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Acquired somatic mutations of isocitrate dehydrogenases 1 and 2 (*IDH1* and *IDH2*) in preleukemic disorders

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ABSTRACT

Mutations of isocitrate dehydrogenase isoform 1 and 2 (*IDH1* and *IDH2*) genes have been identified in glioblastoma and acute myeloid leukemia (AML). However, little is known about the molecular alterations of *IDH* genes in preleukemic disorders with a propensity to transform to AML. We performed polymerase chain reaction–denaturing high performance liquid chromatography (PCR–DHPLC) followed by direct sequencing to detect *IDH* mutations in 237 patients with myeloproliferative neoplasms (MPNs; n = 108), myelodysplastic syndrome (MDS; n = 22), paroxysmal nocturnal hemoglobinuria (PNH; n = 41), and aplastic anemia (AA; n = 66). No *IDH1* R132 and *IDH2* R172 mutations were identified in the entire cohort, whereas *IDH1* G105G allele was detected in 4/108 MPN (3.70%), 2/22 MDS (9.09%), and 2/41 PNH (4.88%) patients. Three *IDH2* R140Q mutations were found in 2/108 MPN (1.85%) and 1/22 MDS (4.54%) patients, while one *IDH2* G145G allele was found in 0.92% (1/108) of MPN patients. Overall, our data suggest that *IDH* mutations are rare in the preleukemic disorders and may not be the major initial step in AML leukemogenesis.

Introduction

Acute myeloid leukemia (AML) is a complex malignant hematologic disease that arises from abnormal expansion and proliferation of unregulated immature hematopoietic cells in the bone marrow [1,2]. Various preleukemic disorders with a transformative potential to evolve into AML are well-recognized, but the exact molecular mechanisms leading to AML development are not yet defined [3–6]. These preleukemic disorders are heterogeneous, including some marrow failure disorders such as aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), and myelodysplastic syndrome (MDS), as well as marrow proliferative disorders, such as chronic myelogenous leukemia (CML) and other myeloproliferative neoplasms (MPNs) [7,8].

Isocitrate dehydrogenases (*IDHs*) are metabolic $NADP^+$-dependent homodimeric enzymes that mainly convert isocitrate into α-ketoglutarate (α-KG) in the Krebs cycle [9]. The *IDH1* isoform is localized in the cytoplasm and peroxisomes, while the *IDH2* and *IDH3* isoforms reside in the mitochondria [10]. Acquired somatic mutations of *IDH1* and *IDH2* genes contribute to abnormal metabolic processes [11–13]. *IDH* mutations have been identified in the majority (>70%) of patients with low-grade gliomas/secondary glioblastoma [14–16], and were recently reported in both de novo AML (5.5–33.0%) [17–21] and secondary AML (sAML) arising from MDS (7.5–25.0%) [22–24] or MPN (9.5–21.6%) [4,7,25]. *IDH* mutations in AML are predominantly associated with normal karyotype (CN-AML) and nucleophosmin (*NPM1*) mutations [18–20,26–30]. The typical *IDH1* mutation affects the evolutionary conserved arginine residue 132 (*IDH1* R132) and the analogous amino acids 172 (*IDH2* R172) and 140 (*IDH2* R140) of the *IDH2* gene [9].

The existence of *IDH* mutations in preleukemic disorders was reported mainly in MDS (3.4–12.3%) and in some cases of MPN (2.6–4.0%), particularly in MPN in blastic transformation [6,7,22,23,25,26,31–37]. The spectrum of *IDH* alterations has been found to be stable during disease evolution in MDS and MPN to sAML [4,7,22–25,32,34]. At present, no study has investigated *IDH* mutations in AA and PNH patients. In the current study, we set out to explore the prevalence of *IDH1* and *IDH2* mutations in various types of preleukemia disorders, including MDS, MPN, AA, and PNH. Clinical and hematologic parameters were also analyzed.

Materials and methods

Preleukemic samples

Preleukemic samples from 237 cases consisting of 108 MPN cases (64 essential thrombocythemia [ET], 36 polycythemia vera [PV], and 8

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primary myelofibrosis [PMF]), 22 MDS, 41 PNH, and 66 AA patients were recruited in the study. Clinical and biological characteristics were collected, including clinical history, complete blood counts, peripheral blood (PB) smear and bone marrow studies. Mononuclear cells were isolated by Ficoll-Hypaque density-gradient centrifugation and subsequently used for molecular analyses. This study was approved by the Ethical Committee for Human Research, Faculty of Medicine Siriraj Hospital, Mahidol University.

IDH mutation identification and analysis

Genomic DNA was extracted using standard phenol–chloroform method or Gentra Puregene Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA amplicons harboring exon 4 of *IDH1* and *IDH2* were amplified by polymerase chain reaction (PCR) using the primer pair as described previously [16]: IDH1f (5′-AGCTCTATATGCCATCACTGC-3′), IDH1r (5′-AACATGCAAAATCACATTATTGCC-3′), IDH2f (5′-AATTTTAGGACCCCCGTCTG-3′), and IDH2r (5′-CTGCAGAGACAAGAGGATGG-3′). PCR reactions were performed in a total volume of 20 μL containing 50 ng of genomic DNA and, PCR master mix consisting of 1× Phusion® HF Buffer (F-520), 200 μM dNTPs, 0.5 μM of each primer, 0.02 U/μL Phusion® DNA polymerase, and Milli-Q water. The PCR was performed in a PerkinElmer Geneamp PCR2400 thermal cycler (Applied Biosystems, Foster City, CA) using the following steps: initial denaturation at 98 °C for 30 s, 35 cycles at 98 °C for 10 s, 60 °C for 30 s and 72 °C for 30 s, and final extension at 72 °C for 5 min [20]. Both amplicons were screened for heterozygous mutations by denaturing high-performance liquid chromatography (DHPLC) on a WAVE 3500HT with DNASep® HT cartridge technology (Transgenomic Inc., Omaha, NE, USA). The optimized condition and temperature were predicted by the Navigator™ software to determine chromatographic peak patterns. A PCR crude sample was injected into the DHPLC column, and the optimal temperature for *IDH1* was 58.5 °C and *IDH2* was 64 °C. Each DHPLC chromatogram was compared with a wild-type reference. The mutational chromatograms were re-amplified in an independent PCR reaction and further subjected to direct sequencing. The sequences were compared with that of the wild-type *IDH1* and *IDH2* cDNAs (GenBank Accession numbers, NM_005896.2 and NM_002168.2, respectively) [28]. *JAK2* mutational analysis was performed according to our previously described method [38].

Statistical analysis

The relationship between *IDH* mutations and various patient characteristics was determined by the student *t*-test, equal variances not assumed for continuous variables, and by Fisher's exact test for categorical variables.

Results

Patterns of DHPLC chromatograms of IDH1 and IDH2 mutations and polymorphisms

Representative abnormal chromatogram patterns that were different from the wild-type profiles by DHPLC are shown in Fig. 1. Three different patterns of abnormal chromatograms were revealed and confirmed by sequencing analysis, including *IDH1* G105G, *IDH2* R140Q, and *IDH2* G145G. The *IDH1* G105G silent polymorphism from GGC to GGT leads to an amino acid substitution at c.315, while the *IDH2* R140Q missense mutation results in a substitution from arginine 140 to glutamine at c.G419A. The *IDH2* G145G silent mutation causes an amino acid exchange from GGG to GGT in codon G145 at c.435, spanning exon 4 on chromosomes 2 and 15. All mutated cases were heterozygous for the mutation.

Frequency and type of IDH1 and IDH2 mutations in preleukemic disorders

In the total cohort of 237 patients, *IDH* missense mutations were found in 1.26% (3/237) of the patients. Three missense *IDH2* mutations restricted at the conserved arginine codon 140 (*IDH2* R140Q) were identified in 2.78% (1/36) of PV patients, 1.56% (1/64) of ET patients, and 4.54% (1/22) of MDS patients. None were detected in PMF, PNH, and AA patients (Table 1). All patients with the missense mutations were heterozygous and retained a wild-type allele. The *IDH1* missense mutation restricted to arginine residue 132 (*IDH1*R 132) was not found in the entire cohort. No *IDH2* R172 missense mutation was identified in the entire cohort, and neither *IDH2* R140Q nor *IDH2* R172 missense mutation was identified in PNH and AA patients. Concurrent *JAK2* V617F mutation and *IDH1* silent mutation (*IDH1* G105G) were found in one *IDH2*-mutated (*IDH2* R140Q) PV patient (female; 56 years old) (Table 2).

Frequency and type of IDH1 and IDH2 polymorphisms in preleukemic disorders

IDH1 and *IDH2* polymorphisms were detected in 3.79% (9/237) and 0.42% (1/237) of the patients, respectively (Table 3). Regarding *IDH1* G105G silent mutations, the highest incidence was detected in MDS patients (9.09%, 2/22) followed by 4.88% in PNH (2/41), and 3.70% in MPN (4/108) patients. Four MPN patients were identified with *IDH1* G105G including 2 PV (5.56%), 1 ET (1.56%), and 1 PMF (12.50%) patients. Furthermore, an *IDH2* G145G silent mutation was detected in one ET patient. No *IDH1* or *IDH2* polymorphisms were identified in AA patients. All clinical and hematologic characteristics of recruited preleukemia patients are summarized in Table 3. Because of the low frequency of *IDH* mutations identified in the entire cohort, we did not statistically calculate any impact in terms of gender, age, hemoglobin or hematocrit levels, white blood cell counts, and platelet counts.

Discussion

Although previous studies have conducted *IDH* mutational analysis of MPN and MDS patients, no reports have ever addressed such mutations in PNH and AA patients. In the present study, we developed a screening DHPLC method followed by sequencing analysis to explore the presence of *IDH* mutations in preleukemic disorders, i.e., MPN, MDS, PNH, and AA patients. The minimal sensitivity of abnormal DHPLC peaks was detected in 3.3–5.0% of the patients, which was higher than genomic DNA direct sequencing (10%). Moreover, we recruited the largest population of PNH and AA patients to detect *IDH* missense mutations. We identified a low occurrence of *IDH* mutations (1.26%, 3/237) in the patients, which was restricted to the *IDH2* R140Q mutation in 2.78% (1/36) of PV, 1.56% (1/64) of ET, 4.54% (1/22) of MDS patients, and none in PMF, PNH, and AA patients. Neither *IDH1* R132 nor its analog residue, *IDH2* R172 mutation, was detected in the entire cohort.

Prior reports from Asia conducted in China and Japan [6,32–34], along with Western reports from the USA [7,25,31,35,37], France [22], and Germany [23,39] are summarized in Table 4. The overall frequency of *IDH* mutations was very low, varying between 0–4.2% for *IDH1* and 0–9.4% for *IDH2*, respectively, in most MPN and MDS Chinese and Western reports. We could have failed to identify *IDH1* missense mutations in our population (0%) because of the small number of MDS patients (22 cases). The frequency of *IDH2* mutations in our cases (1.26%) was also lower than the Chinese/Japanese MDS cases, with a report frequency of 3.4%–6%. The frequency discrepancies among various studies may reflect the variable inclusion criteria of the study samples, the variable sensitivity of the detection assays, the selective inclusion or exclusion of certain *IDH* aberrations, or the true racial difference.

Nevertheless, the fact that no *IDH1* missense mutation was detected in the entire series, the *IDH1* polymorphism provided new information. One silent mutation (*IDH1* G105G) was identified in PMF (12.5%, 1/8),