD tandem duplication and the flow citometry report suggested the presence of a specific pattern of markers which is strongly correlated with NPM1 loss of function mutations.

Regarding ALL patients with down-regulated TET2 gene, no specific mutations in Multiplex PCR and no cytogenetic anomalies were found.

However, we found notable differences when investigated patients demographic data, hemogram values and blood smears morphology.

	TET2 gene normal level of expression	TET2 gene low level of expression
Median WBC in AML pacients	**56.771/mm3**	**68.620/mm3**
Median WBC in AML pacients	**48.115/mm3**	**146.206/mm3**
Blast number blood/ bone marrow	**62%/63.44%**	**67%/90%**

Table 2. Differences between down-regulated TET2 gene group and normal TET2 gene group

The median age in down-regulated TET2 group was 51.6 years while in normal TET2 gene group the median age was 59.5 years. The average leukocyte number (WBC) was higher in patients with down-regulated TET2 gene for both AML and ALL groups. Furthermore, the median bone marrow and blood blasts percentage were also higher in these patients.

Discussions

In our study, the presence of a TET2 gene loss-of-function mutation in patients with AML or ALL is assumed to be associated with particular characteristics like normal kariotype, higher WBC, higher percentage of blasts in blood and bone marrow. Among the AML group with low level of TET2 gene expression, even if the trigger mutation is not known in all cases, we surprinsingly found

an FLT3-ITD and NPM1 association. Also, we identified 3 cases of B-cell ALL with a downregulated TET2 gene. By our knowledge such events are rarely observed, as most ALL cases associated with an underexpressed TET2 gene are in fact T cell ALL.

The impact of a persistent TET2 gene low level and its implication in leukemogenesis is still debatable and larger studies are necessary. However, it is clear that a imbalance of DNA methylation-demethylation cycle due to impaired demethylation affects the tumor suppression response. (11)

There are no current therapies to address TET2 gene loss of function mutations and to restore the methylation adequate levels in critical gene regions.

Although our study does not include a follow up period, it could be interesting to further evaluate this cohort in order to determine if TET2 altered expression changes or not the stratification risk for these patients.

Conclusions:

The epigenetic events are for certain important factors in cancer pathogenesis and their understanding could provide new approaches and therapeutic horizons. Our study highlighted some modifications in clinical and morphological parameters in AML and ALL patients with dysfunctional TET2 gene compared to those with an optimal level of expression.

Conflict of Interest

The authors declare no conflict of interest.

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