Mutation Analysis of Isocitrate Dehydrogenase in Acute Lymphoblastic Leukemia

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Background: Mutations at arginine 132 of isocitrate dehydrogenase 1/2 (*IDH1/2*) have recently been demonstrated to be recurrent gene alterations in acute myeloid leukemia (AML). Subsequently, this mutation was also found in a variety of other hematologic malignancies, including myelodysplastic syndromes, myeloproliferative diseases, and non-Hodgkin lymphoma. Only a few cases were so far identified in acute lymphoblastic leukemia (ALL). To study the *IDH* status in ALL patients, we analyzed 54 adult and 34 pediatric ALL samples' *IDH1/2* gene. **Results:** Three adult cases and no pediatric case with an isocitrate dehydrogenase 1 (*IDH1*) mutation were identified. No isocitrate dehydrogenase 2 (*IDH2*) mutation was identified in the total of 88 samples. The frequency of the *IDH1* mutation in adult ALL was 5.5%. Among the three *IDH1*-mutated patients, two had normal karyotype and expressed the myeloid lineage markers. All three patients with an *IDH1* mutation relapsed or died within 6 months. **Conclusions:** The results suggested that the *IDH1* R132 mutation might be a recurrent gene alteration in ALL; patients carrying the mutation have a trend to aberrantly express myeloid antigen and the mutation may imply a dismal outcome.

Introduction

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) is a neoplastic disorder that is rapidly fatal if untreated. In children, considerable advances in ALL have resulted in most being cured of their disease. By contrast, the majority of adult patients with ALL are not cured, in part, due to an increased frequency of unfavorable genetic alterations (Mullighan and Downing, 2009). This severe malignancy presents great heterogeneity in genetic profiles, clinical features, response to treatment, and prognosis. The diversity of ALL results mainly from mutant genes that are causally implicated in the pathogenesis of the disease. The full complement of genetic alterations responsible for the pathogenesis of ALL is not yet known and has turned into a hotspot interesting many researchers. In recent decades, progress in understanding the molecular basis of the disease not only provides more clues to clarify the mechanism of leukemogenesis, but also allows a more detailed subclassification and more exact prognostic predictions in many patients. In general, BCR/ABL fusion, *MLL-AF4* fusion, and hypodiploidy (<44 chromosomes per leukemic cell) all confer a poor outcome, whereas hyperdiploidy (>50 chromosomes), *TEL-AML1* fusion, and trisomy 4, 10, and 17 are associated with favorable prognosis (Pui *et al.*, 2008). In addition to predicting prognosis, these gene alter-

ations provide the basis for more targeted, risk-adapted therapeutic approaches as well as the development of novel therapies (Pui *et al.*, 2008). For example, the bcr-abl fusion kinase produced by the BCR-ABL fusion gene is a good target of imatinib (also called Gleevec) in treatment (Druker, 2004). γ -Secretase, a multicomponent membrane-associated enzyme, is needed for NOTCH1 signaling through mutant NOTCH receptors in T-cell acute lymphoblastic leukemia, providing an attractive target for therapeutic intervention with a newly developed γ -secretase inhibitor (Paganin and Ferrando, 2011).

Recently, the newly unraveled *IDH* mutation in leukemia brings one more focus in this field. Isocitrate dehydrogenase 1 (*IDH1*) is a cytosolic protein that catalyzes the NADP⁺-dependent carboxylation of isocitrate into α -ketoglutarate. Recently, mutations at arginine 132 of *IDH1* have been revealed in acute myeloid leukemia (AML) by genome-wide screening (Mardis *et al.*, 2009). The *IDH1* mutation occurred with a frequency of 4.4–9.6% in AML (Mardis *et al.*, 2009; Boissel *et al.*, 2010; Green *et al.*, 2010; Ho *et al.*, 2010; Zhang *et al.*, 2011) and up to 16% in cytogenetically normal AML (Paschka *et al.*, 2010). The mutation was demonstrated to be very stable during disease development and might be a potential marker for residual disease monitoring (Chou *et al.*, 2010). Some reports demonstrated that the mutations

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implicate unfavorable outcome in distinct cytogenetic or genetic subgroups (Mardis *et al.*, 2009; Abbas *et al.*, 2010; Boissel *et al.*, 2010; Green *et al.*, 2010; Marcucci *et al.*, 2010; Paschka *et al.*, 2010), whereas in other studies, *IDH1* mutations had no impact on prognosis (Chou *et al.*, 2010; Wagner *et al.*, 2010).

Isocitrate dehydrogenase 2 (*IDH2*) gene encodes a mitochondrial protein homologous to *IDH1* and also catalyzes isocitrate carboxylation. Subsequent to the detection of the *IDH1* mutation, mutations at arginine 140 and 172 of *IDH2* were also detected in AML (Boissel *et al.*, 2010; Marcucci *et al.*, 2010). *IDH2* mutations occurred with a frequency of up to 11% in AML (Abbas *et al.*, 2010; Paschka *et al.*, 2010; Patel *et al.*, 2011; Zou *et al.*, 2010) and were mutually exclusive with mutations in *IDH1* (Marcucci *et al.*, 2010). Some studies showed that the *IDH2* mutation had no impact on the prognosis (Thol *et al.*, 2010), whereas some other studies provided evidence that the mutation had an unfavorable impact on the complete remission rate and overall survival (Boissel *et al.*, 2010; Marcucci *et al.*, 2010).

In addition to AML, the *IDH* mutation was also found in a variety of hematologic malignancies, including myelodysplastic syndromes (Kosmider *et al.*, 2010), myeloproliferative diseases (Pardani *et al.*, 2010; Tefferi *et al.*, 2010), and non-Hodgkin lymphoma (Zou *et al.*, 2010). So far, only a few *IDH*-mutated cases have been identified in ALL. Kang *et al.* (2009) reported one B-acute lymphoblastic leukemia (B-ALL) case with an *IDH1* R132C mutation (1/60; 1.7%). Another single *IDH1* mutation, also R132C, was detected in an analysis of 288 pediatric ALL samples by Andersson *et al.* (2011). Abbas *et al.* (2010) reported an ALL case with an *IDH2* R140Q mutation (1/96; 1.0%). It seemed that the mutation was rare in ALL. With the aim to study the *IDH* status in ALL patients, we analyzed the *IDH1/2* gene in 54 adult and 34 pediatric ALL patients.

Materials and Methods

Patients

Bone marrow samples were obtained at diagnosis from 54 adult (32 men and 22 women) and 34 pediatric ALL patients (20 boys and 14 girls) enrolled in the Institute of Hematology and Blood Disease Hospital, Chinese Academy of Medical Sciences. The adult patients were aged 15–81 years with a median age of 28. The pediatric patients were aged 17 months to 14 years with a median age of 5 years. Informed consent was obtained from all patients and parents according to the Declaration of Helsinki. This study was approved by the Ethics Committee of our institution. Establishment of diagnosis was based on the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues (Vardiman *et al.*, 2009) and the European Group for the Immunological Classification of Leukaemias (EGIL) (Bene *et al.*, 1995).

Nucleic acid isolation

Bone marrow mononuclear cells (BMMNCs) were isolated by density gradient centrifugation over a Ficoll solution (Invitrogen) from 5 mL fresh bone marrow following the instructions of the manufacturer. Genomic DNA was extracted from BMMNCs by using the TIANamp Genomic DNA Kit (Tiangen Biotech). The concentration of DNA was determined by spectrophotometry (NanoDrop Technologies, Thermo Scientific) at 260 nm.

Mutation analysis

The hotspot mutation at codon 132 of *IDH1* was pre-screened by polymerase chain reaction (PCR) and restriction endonuclease-based detection as previously described (Meyer *et al.*, 2010; Zhang *et al.*, 2011) and identified by sequencing confirmation. PCR amplification of a 166-bp fragment overriding codon 132 was carried out using forward primer 5'-TGGGTAAAACCTATCATCATCGT-3' and reverse primer 5'-TTCATACCTTGCTTAATGGGTGT-3'. The last four nucleotides (CGAT) of the forward primer made up one part of the *PvuI* restriction site (CGATCG), and the first two nucleotides (CG) of the wild-type sequence of *IDH1* R132 made up the other part of the *PvuI* restriction site. Consequently, the PCR product of the wild-type *IDH1* carried a *PvuI* restriction site. If substitution mutation occurred in either of the first two nucleotides of *IDH1* R132, then the *PvuI* restriction site would be destroyed. About 2 μ L of the PCR products was digested for 4 h at 37°C using restriction endonuclease *PvuI* and appropriate buffer (New England Biolabs) in a total volume of 20 μ L. The sizes of the cleaved fragments were 142 and 24 bp. Separation on 3% agarose gels allowed detection of the noncleaved fragment of 166 bp and the larger cleaved fragment of 142 bp, whereas the smaller cleaved fragment of 24 bp was invisible. Complete restriction of the PCR product resulted in a single band of 142 bp. Detection of only one signal of 142 bp indicates that the patient carried two wild-type alleles of *IDH1* (Fig. 1). Detection of two signals of 142 and 166 bp indicates a heterozygote with one each of mutated and wild-type allele (Fig. 1). All mutations were determined consequently by cloning into a pMD18-T vector and sequencing (Fig. 2).

For *IDH2* mutational analysis, DNA fragments covering exon 4 were amplified using the primer pair *IDH2*F: AATTTTAGGACCCCGTCTG/*IDH2*R: CTGCAGAGACAAGAGGATGG. The PCR product was directly sequenced as previously described (Marcucci *et al.*, 2010).

Results

Three cases of *IDH1*-mutated ALL were identified in the total of 54 (5.5%) adult patients and no *IDH1* mutation was detected in pediatric patients. No *IDH2* mutation was identified in either the adult or the pediatric cases. The *IDH1* mutation occurred in 5.3% (2/38) of adult B-ALL and 6.25% (1/16) of adult T-acute lymphoblastic leukemia (T-ALL). All the three mutants were heterozygous (Fig. 1). The mutation types were R132H (CGT > CAT; Fig. 2A), R132G (CGT > GGT;

FIG. 1. Prescreening of *IDH1* R132 mutant by endonuclease digestion. M: DL2000; 1: polymerase chain reaction product without digestion; 2: patient 1; 3: patient 2; 4: patient 3 (the three patients were all heterozygous mutant); 5: wild type.

FIG. 2. Sequences of *IDH1* mutations. (A–C) The mutation types of patient 1, 2, and 3. The sequences in the frames were the mutated codon 132 of *IDH1*. The arrow indicates the single-mutated nucleotide.

Fig. 2B), and R132S (CGT > AGT; Fig. 2C). The clinical characteristics of the three patients were described in Table 1.

Discussion

Researchers are focusing on *IDH* mutation since it has been demonstrated to be recurrent gene alteration in AML (Mardis

et al., 2009). Subsequently, the mutation was also detected in myeloproliferative neoplasm (Pardanani *et al.*, 2010; Tefferi *et al.*, 2010), myelodysplastic syndrome (Kosmider *et al.*, 2010), and non-Hodgkin lymphoma (Zou *et al.*, 2010). Only three *IDH*-mutated cases were so far identified in ALL (Kang *et al.*, 2009; Abbas *et al.*, 2010; Andersson *et al.*, 2011). Our observation confirmed the occurrence of *IDH1* mutation in

TABLE 1. CLINICAL CHARACTERISTICS OF THE *IDH1*-MUTATED ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS

	Patient 1	Patient 2	Patient 3
Sex	Female	Male	Male
Age (years)	44	15	53
Symptoms and signs	Metromenorrhagia, dizziness, fatigue, petechia, splenomegaly	Sore throat, fever, adenopathy of cervical lymph nodes, swelling of tonsils, hepatomegaly	Pale, fatigue, chest pain following exercise, paroxysmal throbbing headache
Diagnosis	Pro-B-ALL	Pre-T-ALL	Common-B-ALL
<i>IDH1</i> R132 mutation type	CGT>CAT	CGT>GGT	CGT>AGT
WBC	325, 380/ μ L	1370/ μ L	2920/ μ L
Hg	80 g/dL	84 g/dL	58 g/L
Plt	23,000/ μ L	117,000/ μ L	92,000/ μ L
Blast % in bone marrow	88%	80%	75%
Immunophenotype	CD19, CD22, CD38, cytTdT	cytCD3, CD7, CD8, cTdT, CD13, CD33, CD34, CD38, CD11b	CD19, CD10, CD79a, CD13, CD33, CD65, and HLA-DR
Karyotype	t(4;11)	Normal	Normal
Gene alteration	MLL/AF4	None	None
LDH (reference range 15,220 U/L)	1134 U/L	204 U/L	286 U/L
DFS (months)	6	3	4
OS (months)	18	4	7

IDH1, isocitrate dehydrogenase 1; WBC, white blood cell; Hg, hemoglobin; Plt, platelet; LDH, lactate dehydrogenase; DFS, disease-free survival; OS, overall survival; B-ALL, B-acute lymphoblastic leukemia; T-ALL, T-acute lymphoblastic leukemia.

ALL. Based on this observation, we predict that additional *IDH*-mutated ALL cases will be reported in further studies and *IDH* mutation may be a recurrent gene alteration in ALL.

As for the method to detect the mutation, there are a multitude of means. The most popular method is direct sequencing (Abbas *et al.*, 2010; Andersson *et al.*, 2011). In addition, single-strand conformation polymorphism-based assays (Kang *et al.*, 2009; Zou *et al.*, 2010) and real-time PCR with post-PCR fluorescence melting curve analysis assays (Lin *et al.*, 2011; Noordermeer *et al.*, 2011) were also used. The immunohistochemistry with the anti-*IDH1* R132H antibody (Kato *et al.*, 2009; Byers *et al.*, 2012) allows the observation of *IDH1* R132H-positive cells in the clinical samples and leads to diffused staining by the antibody in almost every single cell of *IDH1* R132H-positive tumor. The restriction digestion method is less expensive and simple. Direct sequencing is simple and fast. However, the limitation of these two methods is that DNA needs to be extracted from samples containing a high proportion of tumor cells, so that if the *IDH1*-mutated tumor cell load was low, then the mutated sample could not be screened out. Therefore, the sensitivity of these two methods is not satisfied. In comparison to DNA sequencing, the restriction digestion method is not applicable to detect other *IDH1* mutations in exon 4 in addition to R132.

In our observation, two *IDH1*-mutated patients (patient 2 and 3) had normal karyotype and expressed the myeloid antigens CD13 and CD33. Interestingly, the pediatric *IDH1*-mutated case reported by Andersson *et al.* (2011) also had a normal karyotype and aberrant myeloid antigen expression of CD13 and CD33. The expression of the myeloid antigens in the *IDH1* mutation carriers suggested that the leukemia clone of *IDH1*-mutated ALL may come from an early stage of hematopoiesis and *IDH1* mutation may be involved in myeloid differentiation. In previous reports, the *IDH1* mutation was demonstrated to be related to an increase of reactive oxygen species (ROS) (Kolker *et al.*, 2002; Ward *et al.*, 2010). It was speculated that the increase of ROS may disturb the quiescence of the hematopoietic stem cells (HSCs) and act as an intracellular trigger for HSC differentiation to the myeloid lineage fate (Tothova and Gilliland, 2007). The expression of myeloid antigens in *IDH1*-mutated ALL observed in our study supported this speculation, which could partially explain how the *IDH1* mutation contributes to the transformation of HSCs.

In our observation, all of the three *IDH1*-mutated patients relapsed or died within 6 months. Although the limited number of mutation carriers allows no final conclusions regarding the prognostic impact of ALL, we speculated that the mutation may imply a dismal outcome. Large scope studies are needed to verify the prognostic significance of the mutation in ALL.

In summary, the *IDH1* mutations may be a recurrent gene alteration in adult ALL (3/54, 5.5%). The mutations occur in both B-ALL (2/38, 5.3%) and T-ALL (1/16, 6.25%). The *IDH1*-mutated ALL patients may have a trend to carry normal karyotype and express myeloid antigen aberrantly. ALL patients harboring the mutation may have unfavorable prognosis. However, the limited number of the mutation carriers identified in this study warranted strong conclusions, and we are expecting further studies to clarify the role of *IDH* in ALL.

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Author Disclosure Statement

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