A gene expression signature for defining aggressive phenotype and prognosis in intermediate-risk acute myeloid leukemia

Ailing Deng[1]

[1] Medicine College of Nankai university, Tianjin, China

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Abstract

Intermediate-risk AML is a group of heterogeneous disease, complete and accurate understanding of their molecular pattern are urgent. We downloaded gene expression profile from TCGA, and performed the differential gene expression analysis of 98 intermediate-risk AML patients according to the different clinical outcome. By studying the overlap of differential genes based on OS and EFS classification, we identified 6 distinct genes (SPANXC, ADAMTS15, C8B, FAM183A, FLJ42875 and PXDN). Only FLJ42875 and PXDN were relatively widely expressed in intermediate-risk AML. Moreover, Q1 (0-25%) of PXDN was associated significantly shortened OS and EFS (P=0.0001, P=0.0032, respectively), while OS and EFS of Q2 (25-50%) of FLJ42875 expression group were significantly longer than that of low FLJ42875 expression patients (P=0.0157, P=0.0074, respectively). In the multivariable model of OS, PXDN Q1 group had a shorter OS and EFS ($P < 0.001$, P =0.009; respectively). While FLJ42875 Q2 group had a tendency of prolonged OS and EFS (P=0.050, P=0.023; respectively). Our results suggest that FLJ42875 and PXDN may play a role in the leukemogenesis of intermediate-risk AML.

Key words: FLJ42875, PXDN, intermediate-risk AML

Introduction

Cytogenetic lesions is used as diagnostic and prognostic markers and also to elucidate the molecular pathogenesis of acute myeloid leukemia (AML) for more than three decades (Mrozek et al. 2004; Rowley 2008). However, nearly half of the AML patients don't have apparent cytogenetic structural abnormalities, they are intermediate-cytogenetic risk (Martelli et al. 2013). However, their clinical outcomes are greatly heterogeneous and lacks effective prognostic indicators at the same time. Some of these patients respond well to chemotherapy, while others not. Therefore, recent studies have focused on improving classification of intermediate-risk AML patients. Mutations, aberrantly expressed genes, microRNAs and changes in DNA methylation are potential prognostic markers. For example, mutations of CEBPA and NPM1 are associated with favorable outcomes (Ahn et al. 2016; Han et al. 2013; Li et al. 2015), whereas mutations of FLT3-ITD, WT1, MLL, TET2 and DNMT3A are associated with an unfavorable clinical outcome (Ley et al. 2010; Mikulasova et al. 2010; Ribeiro et al. 2012; Stirewalt & Radich 2003). High expression of WT1, ERG, and DNMT3B as well as low expression of LEF1 have also been shown to be unfavorable prognostic factor (Metzeler et al. 2009; Metzeler et al. 2012; Schulze et al. 2016; Yi-Ning et al. 2015). However, none of the present classification strategies conform to clinical needs entirely, which suggests that a more complete and accurate understanding of the genetic

and epigenetic alterations associated with the pathogenesis of intermediate-risk AML is crucial.

In order to further identify molecular determinants associated with aggressive behavior of intermediate-risk AML, we downloaded gene expression profile from TCGA, and we compared the gene expression pattern characterized by the distinct clinical outcome.

Methods

Patients and treatment

98 patients with previously untreated intermediate-risk AML (median age, 58 years; range, 21–88 years) were studied, all of them were diagnosed and received treatment according to NCCN guidelines between November of 2001 and March of 2010. 51 patients (52.0%) were aged <60 years (younger patients) and 47 patients (48.0%) were ≥ 60 years (older patients). The diagnosis and risk stratification of AML were based on NCCN Guidelines. All patients were assessed for somtatic mutations such as IDH1, NPM1, FLT3 and gene expression. Clinical, gene expression and somatic mutations profiles of all primary AML cases could be publicly downloaded from the TCGA project via the data portal on 01/10/2015. This research was approved by the Washington University Human Studies Committee (WU HSC #01 -1014), and informed consent from all patients was obtained in accordance with the Declaration of Helsinki(Cancer Genome Atlas Research 2013).

RNA Sequencing analyses

Gene expression data have been previously published. Briefly, gene expression data were obtained using RNA Sequencing. All the design and quality control for this experiment were based on the standard protocols. Patients with OS and EFS values above the median of all patients were subdivided into the OS^{longer} and EFS^{longer} group while others were into $OS^{shorter}$ and $EFS^{shorter}$ group. Gene expression levels were also determined from the normalized data.

Statistical analyses

The time from date of diagnosis to removal from study due to absence of complete remission, relapse or death defined EFS, and the time from date of diagnosis to death due to any cause defined OS. Firstly, we classified the 98 AML patients into two groups based on OS and EFS value respectively. Log-rank Test was used to estimate the association between gene expressions and EFS and OS of the patients, which were further validated using Gehan-Breslow-Wilcoxon test. Student's t-test and multiple hypothesis correction (False Discovery Rate, FDR) was used to identify differences in gene expression profiles. The statistical cutoff values were a fold-change (FC) ≥ 1.5 and an adjusted P-value < 0.05. All analyses were performed using the R3.2.2 software packages and GraphPad Prism 5.0.

Results

Clinical characteristics of primary intermediate-risk AML patients

In the cohort of 98 primary intermediate-risk AML patients, 6 were M0, 25 were M1, 24 were M2, 1 was M3, 24 were M4, 16 were M5, 1 was M7, 1 was not classified. The median age was 58 years, ranging from 21 to 88 years. 51 patients (52.0%) were aged <60 years (younger patients) and 47 patients (48.0%) were ≥ 60 years (older patients).49(50%) patients were male and 49(50%) patients were female. Pre-treatment hemoglobin was 9.629±0.1621g/L, WBC counts was (45.63±5.348)*10^9/L, and PLT counts was (72.60±6.057)*10^9/L. The median OS was 16.35(0-100.5) months, while median EFS was 9.4(0-100.5) months (Table 1).

Table 1. Clinical characteristics of intermediate-risk AML patients.

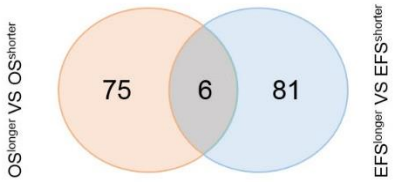
Variable	Intermediate-risk AML patients (n=98)
Age, y(range)	58(21-88)
≥ 60 y, no (%)	47(48.0)
Female	49(50%)
Hemoglobin(g/L)	9.629±0.1621
WBC(10^9/L)	45.63±5.348
PLT(10^9/L)	72.60±6.057
Blast percentage in BM (%)	80.26±2.129
FAB subtype, no (%)	
M0	6(6.1)
M1	25(25.5)
M2	24(24.5)
M3	1(1.0)
M4	24(24.5)
M5	16(16.3)
M7	1(1.0)
Not classified	1(1.0)
Mutation, no (%)	
FLT3	33(33.7)
NPM1	42(42.9)
FLT3+NPM1	21(21.4)
IDH1	10(10.2)

IDH2	12(12.2)
NRAS	9(9.2)
KRAS	4(4.1)

Differential gene expression in primary intermediate-risk AML samples

OS^{longer}, $OS^{shorter}$, EFS^{longer}, $EFS^{shorter}$ groups were subdivided as described above. Normalized RNA Sequencing data was downloaded from TCGA database and differential expression analysis was performed to obtain expression of AML patients. 75 differential genes were detected after the comparison of OS^{longer} and $OS^{shorter}$ groups, while 81 differential genes were discovered in that of EFS^{longer} and $EFS^{shorter}$ groups (Supplemental Table S1 and S2). The overlaps were six genes including SPANXC, ADAMTS15, C8B, FAM183A, FLJ42875 and PXDN (Figure 2). No strong association among them was found.

A



B

Gene	$Log_2FC:OS^{longer}/OS^{shorter}$	P-value	$Log_2FC:EFS^{longer}/EFS^{shorter}$	P-value
ADAMTS15	-1.64824	0.021	-1.6474	0.026
C8B	-2.15338	0.016	-2.52137	0.025
FAM183A	-2.62104	0.047	-2.62104	0.047
FLJ42875	1.530341	0.029	2.195344	0.001
PXDN	-2.97264	<0.001	-1.69152	0.034
SPANXC	3.410173	0.015	3.410173	0.015

Figure 1. Differential genes in intermediate-risk AML patients. (A)Overlap of OS and EFS differential genes. (B)List of differential genes.

Expression of differential genes in AML samples

Next, we analyzed the RNA Sequencing data to obtain the expression of six differential genes mentioned above in intermediate-risk AML patients. As indicated in the Figure 2, SPANXC, ADAMTS15, C8B,

FAM183A were expressed narrowly at a low level in intermediate-risk AML, they could hardly be detected in most patients. However, FLJ42875 and PXDN could be detected widely in intermediate-risk AML patients.

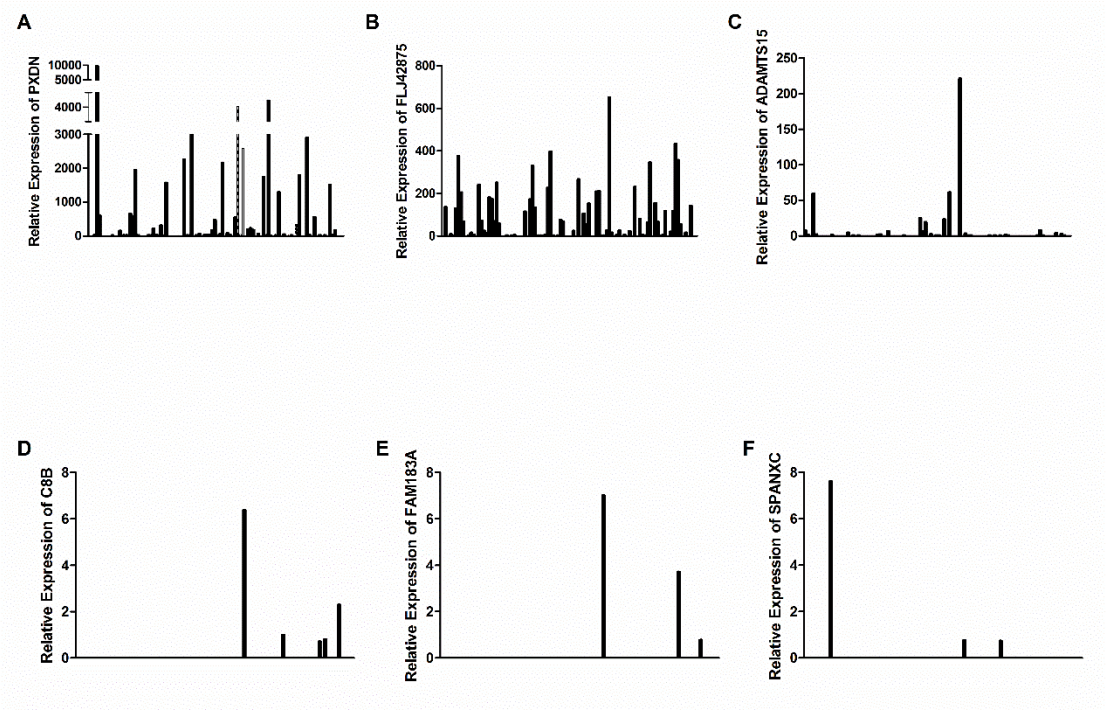


Figure 2. Expression of six differential genes in intermediate-risk AML patients.

Relationship between genes' expression and survival in primary intermediate-risk AML patients

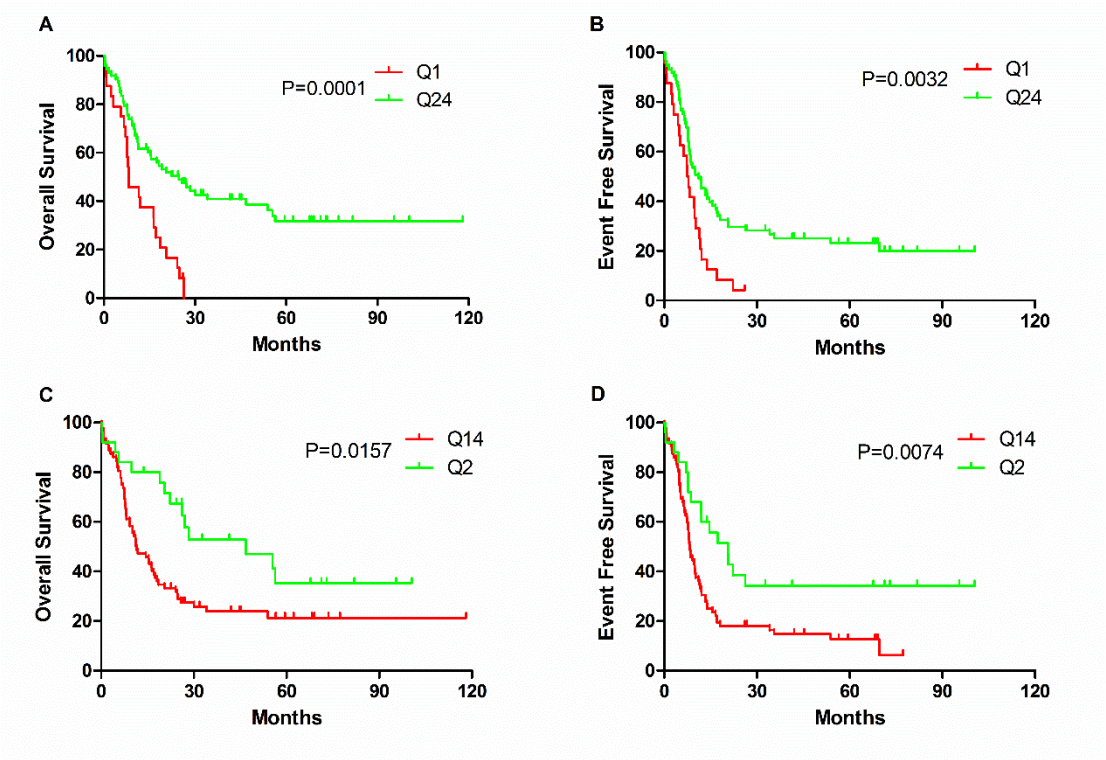


Figure 3. Relationship between gene expression and survival of intermediate-risk AML patients. (A) and (**B**) Kaplan-Meier curves showing the relationship of PXDN expression with Overall Survival and Event Free Survival of intermediate-risk AML patients; (C) and (**D**) Kaplan-Meier curves showing the relationship of FLJ42875 expression with Overall Survival and Event Free Survival of intermediate-risk AML patients.

We performed the survival analysis based on the expression of two widely expressed genes, FLJ42875 and PXDN. Firstly, we subdivided 98 AML patients into four quartiles (Q1: <25%, Q2: 25~50%, Q3: 50~75%, Q4: >75%) based on their expression value. The survival curves showed that PXDN Q1 expression was associated significantly shortened OS and EFS (P=0.0001, P=0.0032, respectively; Figure 3A and Figure 3B). However, OS and EFS of FLJ42875 Q2 expression group were

significantly prolonged than others (*P*=0.0157, *P*=0.0074, respectively; Figure 3C and Figure 3D), the difference was still significant by revalidation of Gehan-Breslow-Wilcoxon test. After adjusting for the impact of several known risk factors, we performed multivariable analyses to determine the prognostic significance of FLJ42875 and PXDN expression. In the multivariable model of OS, PXDN Q1 group had a shorter OS ($P < 0.001$, Table 2). The other factors associated with shorter OS was age more than or equal to 60 years old. While in the multivariable model of EFS, PXDN Q1 group had a shorter EFS (P =0.009, Table 2). FLJ42875 Q2 group had a prolonged OS and EFS (*P*=0.050, *P*=0.023; respectively).

Table 2: Multivariable analysis of PXDN expression with OS and EFS for intermediate-risk AML patients

Variable	OS, n=98		EFS, n=98	
	HR (95% CI)	P value	HR (95% CI)	P value
PXDN(Q1)	2.713(1.589-4.631)	**<0.001**	1.995(1.189-3.345)	**0.009**
Age, ≥60 year	2.655(1.629-4.327)	**<0.001**	2.237(1.419-3.524)	**0.001**
Female	1.155(0.717-1.859)	0.553	1.138(0.730-1.773)	0.569
FLT3 mutation	1.333(0.806-2.204)	0.262	1.602(0.998-2.570)	0.288

Table 3: Multivariable analysis of FLJ42875 expression with OS and EFS for intermediate-risk AML patients

Variable	OS, n=98		EFS, n=98	
	HR (95% CI)	P value	HR (95% CI)	P value
FLJ42875 (Q2)	0.548(0.300-1.000)	**0.050**	0.521(0.297-0.912)	**0.023**
Age, ≥60 year	2.341(1.441-3.806)	**0.001**	2.015(1.288-3.151)	**0.002**
Female	1.186(0.737-1.910)	0.483	1.191(0.764-1.858)	0.569
FLT3 mutation	1.353(0.820-2.230)	0.237	1.685(1.057-2.684)	0.028

Discussion

Nearly half of the AML patients have an intermediate-cytogenetic risk, and their clinical outcome is greatly heterogeneous and lacks effective prognostic indicators at the same time. Some of these patients respond well to chemotherapy, while others not. For this reason, we conducted this analysis to focus on establishing new indicators for complete classification of intermediate-risk.

First, we performed the differential analysis of gene expression profile of 98 intermediate-risk AML patients by using the RNA Sequencing data from TCGA based on different OS and EFS. We identified 6 distinct genes, SPANXC, ADAMTS15, C8B, FAM183A, FLJ42875 and PXDN. Then we detected the overall expression pattern in intermediate-risk AML patients. Only FLJ42875 and PXDN were relatively widely expressed in intermediate-risk AML. At last, we focused on the association of their expression pattern and survival of intermediate-risk AML patients. We found that OS and EFS of high FLJ42875 expression group were significantly shorter than that of low FLJ42875 expression patients (P=0.0409, P=0.0136, respectively), while high PXDN expression was associated significantly prolonged EFS and OS on the contrary (P=0.0185, P=0.0469, respectively). In the multivariable model of OS, PXDN Q1 group had a shorter OS and EFS ($P < 0.001$, P =0.009; respectively). While FLJ42875 Q2 group had a prolonged OS and EFS (P=0.050, P=0.023;

respectively).

PXDN, a member of the larger peroxidase gene family, is an adhesion molecule involved in ECM formation (Peterfi et al. 2014; Tauber et al. 2010). It is one of the target genes of miR-29a. Ectopic overexpression of miR-29a promoted self-renewal of myeloid progenitors and led to AML ultimately in mouse model (Oliveira et al. 2015). What's more, PXDN were diminished in AML (Desmond et al. 2007). In this article, high PXDN expression was associated with favorable prognosis in intermediate-risk AML, it was hypothesized that PXDN downregulation might play a role in the leukemogenesis of intermediate-risk AML.

Researches on FLJ42875 were limited. Only in a case report, upregulation of FLJ42875 by t(1;2)(p36;p21) translocation during evolution of CMML was reported with no further insight into mechanism of this gene(Storlazzi et al. 2008). No biological function especially in hematology malignancies were elucidated. In this article, Q2 of FLJ42875 was associated with favorable prognosis in intermediate-risk AML, it could be that FLJ42875 expression at particular level might predict the prognosis of intermediate-risk AML patients. More researches on this gene are required in the future.

In summary, our results detected two differential genes of which expression were related to the prognosis of primary intermediate AML patients, FLJ42875 and PXDN might be new markers of risk stratification

especially for intermediate-risk AML patients and new targets for clinical therapy. QPCR should be performed to revalidate the RNA Sequencing analysis and making the results more credible. What' more, further studies is needed to elucidate the mechanism behind it.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

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