New Mutations of the *ID1* Gene in Acute Myeloid Leukemia Patients

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Key Words

Inhibitor of DNA binding 1 protein · Mutation · Acute myeloid leukemia

Abstract

Objectives: Overexpression of the inhibitor of DNA binding 1 (ID1) protein is found in many types of cancer. In acute myeloid leukemia (AML), the expression of ID1 is induced by abnormal tyrosine kinases, such as FLT3 and BCR-ABL. High level expression of ID1 is associated with poor prognosis in young patients. We aimed to explore the ID1 mutation and its prognosis in AML patients. **Methods:** Two hundred and sixty-three AML patients were included. Cytogenetic results and *ID1* mutation were compared. The *ID1* gene was amplified by nested PCR, and the mutation was identified by direct sequencing. **Results:** Four new *ID1* mutations (G40C, A124G, A230G, A349G) were identified in the normal karyotype patients. The A349G mutation, located in the nuclear export signal domain of the ID1 protein, was predicted by the in silico method as a damaged protein. Meanwhile, another new mutation, A290G, found in cases with 11q23 deletion, corresponded to the amino acid 97 in the helix 1 position of the ID1 protein. It could interfere with the dimerization of ID1 and EST-1, leading to a disruption of cell proliferation.

Conclusions: In this study, we found 5 mutations in 260 AML patients. *ID1* mutations were not commonly observed in AML. This may differ in other hematologic malignancies. Further studies in other types of hematologic malignancy will help to clarify the importance of ID1 mutations.

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Introduction

The inhibitor of DNA binding 1 (ID1) protein belongs to a family of helix-loop-helix proteins consisting of ID1, ID2, ID3 and ID4. Normally, ID proteins function as negative regulators of helix-loop-helix transcription factors [1]. Expression of the ID1 protein enhances cell proliferation, regulates cell survival and inhibits cellular differentiation in certain cell types such as fibroblast [2], epidermis [3] and Sertoli cells [4]. The *ID1* gene, located on chromosome 20 at 20q11.21 [5], contains 1,239 bp encoding for 2 exons. In normal adult tissues, the expression level of ID1 can be either absent or low. However, the high expression level of ID1 occurs in a variety of benign and malignant tumors, for example lung cancer [6], breast cancer [7], prostate cancer [8], bladder cancer [9] etc. The increased levels of ID1

expression are correlated with aggressive tumor phenotypes and poor prognosis in non-small cell lung cancer [10] and colorectal cancer [11] and are associated with a shorter overall survival and disease-free survival time in breast cancer [12]. In 2008, Suh et al. [13] demonstrated that the suppression of ID1 expression by small interfering RNA in an in vitro study causes cancer cells to be less invasive and more sensitive to chemotherapeutic drugs. In vivo and in vitro studies of hematopoietic malignancies revealed that ID1 can immortalize myeloid progenitors and promote myeloid proliferation diseases. Additionally, the blocking of the *ID1* gene in premature cells leads to the disruption of B-cell development [13]. Also, ID1 is a downstream target of many known oncogenic tyrosine kinases in hematopoietic malignancies such as BCR-ABL, TEL-ABL, FLT3 and PDGFRB [14]. In several previous studies, the important role of ID1 in hematopoietic malignancies was demonstrated [13]. Especially in acute myeloid leukemia (AML), the deletion of the long arm of chromosome 20 is one of the most common chromosome aberrations observed [15]. AML is a heterogeneous neoplasm caused by gene mutations, chromosomal aberrations and deregulation of gene expression. Hence, we aimed to study the mutation of *ID1* in AML patients in comparison with cytogenetic results. The incidence of *ID1* mutation and its possible protein function were analyzed.

Materials and Methods

Patients

A total of 263 AML patients were recruited in this study (fig. 1). They were all diagnosed as AML cases by the morphological study of bone marrow aspirations. Each sample was collected for routine cytogenetic study in our laboratory. Informed consent was obtained from all patients. This study was approved by the Ethics Committee of the Chulabhorn Hospital.

Cytogenetic Study

The chromosome study was performed on fresh bone marrow specimens by culturing for 24 h in 20% FCS-RPMI medium without mitogen stimulation. The chromosomes were stained by the Q-banding method. In each patient, 20 metaphases were analyzed, if possible, and 2 well-banded metaphases were karyotyped. The chromosomal abnormalities were described according to ISCN 2009.

DNA Preparation

After routine chromosome analysis, the 3:1 methanol acetic acid-fixed cells were stored at -20°C . DNA was extracted from these fixed cells using a cell and tissue kit (Gentra systems, Minneapolis, Minn., USA) following the manufacturer's recommended protocol.

Fig. 1. Flow chart of the classification, chromosome analysis and mutation study of the samples.

Direct Sequencing of the *ID1* Exons

Mutations of the *ID1* gene were studied by using the direct sequencing method. The *ID1* exons were amplified by nested PCR using 2 sets of primers designed in our laboratory. The sequences of the outer primers were 5'-TCTCATTCACGTTCTTAAGTGT-3' (forward) and 5'-CGCTTTTCCAGGCTCCT-3' (reverse). The inner primers were 5'-CGTATCTGCTTCGGGCTTCC-3' (forward) and 5'-CCCCCGCATAGCCAGGTA-3' (reverse). The PCR products were subjected to sequencing by Macrogen Inc., Seoul, Korea. Sequence comparisons and mutation discoveries were carried out using the sequencer software Molecular Evolutionary Genetic Analysis.

Mutation and Functional Assessment

Mutations identified in this study were mapped onto the human genome using the NCBI Reference Sequence NP_002156.2. The mutations were analyzed using PolyPhen [16] to assess the potential impact of amino acid substitution on protein function. This tool was run using the online service on the PolyPhen website (<http://genetics.bwh.harvard.edu/pph/index.html>).

Results

A chromosome analysis was performed in all 263 AML patients. No chromosome results were shown in the 17 patients for whom no metaphases were obtained. The

mean age of these patients was 46.89 years (120 males, 143 females). The mean age of male and female patients was 48.14 and 49.94 years, respectively. The patterns of chromosome abnormalities are summarized in table 1. Of these, 28 patients (17 females, 11 males) were under 18 years of age. The mean age of pediatric AML was 10.69 years in females and 13.91 years in males. Chromosome abnormalities in pediatric AML were found in 47.06% (8/17) of female patients and 63.64% (7/11) of male patients. In adult AML, the abnormal chromosomes were found in 47.62% (60/126) of female patients and 39.45% (43/109) of male patients.

The expected PCR products, 617 bp, were successfully detected for all 263 patients. In total, *ID1* mutations were identified in 2.66% (7/263) of the samples. Two of them were silent mutations which have been previously reported (<http://www.ncbi.nlm.nih.gov/SNP/>). Five new mutations were identified (table 2). When the mutations were found, resequencing of the same specimen with both forward and reverse direction was repeated. The new mutations were found in 4 patients with normal chromosome patterns. Only 1 mutation was found in 1 patient with 11q23 deletion (ID: 467). The 5 new mutations (G40C, A124G, A230G, A290G, A349G) were mapped onto the human genome NCBI Reference Sequence NP_002156.2 database dbSNP (HG19). All of these mutations were located in *ID1* gene coding regions, and all of them were nonsynonymous mutations, identified as new mutations of the *ID1* gene. Furthermore, we analyzed the novel mutations using the PolyPhen software for functional prediction. The software prediction showed that 4 mutations (G40C, A124G, A230G, A290G) are likely to affect the protein function. Only 1 mutation (A349G) has a potential benign activity, as shown in table 2.

Discussion

The relationship between *ID1* expression and progressive cancer development has been shown in several studies. The high levels of *ID1* expression are related to an aggressive phenotype and a poor clinical outcome in many types of cancer [17–19]. Previous observations also showed that *ID1* overexpression was found in the aggressive type of AML [20].

According to the single nucleotide polymorphism database, only few mutations of the *ID1* gene in AML patients have been identified, and therefore we investigated the mutation rate of the *ID1* gene in AML pa-

Table 1. Frequency of common chromosome aberrations in the present study

Chromosome abnormalities ¹	Males	Females
Monosomy 7	5	3
Trisomy 8	8	12
Trisomy 21	3	9
t(8;21)(q22;q22)	8	5
t(9;22)(q34;q11.2)	2	5
Rearrangement of 11q23	3	11
t(15;17)(q24;q21)	3	9
Inv(16)(p12q13)	2	2

¹ Sole chromosome abnormalities.

tients. In our study, mutations of *ID1* in AML patients were found in 2.66% of the samples; 1 of them was with 11q23 deletion. That patient's karyotype was 46,XX,del(11)(q23). Most of the *ID1* mutations were observed in patients with normal karyotype. Five of the 6 mutations were identified as new mutations. Four of these new mutations were nonsynonymous, and they were predicted by a known algorithm to likely alter the protein function. The mutations of the amino acid positions 77 and 97 are located in the helix 1 area of the *ID1* protein [21], while the amino acid position 117 is within the nuclear export signal motif [22]. A study of the *ID1* interaction using the in silico method showed that the H97 has a strong interaction with the K381 of ETS-1 [23], a member of the E26 protein family. The dimerization of *ID1* and ETS-1 shuts down the CDKN2A (p16INK4a) expression. Under normal circumstances, ETS-1 binds to the promoter region of CDKN2A and induces apoptosis. As shown in ovarian cancer, the suppression of CDKN2A expression promotes abnormal cell proliferation [24]. The peptide aptamer *ID1*/3-PA7 was designed to inhibit the binding of *ID1* and ETS-1 and allows the transcription of CDKN2A [23], triggering cell cycle arrest and apoptosis of malignant cells. The characterization of *ID1* mutations may provide more insight into drug interactions in the treatment of cancer [25].

From the above data, we hypothesized that the up-regulated *ID1* gene expression may represent a secondary mechanism to the stimulation of the fusion gene commonly found in AML. Other possible mechanisms may include the mutation of the *ID1* gene itself that could alter the *ID1* protein function. So, the *ID1* gene mutation should be more commonly found in patients

Table 2. Position and type of mutation in the *ID1* gene identified in this study

Sample No.	Sequence	Amino acid change		Type	Nucleotide (protein) position	In silico functional prediction	Karyotype
		ref.	var.				
321	gccgccgcg[g/c]gccccagctgcgcgctg	G [GGC]	R [CGC]	new SNP No. 1	40 (14)	probably damaging	normal
936	gtggcc[a/g]tctcgcgtgcgccggggcgcc	I [ATC]	V [GTC]	new SNP No. 2	124 (42)	probably damaging	normal
431	tgttactcacgctca[a/g]ggagctggtgccacc	K [AAG]	R [AGG]	new SNP No. 3	230 (77)	probably damaging	normal
467	ggagattctccagc[a/g]cgtcatcgactacat	H [CAC]	R [CGC]	new SNP No. 4	290 (97)	probably damaging	del(11)(q23)
873	cggaatccgaagtggga[a/g] cccccgggggccgagggt	T [ACC]	A [GCC]	new SNP No. 5	349 (117)	benign	normal
858	tgtctgtctgagcagag[c/t]gtggcca	S [AGC]	S [AGT]	known SNP	117 (39)	–	normal
1,374	tgtctgtctgagcagag[c/t]gtggcca	S [AGC]	S [AGT]	known SNP	117 (39)	–	normal

with a normal karyotype. This could be confirmed by using experimental functional assays to determine the effect of each mutation. The high expression level of *ID1* may not be an early event in the tumorigenesis pathway.

Nevertheless, *ID1* mutations may not be considered common in AML patients. Most of the *ID1* mutations in this study were found in patients with a normal karyotype. The *ID1* mutation is rare in AML patients, but this

may differ in other hematologic malignancies, including acute lymphocytic leukemia, multiple myelomas and myelodysplasia, and needs to be further clarified.

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