

Concurrent Evaluation of the Expression and Methylation of Secreted Frizzled-Related Protein 2 along with Beta-Catenin Expression in Patients with non-M3 Acute Myeloid Leukemia

Fatemeh Mirzaeyan¹, MSc; Bahram Chahardouli², PhD; Amin Mirzaeian³, MSc; Nasrin Alizad Ghandforoush², MSc; Kamran Alimoghaddam², MD; Shahrabano Rostami², PhD

¹Department of Hematology and Blood Banking, School of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran;

²Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran;

³Department of Immunology, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

Correspondence:

Shahrabano Rostami, PhD;
Hematology-Oncology and Stem Cell Transplantation Research Center, Shariati Hospital, Kargar Ave., Jalal-e-Al-e-Ahmad Hwy., Postal code: 14117-13135, Tehran, Iran
Tel: +98 21 84902626
Fax: +98 21 88004140
Email: sh-rostami@sina.tums.ac.ir
Received: 27 November 2019
Revised: 06 January 2020
Accepted: 12 January 2020

What's Known

- Wnt signaling is critical for the development of many malignancies, including acute myeloid leukemia. Research has shown the importance of the expression or methylation of secreted frizzled-related protein 2 as an antagonist and beta-catenin as a critical mediator of this pathway.

What's New

- We investigated the status of both secreted frizzled-related protein 2 expression and methylation simultaneously, and their correlation with beta-catenin expression in Iranian patients. We showed that evaluating beta-catenin expression might be valuable in predicting complete remission in patients with non-M3 acute myeloid leukemia.

Abstract

Background: Wnt signaling is a critical pathway for the development of acute myeloid leukemia (AML). Some studies have evaluated the expression or methylation of secreted frizzled-related protein 2 (*SFRP2*) as an antagonist and beta-catenin (*β-catenin*) as a critical mediator of this pathway. Since we found no comprehensive study on these genes in Iran, we aimed to investigate the status of both *SFRP2* expression and methylation, and also *β-catenin* expression, in conjunction with clinical characteristics, in Iranian patients with *de novo* non-M3 AML.

Methods: The methylation and expression of *SFRP2* were determined in 188 patients with primary non-M3 AML and 60 healthy controls, who were referred to Shariati Hospital, Tehran, Iran, between January 2017 and February 2019. The methylation-specific polymerase chain reaction (PCR) and real-time quantitative PCR were used, respectively. The expression of *β-catenin* was explored via real-time quantitative PCR. Statistical analysis was performed using the Mann-Whitney *U* test (SPSS software, version 23). A *P* value of less than 0.05 (2-tailed) was considered significant.

Results: *SFRP2* mRNA showed a significant decline in the AML group compared with the controls (*P*<0.001). The hypermethylation of the *SFRP2* promoter occurred in 25.5% (48/188) of the cases. *SFRP2* expression exhibited a negative correlation with the white blood cell count (*P*=0.003). The expression of *β-catenin* increased significantly in the patients in comparison with the controls (*P*<0.0001), and a significant difference was observed between the patients, who achieved complete remission and those, who did not (*P*=0.046).

Conclusion: The findings of this study showed that alterations in *SFRP2* and *β-catenin* expression can be used as a potential biomarker for differentiating patients with new non-M3 AML from the controls. Additionally, an evaluation of *β-catenin* expression may be valuable in predicting complete remission in patients with non-M3 AML.

Please cite this article as: Mirzaeyan F, Chahardouli B, Mirzaeian A, Alizad Ghandforoush N, Alimoghaddam K, Rostami S. Concurrent Evaluation of the Expression and Methylation of Secreted Frizzled-Related Protein 2 along with Beta-Catenin Expression in Patients with non-M3 Acute Myeloid Leukemia. Iran J Med Sci. 2021;46(3):180-188. doi: 10.30476/ijms.2020.84316.1396.

Keywords • *SFRP2* protein • Humans • Leukemia • Myeloid • Acute • beta Catenin • Wnt signaling pathway

Introduction

Acute myeloid leukemia (AML) is a heterogeneous blood cancer characterized by the clonal disorders of hematopoietic progenitor

cells.^{1, 2} Genetic and epigenetic abnormalities are considered to be a critical player in the pathogenesis of AML.^{3, 4} Aberrant promoter hypermethylation is one of many epigenetic aberrations that contribute to leukemogenesis.⁵

In recent years, a considerable number of signaling pathways have been recognized and indicated as important factors for the regulation of cellular processes. The Wnt signaling pathway is one of them with a key role in hematopoietic cell fate. Based on numerous studies, the abnormal activation of the Wnt signaling pathway is indicated as the pathogenesis of AML, given its critical roles in differentiation, proliferation, cell adhesion, and migration.^{6, 7} Many instances of Wnt signaling dysregulation have been detected in various cancers, including AML. Wnt signaling comprises the canonical pathway (*beta-catenin* [*β-catenin*]-dependent) and noncanonical pathways (*β-catenin*-independent).⁸⁻¹⁰

In the absence of Wnt signaling, the phosphorylated form of *β-catenin* is degraded by ubiquitination, and the cytoplasmic levels of protein remain low. With the activation of the Wnt pathway, the phosphorylation and degradation of *β-catenin* are inhibited, leading to its accumulation in the cytoplasm, and transport into the nucleus. Nuclear non-phosphorylated *β-catenin* is the downstream effector of canonical Wnt signaling and mediates the expression of several genes, including *cyclin D1* and *c-Myc*.¹¹

Several families of Wnt signaling antagonists such as secreted frizzled-related proteins (SFRPs), Dickkopf (Dkk) proteins, and Wnt inhibitory factor 1 act as modulators of the Wnt signaling cascade through the inhibition of Wnt proteins.^{9, 12} In humans, SFRPs consist of five members, and have been implicated as the largest family among Wnt antagonists. The aberrant methylation of *SFRP* genes, which was associated with abnormal Wnt signaling activation,^{13, 14} was demonstrated in AML.¹⁵ Four out of five *SFRP* genes (*SFRP1*, *SFRP2*, *SFRP4*, and *SFRP5*) contain dense CpG islands around their promoter regions. Previous studies have reported that *SFRP* genes, except for *SFRP3* are silenced by promoter hypermethylation in various malignancies, including AML.^{13, 16} Moreover, it has been indicated that Wnt signaling can also be activated by mutant fms-like tyrosine kinase 3 (FLT3).^{17, 18} In total, these findings show that Wnt signaling aberration involves multiple mechanisms, and is a common dysregulated pathway in various cancers. Furthermore, some studies have proposed that the hypermethylation of the *SFRP* promoter is an adverse risk factor for survival in patients with AML.^{6, 18, 19}

Accordingly, in the present study, we aimed to

investigate the status of *SFRP2* expression and methylation simultaneously and explore their clinical significance besides their correlation with the *β-catenin* expression as the most important mediator of the canonical Wnt signaling pathway, in Iranian patients with *de novo* non-M3 AML.

Patients and Methods

Patients and Samples

In this cross-sectional study, written informed consent was obtained before bone marrow specimens were collected from 188 patients with *de novo* non-M3 AML (98 male and 90 female patients), who were referred to the Hematology-Oncology and Stem Cell Transplantation Research Center of Shariati Hospital (Tehran, Iran) between January 2017 and February 2019. Based on previous studies and via the Cochran formula, the sample size was determined.²⁰ The diagnosis and classification of patients with *de novo* AML were made according to the criteria of the French-American-British (FAB) classification systems and the World Health Organization (WHO) (blast \geq 20%). Patients, who had a history of other malignancies, myelodysplastic syndromes, and treatment with cytostatic drugs (e.g., steroids) were excluded from the study. The main clinical and laboratory features of the patients are summarized in table 1. Sixty age- and sex-matched healthy controls with no current morbidity or history of serious diseases were included in the study. All the patients received standard 3+7 induction chemotherapy, comprising idarubicin (Pfizer, Australia; 12 mg/m²) for three days plus cytarabine (Abbvie, Australia; 100 mg/m²) for seven days. The project was approved by the Ethics Committee of Tehran University of Medical Sciences (code: IR.TUMS.REC.1395.2313).

DNA Extraction, Bisulfite Modification, and Methylation-Specific Polymerase Chain Reaction (MSP)

Genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). DNA was bisulfite converted using the EpiTect Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. MSP was carried out using *SFRP2* promoter methylation and non-methylation-specific primer pairs (table 2) designed in previous studies.²¹ MSP was carried out for each sample in a final reaction volume of 20 μ L, containing 0.4 μ M of primers, 10 μ L of Taq DNA Polymerase Master Mix RED (10 μ L) (Ampliqon, Stenhusgervej, Denmark), and 50 ng of bisulfite-treated DNA.

Table 1: Clinical characteristics of the study patients with acute myeloid leukemia based on the expression of *SFRP2* and methylation status

Patients' Parameter(s)		Status of <i>SFRP2</i> Expression			Status of <i>SFRP2</i> Methylation			
		Low (n=136)	High (n=52)	Total (n=188)	P value	M (120)	U (68)	P value
Sex,	Male	82	16	98	0.006	62	36	0.746
	Female	54	36	90		58	32	
Median age, year (range)		43 (14-90)	54 (1-87)	45 (1-90)	0.001	50 (1-90)	42 (14-64)	0.167
Median WBC, ×10 ⁹ /L (range)		25.41 (0.57-290.00)	6.82 (0.98-62.81)	20.62 (0.57-290.00)	0.003	14.31 (0.82-16.12)	60.00 (0.57-290.00)	0.083
Median hemoglobin, g/L (range)		79.00 (50.00-150.24)	84.21 (40.20-120.16)	80.11 (40.20-150.24)	0.436	82.32 (40.20-150.24)	76.20 (50.00-110.33)	0.218
Median platelets, ×10 ⁹ /L (range)		17.00 (1.54-198.22)	53.10 (13.08-369.18)	40.12 (1.54-369.18)	0.191	34.51 (1.54-369.18)	43.00 (1.89-198.71)	0.175
Age (year)	<60	116	34	150	0.009	83	67	0.675
	≥60	20	18	38		37	1	
BM blasts, % (range)		86.51 (28.20-97.78)	79.54 (30.1-97.78)	82.00 (28.20-97.78)	0.295	82.00 (28.20-97.78)	87.43 (30.10-97.78)	0.307
FAB type, n (%)	M0	10 (7.3)	0(0)	10 (5.3)	0.01	8 (6.7)	2 (2.9)	0.350
	M1	30 (22.1)	12 (23.1)	42 (22.3)		26 (21.7)	16 (23.5)	
	M2	41 (30.1)	12 (23.1)	53 (28.2)		34 (28.3)	19 (28.0)	
	M4	28 (20.6)	25 (48.1)	53 (28.2)		33 (27.5)	20 (29.4)	
	M5	13 (9.6)	1 (1.9)	14 (7.4)		10 (8.3)	4 (5.9)	
	M6	6 (4.4)	2 (3.8)	8 (4.3)		4 (3.3)	4 (5.9)	
	Unclassified	8 (5.9)	0(0)	8 (4.3)		5 (4.2)	3 (4.4)	
Gene mutation, n (%)	<i>NPM1</i>	25(18.38)	15 (28.84)	40 (21.27)	0.891	30 (25.00)	10 (14.7)	0.283
	<i>FLT3-ITD</i>	24 (17.64)	16 (30.76)	48 (25.53)	0.443	32 (26.67)	16 (23.52)	0.421
Complete remission n(%)		94 (69.12)	38 (73.07)	132 (70.21)	0.109	32 (26.67)	23 (33.82)	0.236
<i>Beta-catenin</i> expression				0.238				0.843

WBC: White blood cell; BM: Bone marrow; FAB: French–American–British; *SFRP2*: Secreted frizzled-related protein 2; *NPM1*: Nucleophosmin 1; *FLT3-ITD*: FMS-like tyrosine kinase 3-internal tandem duplication

Table 2: Primer sequences used for real-time quantitative polymerase chain reaction and methylation-specific polymerase chain reaction

Primer	Sequence (5'-3')	Product Size (bp)	Annealing Temperature (°C)
Expression			
<i>Beta-catenin</i> -F	CATCTACACAGTTTGATGCTGCT	150	60
<i>Beta-catenin</i> -R	GCAGTTTTGTCAGTTCAGGGA		
<i>SFRP2</i> -F	TAGACGAGACCATCCAGCCA	176	60
<i>SFRP2</i> -R	CCTTTGGAGCTTCCTCGGT		
<i>ABL</i> -F	TGGAGATAAACTCTAAGCATAACTAAAGG	124	60
<i>ABL</i> -R	GATGTAGTTGCTTGGGACCCA		
Methylation			
<i>SFRP2</i> -M-F	GGGTCGGAGTTTTTCGGAGTTGCGC	138	62
<i>SFRP2</i> -M-R	CCGCTCTCTTCGCTAAATACGACTCG		
<i>SFRP2</i> -U-F	TTTTGGGTTGGAGTTTTTTGGAGTTGTGT	145	64
<i>SFRP2</i> -U-R	AACCCACTCTCTTCACTAAATACAACCTCA		

M: Methylated; U: Unmethylated; F: Forward; R: Reverse; *SFRP2*: Secreted frizzled-related protein 2

MSP was performed on the Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Polymerase chain reaction (PCR) conditions were as follows: initial denaturation for three minutes at 94 °C, followed by 40 cycles of 30 seconds at 94 °C, 30 seconds at 64 °C, 30 seconds at 72 °C, and final elongation for seven minutes at 72 °C. Unmethylated and completely methylated DNAs, contained in the EpiTect PCR Control DNA Kit (QIAGEN, Hilden, Germany), were used as positive controls. Electrophoresis

on a 2.5% agarose gel was done for the identification of the MSP product.

Flow Cytometric Analysis

Normal and leukemic mononuclear cells were isolated from bone marrow using Histopaque (Sigma, St Louis, USA) density-gradient centrifugation.²² After separation, the mononuclear cells were stained with a panel of fluorescent-conjugated monoclonal antibodies (Dako, Glostrup, Denmark), including CD45,

CD34, CD38, CD13, CD33, CD14, CD64, CD10, HLA-DR, CD19, CD20, and isotype controls, in accordance with the manufacturer's instructions as described previously.²³ The Flomax software (Version: 2.6; PARTEC, Nuremberg, Germany) was used for data analysis.

RNA Extraction and Reverse Transcription and Real-time Quantitative Polymerase Chain Reaction

Total RNA was isolated from mononuclear cells using the TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and reverse-transcribed into complementary DNA using the PrimeScript RT Reagent Kit (Takara Bio, Tokyo, Japan). Real-time quantitative PCR was carried out for each sample in a final reaction volume of 20 μ L, consisting of 0.4 μ M of primers, 10 μ L of SYBR Premix Ex Taq II, 0.4 μ L of 50x ROX (Takara Bio, Tokyo, Japan), and 50 ng of complementary DNA. The primer sequences for β -catenin and *SFRP2* (target genes), and *ABL1* (reference gene) are listed in (table 2). Real-time quantitative PCR was performed on StepOnePlus (Applied Biosystems, Foster City, CA, USA). The PCR program was carried out at 95 °C for 10 minutes, followed by 40 cycles at 95 °C for 15 seconds, 59 °C for 20 seconds, and 72 °C for 25 seconds. The mRNA expression level of the *SFRP2* gene was calculated relative to the expression of the reference gene using the $2^{-\Delta Ct}$ formula.

Gene Mutation Detection

FLT3-ITD and *NPM1* mutations were detected

by fragment analysis as reported previously.²⁴

Statistical Analysis

The Mann–Whitney *U* test and the Kruskal–Wallis test were applied for the comparison between two and multiple groups, respectively. The Spearman correlation coefficient was utilized to study the correlations between different values. The SPSS software, version 23, (SPSS, Chicago, IL, USA) and GraphPad Prism, version 5, (GraphPad Software, Inc., La Jolla, CA, USA) were employed for the analyses. The receiver operating characteristic curve (ROC) and the area under the ROC curve (AUC) were drawn upon to assess the diagnostic value of *SFRP2* and β -catenin expression in differentiating patients with non-M3 AML from healthy controls. A *P* value of less than 0.05 (2-tailed) was considered to be statistically significant.

Results

Expression and Methylation of *SFRP2* in Patients with non-M3 Acute Myeloid Leukemia

The level of *SFRP2* expression was investigated in 188 patients with non-M3 AML and 60 normal controls. The *SFRP2* level showed a significant decline in the AML group compared with the control group (figure 1A). Additionally, the methylation of *SFRP2* promoter regions was analyzed (figure 2). The *SFRP2* promoter was fully methylated in 25.5% (48/188) of the patients, partially methylated in 38.3% (72/188), and unmethylated in 36.2% (68/188).

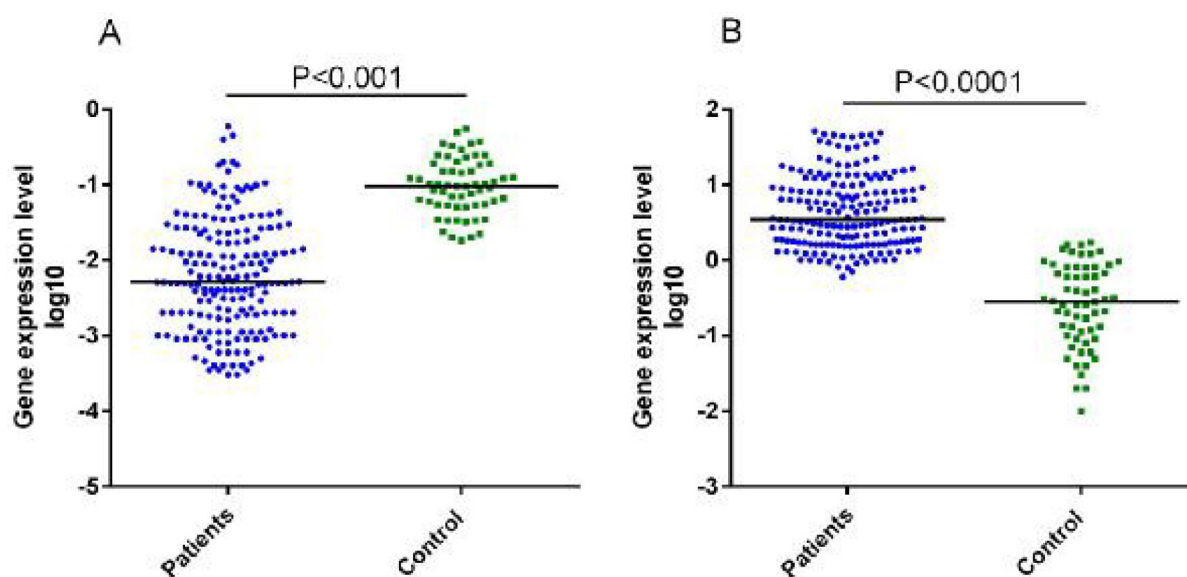


Figure 1: Relative expression levels of *secreted frizzled-related protein 2* (*SFRP2*) (A) and (B) *beta-catenin* (β -catenin) are illustrated between patients with non-M3 acute myeloid leukemia (AML) compared with healthy controls. The solid line represents the median of $2^{-\Delta Ct}$ of the expression of each gene. The *SFRP2* mRNA level decreased, and the β -catenin mRNA level increased significantly in patients with non-M3 AML compared with controls.

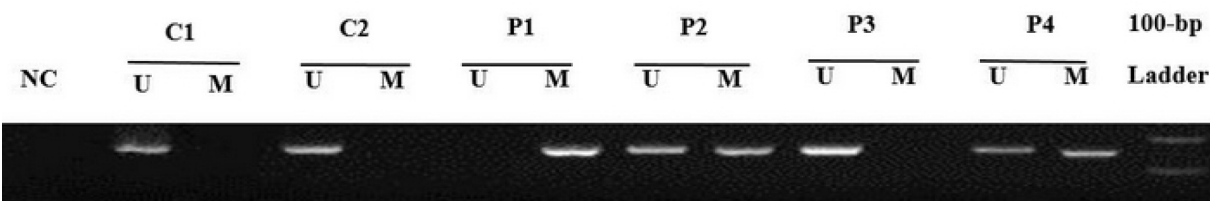


Figure 2: Methylation analysis of the *secreted frizzled-related protein 2 (SFRP2)* promoter in four patients with non-M3 acute myeloid leukemia and two healthy controls shows that the *SFRP2* promoter was fully methylated in Patient 1, partially methylated in Patients 2 and 4, and unmethylated in Patient 3. *SFRP2* methylation was found in neither of the two controls. NC, Negative control; P, Patient; C, Control; M, Methylated; U, Unmethylated

SFRP2 methylation was found in none of the 60 (0%) controls. Collectively, the methylation of the *SFRP2* gene occurred in 63.8% (120/188) of the patients with AML.

Value of SFRP2 and β-catenin Expression for Differentiating Patients with Non-M3 Acute Myeloid Leukemia from Controls Using the Receiver Operating Characteristic Curve

The expression of *SFRP2* and *β-catenin* was evaluated using the ROC curve to determine whether it was useful as a potential diagnostic marker for *de novo* AML. It was concluded that the level of *SFRP2* and *β-catenin* expression could be helpful as a potential diagnostic biomarker for differentiating patients with non-M3 AML from controls with an AUC of 0.861 (95% CI: 0.785 to 0.936; $P < 0.001$) for *SFRP2*, and an AUC of 0.88 (95% CI: 0.79 to 0.97; $P < 0.001$) for *β-catenin* (figure 3). At a cutoff value of 5.85% for *SFRP2*, the sensitivity and the specificity were 75% and 88%, respectively. The cutoff value of *β-catenin* was 0.3%, and the sensitivity and the specificity were 98% and 70%, respectively.

Impact of Different SFRP2 Expression and Methylation on the Clinical and Laboratory Characteristics of Acute Myeloid Leukemia

The expression of *SFRP2* was evaluated based on a cutoff value of 5.85%. Low *SFRP2* ($\leq 5.85\%$) and high *SFRP2* ($> 5.85\%$) expression levels, as well as the methylation status of the *SFRP2* gene, were evaluated in two groups: methylated and unmethylated. The results revealed no significant differences in the hemoglobin level, the platelet count, the percentage of CD34+, the percentage of blasts in bone marrow, and gene mutations between the two groups of high and low *SFRP2* expression levels. However, there was a negative correlation between the white blood cell count (WBC) and *SFRP2* expression ($P = 0.003$). A low *SFRP2* expression level was found more frequently in male subjects ($P = 0.001$). *SFRP2* expression was different between AML subtypes ($P = 0.001$). Additionally, a significant difference in terms of *SFRP2* expression was detected between the two age groups of younger than 60 and at least 60 years old ($P = 0.009$) (table 1). After the evaluation of methylation status, no significant difference was found in gender, age, WBC, the hemoglobin level, the platelet count, the percentage of blasts in bone marrow, the percentage of CD34+, the FAB classification, and the gene mutations between the methylated and unmethylated groups (table 1).

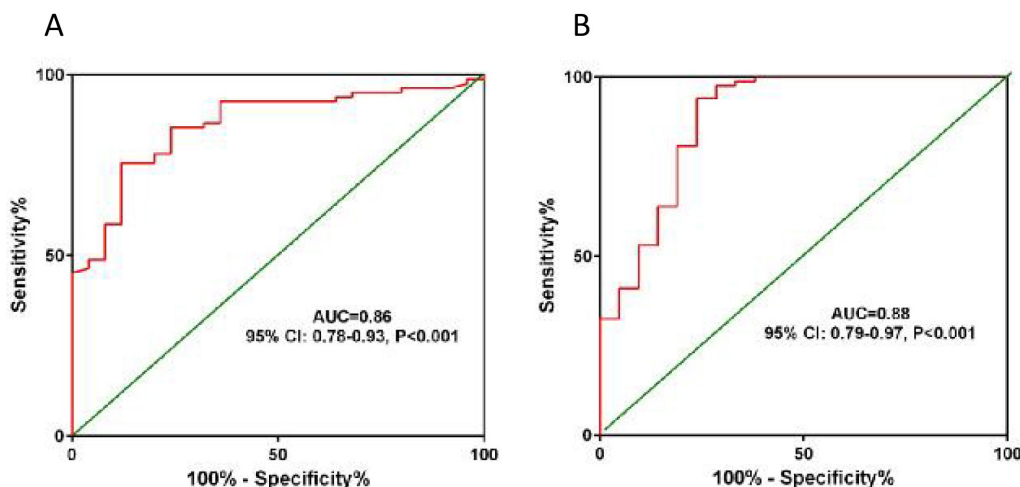


Figure 3: Receiver operating characteristic curve analysis shows that the expression of *secreted frizzled-related protein 2 (SFRP2)* (A) and *beta-catenin* (B) can be used as a potential diagnostic biomarker for discriminating between patients with non-M3 acute myeloid leukemia and controls.

Correlation between the Expression and Methylation of *SFRP2* in Patients with Acute Myeloid Leukemia

The relationship between the expression and methylation of *SFRP2* was analyzed. The results demonstrated no significant difference concerning *SFRP2* expression between the methylated and unmethylated *SFRP2* groups ($P=0.635$).

Correlation between *SFRP2* Expression and Methylation and β -catenin Expression

The results showed that the β -catenin expression level rose significantly in the AML group in comparison with the control group ($P<0.0001$) (figure 1B). The expression of β -catenin was not significantly different between the methylated and unmethylated groups. No correlation was found between β -catenin expression and *SFRP2* expression. Furthermore, β -catenin expression was significantly different between the patients, who achieved complete remission and those, who did not ($P=0.046$).

Discussion

The critical role of the Wnt signaling pathway in the cellular processes of the differentiation and proliferation of hematopoietic progenitors has been determined. The dysregulation of this pathway plays a role in leukemogenesis. The epigenetic inactivation of the Wnt pathway inhibitors has been indicated as an important explanation for the Wnt pathway activation. *SFRP2* hypermethylation is implicated in various cancers such as breast cancer,⁹ gliomas,¹² gastric cancer,¹³ and pituitary adenomas.¹⁴ In this study, we explored *SFRP2* methylation and expression simultaneously, and evaluated their correlation with β -catenin expression and clinical features in Iranian patients with non-M3 AML. We assessed the results in conjunction with clinical characteristics and treatment responses. In our study, the percentage of the aberrant methylation of at least one *SFRP2* gene was 63.8% (120/188), while no methylation was reported in the controls. Previous studies have reported various frequencies of *SFRP2* hypermethylation events in patients with AML (17%–66%). Nonetheless, our result (25.5%) is comparable with that reported by Shen and others²⁵ (25.4%) and Guo and others²⁶ (27.3%). Therefore, the methylation of these genes may contribute to the onset of AML.

We could not evaluate our patients' karyotype in our study; nevertheless, *SFRP2* methylation has been reported in adults with core-binding factor leukemia with poor prognosis¹⁶ as well as

in patients with intermediate-karyotype leukemia younger than 60 years of age.⁶

We found no correlation between *SFRP2* hypermethylation and the age or gender of the patients. Some researchers have shown that the aberrant *SFRPs* promoter methylation is correlated significantly with an increase in age^{16,26} and male gender.^{21,26}

Our results also demonstrated no correlation between the methylation and expression of *SFRP2*. This result is consistent with that reported by Guo and others.^{26,27} Still interestingly, they detected a negative correlation between *SFRP1* expression and methylation in patients with AML. In contrast to our results, in a study by Jost and others, there was an association between *SFRP2* hypermethylation and transcriptional downregulation.¹⁹

In contrast to Guo and others,²⁷ we assessed *SFRP2* expression in all the study patients after diagnosis not after achieving complete remission. Guo and colleagues suggested that *SFRP2* expression could be potentially used as a biomarker for disease monitoring in patients with cytogenetically normal AML after complete remission.²⁷ Consequently, our results could not be used for disease monitoring. In the same line with Guo and others,²⁷ however, we concluded that *SFRP2* expression could be drawn upon as a diagnostic biomarker.

In our study, complete remission after 7+3 induction chemotherapy was achieved in 132 out of 188 patients (70.2%). Nonetheless, we did not detect any significant association between the hypermethylation of *SFRP2* and the percentage of complete remission. In accordance with our result, some studies have found no prognostic factor for aberrant methylation in the *SFRP2* promoter alone, and they have shown that the concurrent methylation of Wnt antagonists is needed.^{6,26,28} Griffiths and others pointed out that patients suffering from AML with *SFRP2* and *SFRP5* methylation at the time of diagnosis had an increased risk of relapse, and *SFRP2* methylation was associated with a higher risk for death.¹⁵ Valencia and others reported that the methylation of *SFRPs* and *DKKs* was associated with an adverse prognosis in young patients suffering from AML with intermediate-risk cytogenetics.⁶ Unfortunately, we only evaluated the hypermethylation of *SFRP2* and not that of other mediators, and our information about prognosis is not complete yet. These results indicate that the aberration of several mediators simultaneously is important for the prediction of prognosis in patients.

Interestingly, in contrast to Guo and others,²⁷ we found a negative correlation between WBC

and *SFRP2* expression. Our results revealed low *SFRP2* expression levels more frequently in our male patients. *SFRP2* expression was different between AML subtypes, and we found a significant difference vis-à-vis *SFRP2* expression between the two age groups of younger than 60 and at least 60 years old. In general, different results have been obtained in various studies, which may be due to racial differences, environmental conditions, and different methods of testing and sampling.

As has been indicated, β -catenin plays a role as the main mediator of the Wnt signaling pathway,^{11, 28} and its accumulation in the cytoplasm/nucleus is critical for the activation of this pathway. The overexpression of β -catenin has been previously reported in blast crisis-CML CD34+ progenitors.²⁹ Wang and others analyzed the relationship between the expression levels of β -catenin and the methylation status of Wnt antagonist genes in gastric cancer, and detected no association between them, which is in concordance with our findings.²⁸ It appears that as an important Wnt antagonist, *SFRP2* hypermethylation alone cannot affect the Wnt signaling pathway and ultimately β -catenin expression. Ysebaert and others indicated that β -catenin expression did not affect complete remission, but could be considered as an independent prognostic factor for both poor event-free survival and shortened overall survival.³⁰

We showed that β -catenin expression levels increased significantly in the non-M3 AML group by comparison with the control group. In contrast to the study by Ysebaert and others,³⁰ we found a significant difference in the rates of complete remission, but we did not evaluate event-free survival and overall survival in our research. The overexpression of β -catenin is regarded as an independent adverse prognostic factor.³¹ Some studies have suggested that β -catenin inhibition could be a treatment option at least in some subtypes, but it cannot be a universal target in all patients with AML.^{32, 33} These data can be utilized in designing personalized treatment procedures. So, indubitably, further research in this field seems necessary.

Conclusion

Our results showed that alterations in *SFRP2* expression could be used as a potential biomarker for differentiating between patients with *de novo* non-M3 AML and controls. However, its use as a prognostic factor alone requires complete information on relapse and overall survival. Moreover, an evaluation of β -catenin expression

may be valuable in predicting the achievement of complete remission in patients with non-M3 AML. We suggest that further studies be performed on β -catenin at the level of gene and protein expression in patients with AML.

Acknowledgment

This manuscript is a part of a PhD thesis written by Fatemeh Mirzaeian and was approved by the Research Council of Tehran University of Medical Sciences (94-04-31-29976). The authors wish to thank the staff members of the Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, for all their support.

Conflict of Interest: None declared.

References

- Grove CS, Vassiliou GS. Acute myeloid leukaemia: a paradigm for the clonal evolution of cancer? *Dis Model Mech.* 2014;7:941-51. doi: 10.1242/dmm.015974. PubMed PMID: 25056697; PubMed Central PMCID: PMC4107323.
- Siveen KS, Uddin S, Mohammad RM. Targeting acute myeloid leukemia stem cell signaling by natural products. *Mol Cancer.* 2017;16:13. doi: 10.1186/s12943-016-0571-x. PubMed PMID: 28137265; PubMed Central PMCID: PMC5282735.
- Goldman SL, Hassan C, Khunte M, Soldatenko A, Jong Y, Afshinnekoo E, et al. Epigenetic Modifications in Acute Myeloid Leukemia: Prognosis, Treatment, and Heterogeneity. *Front Genet.* 2019;10:133. doi: 10.3389/fgene.2019.00133. PubMed PMID: 30881380; PubMed Central PMCID: PMC6405641.
- Kostroma, II, Gritsaev SV, Sidorova ZY, Tiranova SA, Svitina SP, Drizhun YS, et al. Aberrant methylation of the promoter regions of the SOX7 and p15INK4b genes and Wnt signaling pathway antagonists in patients with acute myeloid leukemias. *Ter Arkh.* 2016;88:31-6. doi: 10.17116/terarkh201688731-36. PubMed PMID: 27459612.
- Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. *Nat Rev Cancer.* 2008;8:387-98. doi: 10.1038/nrc2389. PubMed PMID: 18432252.
- Valencia A, Roman-Gomez J, Cervera J, Such E, Barragan E, Bolufer P, et al. Wnt signaling pathway is epigenetically regulated by methylation of Wnt antagonists in acute

- myeloid leukemia. *Leukemia*. 2009;23:1658-66. doi: 10.1038/leu.2009.86. PubMed PMID: 19387464.
- 7 Grainger S, Traver D, Willert K. Wnt Signaling in Hematological Malignancies. *Prog Mol Biol Transl Sci*. 2018;153:321-41. doi: 10.1016/bs.pmbts.2017.11.002. PubMed PMID: 29389522; PubMed Central PMCID: PMC5972548.
 - 8 Wang H, Fan R, Wang XQ, Wu DP, Lin GW, Xu Y, et al. Methylation of Wnt antagonist genes: a useful prognostic marker for myelodysplastic syndrome. *Ann Hematol*. 2013;92:199-209. doi: 10.1007/s00277-012-1595-y. PubMed PMID: 23093371.
 - 9 Veeck J, Bektas N, Hartmann A, Kristiansen G, Heindrichs U, Knuchel R, et al. Wnt signalling in human breast cancer: expression of the putative Wnt inhibitor Dickkopf-3 (DKK3) is frequently suppressed by promoter hypermethylation in mammary tumours. *Breast Cancer Res*. 2008;10:R82. doi: 10.1186/bcr2151. PubMed PMID: 18826564; PubMed Central PMCID: PMC2614517.
 - 10 Zhan T, Rindtorff N, Boutros M. Wnt signaling in cancer. *Oncogene*. 2017;36:1461-73. doi: 10.1038/onc.2016.304. PubMed PMID: 27617575; PubMed Central PMCID: PMC5357762.
 - 11 Rao TP, Kuhl M. An updated overview on Wnt signaling pathways: a prelude for more. *Circ Res*. 2010;106:1798-806. doi: 10.1161/CIRCRESAHA.110.219840. PubMed PMID: 20576942.
 - 12 Majchrzak-Celinska A, Slocinska M, Barciszewska AM, Nowak S, Baer-Dubowska W. Wnt pathway antagonists, SFRP1, SFRP2, SOX17, and PPP2R2B, are methylated in gliomas and SFRP1 methylation predicts shorter survival. *J Appl Genet*. 2016;57:189-97. doi: 10.1007/s13353-015-0312-7. PubMed PMID: 26337424; PubMed Central PMCID: PMC4830852.
 - 13 Chiurillo MA. Role of the Wnt/beta-catenin pathway in gastric cancer: An in-depth literature review. *World J Exp Med*. 2015;5:84-102. doi: 10.5493/wjem.v5.i2.84. PubMed PMID: 25992323; PubMed Central PMCID: PMC4436943.
 - 14 Ren J, Jian F, Jiang H, Sun Y, Pan S, Gu C, et al. Decreased expression of SFRP2 promotes development of the pituitary corticotroph adenoma by upregulating Wnt signaling. *Int J Oncol*. 2018;52:1934-46. doi: 10.3892/ijo.2018.4355. PubMed PMID: 29620167; PubMed Central PMCID: PMC5919716.
 - 15 Griffiths EA, Gore SD, Hooker C, McDevitt MA, Karp JE, Smith BD, et al. Acute myeloid leukemia is characterized by Wnt pathway inhibitor promoter hypermethylation. *Leuk Lymphoma*. 2010;51:1711-9. doi: 10.3109/10428194.2010.496505. PubMed PMID: 20795789; PubMed Central PMCID: PMC4000011.
 - 16 Cheng CK, Li L, Cheng SH, Ng K, Chan NP, Ip RK, et al. Secreted-frizzled related protein 1 is a transcriptional repression target of the t(8;21) fusion protein in acute myeloid leukemia. *Blood*. 2011;118:6638-48. doi: 10.1182/blood-2011-05-354712. PubMed PMID: 22031861.
 - 17 Tickenbrock L, Schwable J, Wiedehage M, Steffen B, Sargin B, Choudhary C, et al. FIt3 tandem duplication mutations cooperate with Wnt signaling in leukemic signal transduction. *Blood*. 2005;105:3699-706. doi: 10.1182/blood-2004-07-2924. PubMed PMID: 15650056.
 - 18 Jiang J, Griffin JD. Wnt/beta-catenin Pathway Modulates the Sensitivity of the Mutant FLT3 Receptor Kinase Inhibitors in a GSK-3beta Dependent Manner. *Genes Cancer*. 2010;1:164-76. doi: 10.1177/1947601910362446. PubMed PMID: 21779446; PubMed Central PMCID: PMC3092187.
 - 19 Jost E, Schmid J, Wilop S, Schubert C, Suzuki H, Herman JG, et al. Epigenetic inactivation of secreted Frizzled-related proteins in acute myeloid leukaemia. *Br J Haematol*. 2008;142:745-53. doi: 10.1111/j.1365-2141.2008.07242.x. PubMed PMID: 18537968.
 - 20 Naing NN. Determination of sample size. *Malays J Med Sci*. 2003;10:84-6. PubMed PMID: 23386802; PubMed Central PMCID: PMC3561892.
 - 21 Ghasemi A, Rostami S, Chahardouli B, Alizad Ghandforosh N, Ghotaslou A, Nadali F. Study of SFRP1 and SFRP2 methylation status in patients with de novo Acute Myeloblastic Leukemia. *Int J Hematol Oncol Stem Cell Res*. 2015;9:15-21. PubMed PMID: 25802696; PubMed Central PMCID: PMC4369229.
 - 22 Gharagozlou S, Kardar GA, Rabbani H, Shokri F. Molecular analysis of the heavy chain variable region genes of human hybridoma clones specific for coagulation factor VIII. *Thromb Haemost*. 2005;94:1131-7. doi: 10.1160/TH05-06-0445. PubMed PMID: 16411384.
 - 23 Asgarian Omran H, Shabani M, Shahrestani T, Sarafnejad A, Khoshnoodi J, Vossough P, et al. Immunophenotypic subtyping of

- leukemic cells from Iranian patients with acute lymphoblastic leukaemia: association to disease outcome. *Iran J Immunol.* 2007;4:15-25. doi: IJlv4i1A2. PubMed PMID: 17652839.
- 24 Ardestani MT, Kazemi A, Chahardouli B, Mohammadi S, Nikbakht M, Rostami S, et al. FLT3-ITD Compared with DNMT3A R882 Mutation Is a More Powerful Independent Inferior Prognostic Factor in Adult Acute Myeloid Leukemia Patients After Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective Cohort Study. *Turk J Haematol.* 2018;35:158-67. doi: 10.4274/tjh.2018.0017. PubMed PMID: 29786546; PubMed Central PMCID: PMC6110452.
- 25 Shen JZ, Xu CB, Fu HY, Wu DS, Zhou HR, Fan LP. Methylation of secreted frizzled related protein gene in acute leukemia patients in China. *Asian Pac J Cancer Prev.* 2011;12:2617-21. PubMed PMID: 22320963.
- 26 Guo H, Zhang TJ, Wen XM, Zhou JD, Ma JC, An C, et al. Hypermethylation of secreted frizzled-related proteins predicts poor prognosis in non-M3 acute myeloid leukemia. *Onco Targets Ther.* 2017;10:3635-44. doi: 10.2147/OTT.S136502. PubMed PMID: 28790854; PubMed Central PMCID: PMC5530859.
- 27 Guo H, Lin J, Wen XM, Yang J, Qian W, Deng ZQ, et al. Decreased SFRP2 expression is associated with intermediate and poor karyotypes in de novo acute myeloid leukemia. *Int J Clin Exp Pathol.* 2014;7:4695-703. PubMed PMID: 25197341; PubMed Central PMCID: PMC4152031.
- 28 Wang H, Duan XL, Qi XL, Meng L, Xu YS, Wu T, et al. Concurrent Hypermethylation of SFRP2 and DKK2 Activates the Wnt/beta-Catenin Pathway and Is Associated with Poor Prognosis in Patients with Gastric Cancer. *Mol Cells.* 2017;40:45-53. doi: 10.14348/molcells.2017.2245. PubMed PMID: 28152305; PubMed Central PMCID: PMC5303888.
- 29 Zhou H, Mak PY, Mu H, Mak DH, Zeng Z, Cortes J, et al. Combined inhibition of beta-catenin and Bcr-Abl synergistically targets tyrosine kinase inhibitor-resistant blast crisis chronic myeloid leukemia blasts and progenitors in vitro and in vivo. *Leukemia.* 2017;31:2065-74. doi: 10.1038/leu.2017.87. PubMed PMID: 28321124; PubMed Central PMCID: PMC5628102.
- 30 Ysebaert L, Chicanne G, Demur C, De Toni F, Prade-Houdellier N, Ruidavets JB, et al. Expression of beta-catenin by acute myeloid leukemia cells predicts enhanced clonogenic capacities and poor prognosis. *Leukemia.* 2006;20:1211-6. doi: 10.1038/sj.leu.2404239. PubMed PMID: 16688229.
- 31 Gandillet A, Park S, Lassailly F, Griessinger E, Vargaftig J, Filby A, et al. Heterogeneous sensitivity of human acute myeloid leukemia to beta-catenin down-modulation. *Leukemia.* 2011;25:770-80. doi: 10.1038/leu.2011.17. PubMed PMID: 21339756; PubMed Central PMCID: PMC4289854.
- 32 Yeung J, Esposito MT, Gandillet A, Zeisig BB, Griessinger E, Bonnet D, et al. beta-Catenin mediates the establishment and drug resistance of MLL leukemic stem cells. *Cancer Cell.* 2010;18:606-18. doi: 10.1016/j.ccr.2010.10.032. PubMed PMID: 21156284.
- 33 Siapati EK, Papadaki M, Kozaou Z, Rouka E, Michali E, Savvidou I, et al. Proliferation and bone marrow engraftment of AML blasts is dependent on beta-catenin signaling. *Br J Haematol.* 2011;152:164-74. doi: 10.1111/j.1365-2141.2010.08471.x. PubMed PMID: 21118196.