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FMS-LIKE TYROSINE KINASE (FLT3) GENE ITD MUTATION IN ACUTE MYELOID LEUKEMIA

MUTACIJA GENA FLT3 PRI AKUTNI MIELOBLASTNI LEVKEMIJI

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Abstract – Background. *FLT3 is a class III receptor tyrosine kinase expressed in normal stem cells and blasts of myeloid leukemia. Internal tandem duplication (ITD) of the FLT3 gene affecting the exons 14 and 15 leads to ligand-independent FLT3 dimerization and constitutive activation. This stimulates proliferation and induces inhibition of apoptosis which contributes to leukemogenesis. We have screened a panel of acute myeloid leukemia (AML) patients for the occurrence of FLT3/ITD mutation and correlated this mutation to patients' survival and basic hematological parameters.*

Methods. *RT-PCR for ITD in exons 14 and 15 of FLT3 gene was done on bone marrow samples of 67 AML patients at diagnosis.*

Results. *There was a 16.4% incidence of FLT3/ITD mutation in the cohort of examined patients. By cytognetic subgroups there were 2/6 t(15;17) and 1 of 4 t(8;21) positive patients. The rest had normal and 2 had complex karyotype. Majority were of FAB M2 or M4 phenotype. For a subset of patients taken into comparative survival analysis there was a clear disadvantage for FLT3/ITD patients. No difference was found for basic hematological parameters between two groups.*

Conclusions. *As it is evident today that FLT3/ITD is the single most common genetic abnormality in AML that also presents unfavorable clinical prognostic marker, it should be included in molecular diagnostic testing of acute myeloid leukemia.*

Izvleček – Izhodišča. *FLT3 je recepTOR razreda III za tirozin kinazo. Nahaja se v normalnih matičnih celicah in levkemičnih celicah pri aktuni mieloblastni levkemiji (AML). Notranja tandemska podvojitev (ITD) gena FLT3 na eksonih 14 in 15 vodi do nastanka neodvisnih dimerov in aktivacije s posledično proliferacijo in inhibicijo apoptoze, kar v končni fazi vodi do leVkemogeneze. Pregledali smo vzorce serije bolnikov z AML za prisotnost mutacije FLT3/ITD in ugotavljali morebitno povezavo med prisotnostjo mutacije s klinično sliko in potekom bolezni.*

Metode. *Z metodo verižne reakcije s polimerazo v realnem času (RT-PCR) smo preiskali 67 vzorcev kostnega mozga bolnikov z AML za prisotnost ITD na eksonih 14 in 15 gena FLT3.*

Rezultati. *V skupini pregledanih vzorcev je bila incidenca FLT3/ITD mutacije 16,4%. Mutacijo smo ugotovili pri 2 do 6 bolnikIH s translokacijo t (15;17), pri 1 od 4 s translokacijo kariotip. Večina je imela fenotip AML M2 in M4 po FAB razdelitvi. Pri bolnikih z mutacijo je bolezen kazala slabši potek in preživetje, ni pa bilo nobenih razlik v osnovnih hematoloških parametrih med skupinami z mutacijo in brez.*

Zaključki. *Znano je, da je mutacija FLT3/ITD najpogostejša samostojna mutacija pri bolnikih z AML in predstavlja neugoden dejavnik napovedi poteka bolezni. Zaradi tega bi morali to preiskavo vključiti v redno molekularno diagnostično preiskovanje bolnikov z AML.*

Introduction

Deregulated tyrosine kinase activity has been implicated in the pathogenesis of myeloid malignancies. Recently a FMS-like tyrosine kinase-3 (FLT3) gene, a member of the PDGF-R subfamily has been recognized as an important molecule in acute myeloid leukemia. FLT3 is primarily expressed in hematopoietic stem cells, where it plays a role in hematopoiesis. It is activated by ligand binding that causes its dimerisation and activation and receptor phorphorylation (1). Activation of FLT3 leads to cell proliferation and activation. An activating somatic mutation in the form of internal tandem duplication in the juxtamembrane region of the gene has been described (2). Since the identification of this mutation, and another, D835 activation loop domain mutation, a wealth of reports has emerged suggesting that FLT3 mutation is the single most common molecular of genetic abnormality in acute myeloid leukemia with direct clinical impact on the disease outcome. An average frequency of approximately 20% for FLT3/ITD and 7% for D835 has been reported in the literature, associated more frequently with standard risk cytogenetics, PML/RARα rearrangement, less frequent with core binding factor leukemia,

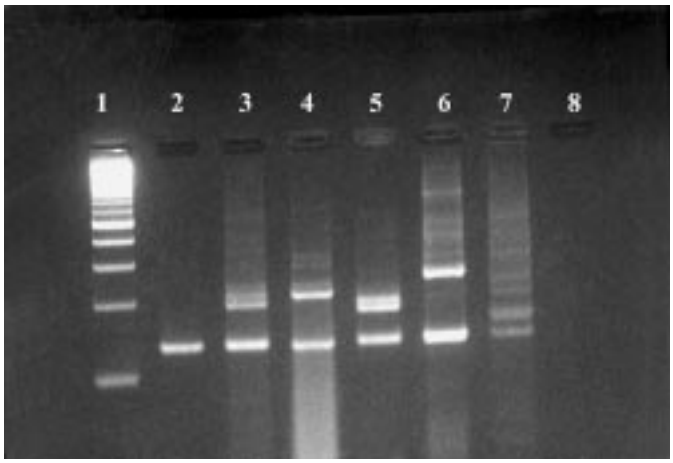

Figure 1. *Detection of FLT3/ITDs by PCR. Lane 1: Molecular weight marker 100 bp lader; Lane 2: wild type FLT3 amplicon; Lanes 3-6: FLT4/ITD positive cases. Note that additional bands varie in size (arrows). Lane 8: blank, negative control with no DNA.*

secondary or pediatric AML. Except of elderly AML presence of FLT3/ITD is associated with shorter survival.
In the present study we have examined the panel of 67 AML patients for FLT3/ITD mutation and have determined its prognostic significance and correlation to basic hematological data in a subset of them.

Materials and methods

Patient samples

Bone marrow at diagnosis was obtained from 67 AML patients treated at the Department of hematology, Merkur University Hospital in Zagreb, Croatia. All patient related procedures were in accordance with the Helsinki declaration and accepted ethical standards. Prognostic impact of FLT3/ITD mutation was tested on a subset of 28 patients, median age 53, range 19–85 years. Mutation positive and negative patients in analysis did not differ by age or sex distribution.

PCR amplification of FLT3/ITD

Exons 14 and 15 were amplified using primers FLT3F 5'-caatttaggtatgaaagcc-3' FLT3R 5'-caaactctaaattttctct-3', as designed by the molecular genetics working group of European Organization for Research and Treatment of Cancer (EORTC). PCR reaction was performed in 25 ml reaction buffer, containing 1ml of cDNA, 10pmol of each primer, 0,1 mM dNTP, 5mM $MgCl_2$ and 1U of Taq DNA polymerase (Applied Biosystems). PCR was performed with cycling conditions of initial denaturation step at 94 ^{0}C for 5 min, 36 cycles of 94 ^{0}C for 30 sec., 55 ^{0}C for 1 min and 72 ^{0}C for 1 min, with a final extension step of 72 ^{0}C for 7 minutes. Unmutated PCR products were seen as 130 bps products on 3,5% agarose gel. Samples showing additional longer PCR products were considered FLT3/ITD+. All mutation suspected samples were repeatedly tested for confirmation.

Statistical analysis

Curves for overall survival for ITD+, and FLT3/WT patients were calculated by the Kaplan-Meier, and Wilcoxon test for difference between groups was calculated. All statistical analyses were performed by using MedCalc statistical package (MedCalc software, Mariakerke, Belgium).

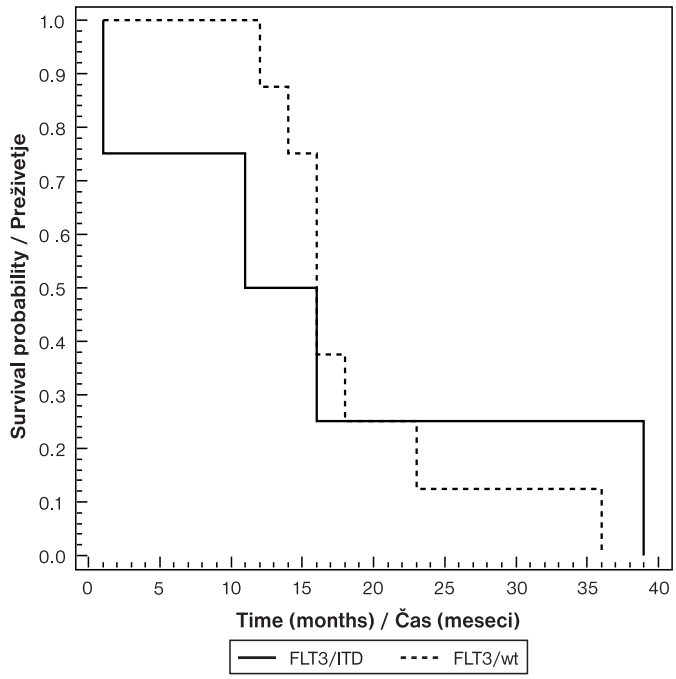

Figure 2. *Kaplan-Meier survival curve for FLT3/ITD+ (full line, 1) and LFT3/wt patients (dashed line, 2).*

Results

Eleven of 67 AML patients (16.4%) were shown to have FLT3/ITD mutation in the form of additional larger gene amplicon by PCR. The sizes of ITD varied between 30 and approximately 100 bp (Figure 1). All positive patients also expressed the unmutated allele.
Among the mutated patients there were 2 PML patients, 1 with core binding factor leukemia and others had either normal standard cytogenetics or complex abnormalities (2 patients). Morphological classification and hematological data of positive patients are given in Table 1. For the subset of 28 tested patients, 11 mutated and 17 non-mutated we calculated overall survival that showed trend for worse outcome for FLT3/ITD+ patients but with the given sample size the difference was not significant (Figure 2). However, median survival for FLT3/ITD+ patients was 3 months vs. 16 months for non-mutated patients ($p = 0.0431$). Two groups did not differ in the grade of leukocytosis, hemoglobin or platelet counts at diagnosis.

Discussion

Since the discovery of the existence of FLT3/ITD and D835 mutations in myeloid leukemia some 7 years ago there is an increasing body of research results accumulating that is suggesting an important role of aberrantly expressed FLT3 gene in the biology and clinical behavior of the disease. Our results fit into so far reported frequencies of this molecular event in AML. For adult AML frequencies from 17.3% (3) to higher 26.2% (4). Recent review by Levis et al. (5) summarizes the results of multiple studies and confirms some of the impressions had from individual reports. For instance, in pediatric AML incidence of FLT3/ITD is lower than in adults (from 6–15%) (6). Normal cytogenetic or t(15;17) AML have higher incidence than core-binding factors leukemia (t(8;21) and inv(16)). Secondary AML has less of this mutation than primary tumors and poor risk cytogenEtics is not associated frequently with FLT3 mutation (5).

Table 1. *Clinical data of FLT3/ITD-positive patients.*
Razpr. 1. *Klinični podatki o FLT3/ITD pozitivnih bolnikih.*

Age/Sex Starost/Spol	FAB	Cytogenetics Citogenetika	FLT3/ITD size (bp)	WBC $\times 10^9$/L	Outcome Izid
30, M	M2	45, x,–y, t(8;21)	70	76.3	A, Allo BMT
40, F	M3	46, xx, t(15;17)	70	34.0	D
49, M	M3	46, xy, t(15;17)	70	5.4	A
57, M	M2	normal	70	35.0	A
58, M	M2	49, xy, del(9) (q13; q22) +11, +13, +14	80	7.4	D
43, M	M2	normal	80	485.0	D
52, F	M1+CLL	normal	30	127.0	D
65, F	M4	normal	30	26.11	D
60, F	M4	46, xx, der(19), t(17; 19)/ 48, xx, +8, +13	100	18.6	D
85, F	M1	normal	30	1.2	D
69, M	M2	normal	30	48.11	A

A = alive, D = dead

Prognostic impact of FLT3 mutation today is also believed to be clear: it does adversely affect survival. Adult and pediatric AML patients with FLT3 mutation live worse than non-FLT3 mutated patients (5, 7). Our results are in accordance with this understanding. Overall incidence of FLT3/ITD mutation in a panel of 72 consecutive leukemia we screened was 16.4%. We had 2 FLT3/ITD+ cases of 6 t(15;17) promyelocytic leukemia (30%). There was one t(8;21) AML with mutated FLT3 of 4 with the same karyotype. Interestingly, among our positive cases there were 2 with complex karyotypes both of which were including an extra copy of chromosome 13, to which FLT3 maps. Both patients had relapsing and resistant disease. In our assay we could only register somewhat longer ITDs in those patients but it was not possible to relate to the gene expression level itself.

In conclusion, our results confirmed the significant presence of FLT3/ITD mutation in primary adult AML that was also associated with poorer clinical performance of the disease. Based on the numerous studies with similar experience one can expect the molecular testing for FLT3/ITD and possibly D835 mutation to be introduced into the routine molecular diagnostic workup of acute myelogenous leukemia. An additional argument for this will certainly be targeting of FLT3 receptor by the new generation of kinase inhibitors that will as drugs enter clinical practice in the near future.

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