Prognostic Significance of Secreted Frizzled-Related Protein 2 Expression in Cytogenetically Normal Primary Acute Myeloid Leukemia

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Abstract: *Background:* Deregulation of secreted frizzled-related protein 2 (sFRP2) has been found in many types of cancer. However, the pattern of sFRP2 expression in acute myeloid leukemia (AML) is still unclear. *Methods:* This study aimed to validate the prognostic significance of sFRP2 expression in 54 older patients with cytogenetic normal acute myeloid leukemia (CN-AML) using real-time quantitative polymerase chain reaction. *Results:* sFRP2 expression was decreased markedly in patients compared with controls ($P < 0.001$). No correlation was found between sFRP2 gene expression and WBCs, hemoglobin, platelets, FAB type, NMP1 and FLT3/ITD mutations at diagnosis. All patients were treated with standard induction chemotherapy. Patients with high sFRP2 expression had higher incidence of complete remission rate ($P = 0.04$) and better overall survival ($P = 0.026$). Multivariate analysis revealed that high sFRP2 expression was a prognostic factor for older patients with CN-AML. *Conclusions:* This study demonstrated that sFRP2 gene expression at diagnosis had an impact on outcome of elderly CN-AML patients.

Key Indexing Terms: Expression; Prognosis; sFRP2; AML. **[Am J Med Sci 2015;350(5):369–373.]**

Acute myeloid leukemia (AML) is a cytogenetically and molecularly heterogeneous disease characterized by uncontrolled proliferation of abnormal myeloid precursors.[1,2] The prognosis of older patients who aged more than 60 years remains poor, with complete remission (CR) rates of 40% to 60% and long-term overall survival (OS) rates of 5% to 16%.[3–5] The reasons for the poor outcome of this older patient population may not only relay on clinical comorbid conditions, high-risk cytogenetics and poor performance status but also to the presence of specific molecular genetic alterations, including gene mutations and changes in gene expression.[6]

Cytogenetic aberrations have been reported as the most important prognostic factors for survival and response to therapy in AML, allowing the identification of molecular markers that have greatly advanced the understanding of leukemogenesis.[7] These cytogenetic aberration include covalent histone modification pattern, aberrant promoter hypermethylation and miRNA expression.[8] However, patients are usually associated with cytogenetic normal acute myeloid leukemia (CN-AML), which is best characterized molecularly.[1,7] Despite the relatively large number of patients presenting with this feature, few studies have investigated the prognostic significance of molecular markers in this age group.

In recent years, epigenetic disorders including methylation of tumor suppressor genes like secreted frizzled-related proteins (sFRPs) family genes have also been shown to play a role in AML pathogenesis. These alterations may lead to differentiation and apoptosis arrest in leukemic blasts as well as increase in proliferation and self-renewal.[9] Recently, a critical role in adult stem cell biology has been identified for the Wnt pathway, an evolutionarily highly conserved signaling cascade critical for normal hematopoiesis.[10,11]

Wnts regulate multiple signaling pathways through both canonical mechanism (β-catenin dependent) and noncanonical mechanism (β-catenin indepedent).[12] These pathways are critical for cell fate determination, proliferation, migration, polarity and gene expression.[13–15] Activation of the Wnt signaling pathway has been implicated in the pathogenesis of many tumors and in leukemia.[16] Signal transduction by Wnts is tightly regulated by several families of extracellular modulators among which sFRPs are the largest family.[17] Interestingly, previous studies reported that epigenetic silencing of sFRP was associated with aberrant Wnt activation in AML.[18,19] It has been shown also that methylation of Wnt inhibitory genes, including sFRP1, 2, 4, 5 and DKK1, 3, occurs in AML and is associated with adverse prognosis within a subset of newly diagnosed, young patients with intermediate-risk karyotype AML.[20]

Methylation of sFRP2 promoter has been identified as a poor prognostic factor in core binding factor AML.[21] However, the pattern of sFRP2 expression and its prognostic role in AML remain unclear to date. Therefore, this study is aimed to evaluate the clinical significance of sFRP2 expression in older patients with *de novo* CN-AML.

MATERIALS AND METHODS

Patients

sFRP2 was assessed in a cohort of 54 (29 men and 25 women) patients aged 60 years or older with newly diagnosed with CN-AML in the Oncology Center of Mansoura University (OCMU). Normal blood samples included peripheral blood mononuclear cells derived from 35 controls (18 men, 17 women) who were healthy blood donors with normal laboratory findings, and no history of malignancies were selected to closely match the age and sex of the cases. All included, patients were receiving the same treatment protocol approved by the Oncology Team of Mansoura University Hospital. Patients received the standard 3 + 7 induction chemotherapy protocol.[22] Consolidation therapy is composed of one to two courses of cytosine arabinoside. Patients were followed up every three months with clinical examination and regular bone marrow (BM) aspiration every 21 days to confirm remission. All patients were classified according to morphologic and immunophenotypic (FAB) criteria. A panel of monoclonal antibodies designed against myeloid lineage-specific antigens

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including CD33, CD11b, CD15, CD14, CD64, CD117 and MPO and nonspecific antigens including CD34 and HLA-DR based on previous studies was performed.[16,23] The study was approved by the Institutional Review Board at the Mansoura University Hospital. CR was defined as recovery of morphologically normal BM and blood and no circulating leukemic blasts or evidence of extramedullary leukemia. Relapse was defined by ≥5% BM blasts, circulating leukemic blasts or development of extramedullary leukemia.

Methods

Cytogenetic Analysis

BM cells were harvested directly before chemotherapy. The metaphase chromosomes were banded by the G-banding techniques and Karyotype according to the International System for Human Cytogenetic Nomenclature.[24]

Mutational Markers

FLT3 (internal tandem duplications) and NMP1 mutations were analyzed as previously described.[25,26]

FLT3-ITD. Samples from patients were analyzed for mutation in Exons 14, 15 of the FLT3 gene using genomic polymerase chain reaction (PCR) method.[27]

NPM1. Analysis of NPM exon 12 mutations was performed as described by Falini et al.[28]

Quantitative Real-Time PCR

Leukemia peripheral mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation. High-quality RNA was extracted using the Rneasy Mini kit in accordance with the manufacturer's instructions (Qiagen, Valencia, CA). The concentration, the quality and purity of RNA were known by measuring by ultraviolet spectrophotometer at 260/280 nm. cDNA synthesis reaction was performed with 25 μL of total RNA, 2.5 μL reverse transcriptase, 5 μL RT, 4 μL dNTPs, 5 μL random primers and 8.5 of water. Then, it was incubated at 42°C for 1 hour. The reaction was inactivated by heating 95°C for 5 minutes. Real-Time quantitative PCR was performed in a MicroAmp optical 96-well plate with 10 μL of the cDNA solution, 1.0 μL of forward primer, 1.0 μm of reverse primer, 10 μL of dH2O, 0.5 μL of probe and 25 μL of universal master mix (Qiagen). sFRP2 was amplified using the primer pair of 5′-TGAGTGCGACCGTTTCC C-3′ (forward) and 5′-GAGCCA-CAGCACCGATTT-3′ (reverse) with expected products of 298 bp. The expression levels of sFRP2 and the endogenous housekeeping gene ABL as a reference were quantified using quantitative real-time PCR analysis (TaqMan) on an ABI prism 7,700 sequence detection system. The relative expression level of sFRP2 was measured by the comparative cycle threshold method.[29]

Statistical Analysis

SPSS software package (Version 15.0 for Windows; SPSS) was used for calculations. To compare sFRP2 expression between different patient groups; the Mann-Whitney's *U*-test were used. sFRP2 expression was categorized using its median expression among all AML patients as threshold value (0.20). OS was defined as the time interval between the date of diagnosis and the date of death or last follow-up. Disease-free survival (DFS) was defined as the time interval from the date of initial diagnosis to the date of disease progression, death from any cause or last follow-up evaluation. Cox's regression assay was performed including all variables with $P < 0.05$.

TABLE 1. Expression level of sFRP2 in patients and controls

	sFRP2 expression		
	Median	**Range**	***P***
Patients (n = 54)	0.20	0.09–1.24	0.001
Controls (n = 35)	1.30	0.25–3.70	

sFRP2, secreted frizzled-related protein 2.

RESULTS

As shown in Table1, the sFRP2 expression in CN-AML patients ranged from 0.09 to 1.24, with a median value of 0.20, whereas the sFRP2 expression in the control group ranged from 0.25 to 3.70, with a median value of 1.30. The study showed a statistically highly significant decrease in the relative sFRP2 expression in the CN-AML patients compared with the control group with a *P* value of 0.001. The patients were divided into two groups with low or high levels of sFRP2 levels using the median sFRP2 expression value of whole cohort of CN-AML patients (0.20) as cutoff value.

TABLE 2. Pretreatment characteristics in CN-AML patients according to sFRP2 expression levels

	Low sFRP2 (n = 29)	**High sFRP2 (n = 25)**	***P***
Age, yr			
Median	64	62	0.39
Range	60–67	60–66	
Sex			
Male	15	14	0.72
Female	13	12	
Laboratory parameters			
WBCs, $\times 10^9$/L			0.15
Median	25.4	7.6	
Range	1.0–82.4	1.0–69.5	
Hemoglobin, g/dL			0.42
Median	8.6	9.2	
Range	7.5–11.5	8.3–12.7	
Platelets, $\times 10^9$/L			0.58
Median	72.6	70.5	
Range	19.5–295.2	25.0–315.6	
Percentage of BM blast			0.27
Median	52	49	
Range	6–90	7–86	
FAB			
M0	3	2	0.46
M1	5	3	
M2	9	9	
M4	7	6	
M5	5	5	
M6	0	0	
Genetic mutation, n (%)			
FLT3/ITD	5 (17.2)	3 (12.0)	0.62
NPM1	4 (13.7)	3 (12.0)	

BM bone, bone marrow; CN-AML, cytogenetic normal acute myeloid leukemia; sFRP2, secreted frizzled-related protein 2.