Biochemical and Biophysical Research Communications 402 (2010) 378–383

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

IDH1 and *IDH2* mutations are frequent in Chinese patients with acute myeloid leukemia but rare in other types of hematological disorders

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ARTICLE INFO

Article history:
Received 5 October 2010
Available online 12 October 2010

Keywords:
IDH1
IDH2
Mutation
Hematological malignancies
Chinese

ABSTRACT

Frequent mutations in the isocitrate dehydrogenase 1 and 2 genes (*IDH1* and *IDH2*) have been identified in gliomas and acute myeloid leukemia (AML). Our aim is to assess whether *IDH* mutations were presented in Chinese patients with various hematological disorders. In this study, we screened the *IDH1* and *IDH2* mutations in a cohort of 456 Chinese patients with various hematological malignancies and disorders. We found three missense (p.R132C, p.R132G, and p.I99M; occurred in five patients) and one silent mutation (c.315C>T; occurred in two patients) in the *IDH1* gene and two missense mutations (p.R140Q and p.R172K; occurred in four AML patients) and one silent mutation (c.435G>A) in the *IDH2* gene. Except for one non-Hodgkin lymphoma (NHL) patient harboring *IDH1* mutation p.R132C, all *IDH1* and *IDH2* missense mutations were observed in patients with AML. Intriguingly, the *IDH2* mutation p.R140Q and novel *IDH1* mutation p.I99M co-occurred in a 75-year-old patient with AML developed from myelodysplastic syndromes (MDS). The frequency of *IDH1* and *IDH2* missense mutations in Chinese AML patients reached 5.9% and 8.3%, respectively. Our results supported the recent findings that *IDH* gene mutations were common in AML. Conversely, *IDH* mutations were rather rare in Chinese patients with other types of hematological disorders.

1. Introduction

Hematological malignancies are a heterogeneous group of malignant neoplasms that are originated from bone marrow. They are comprised of distinct subtypes of leukemia, lymphoma and multiple myeloma (MM), which all together caused approximately 480,000 deaths per year worldwide [1]. To date, it is generally accepted that genetic factors play important roles in the pathogenesis of hematological malignancies [2]. A great number of genetic alterations have been suggested to be associated with the development of hematological malignancies and this have greatly improved the diagnosis, prevention, and treatment of such disorders [2,3]. Nevertheless, there are many cases with hematological malignancies that do not harbor any of the well-established cancer-related genetic alterations, emphasizing the necessity of identifying new potential genes responsible for these disorders.

Recently, frequent point mutations in the evolutionarily conserved 132nd codon in exon 4 of the *IDH1* gene were reported in central nervous system tumors and AML [4,5]. Subsequent studies confirmed these pioneer observations and such mutations were also found in acute lymphoblastic leukemia (ALL), myeloproliferative neoplasms (MPN), and myelodysplastic/myeloproliferative disease (MDS/MPD) patients at variable frequencies [6–12]. Till now, a total of 13 types of *IDH1* mutations were identified (c.211G>A, p.V71I; p.G97D; c.315C>T; c.367G>A, p.G123R; c.368C>A, p.G123E; c.392_396delinsTCCTG, p.G131_R132delinsVL; c.395G>A, p.R132H; c.394C>T, p.R132C; c.394C>A, p.R132S; c.394C>G, p.R132G; c.395G>T, p.R132L; c.394_395delinsGT, p.R132V; and p.R132P). Empirically, for central nervous system tumors or certain types of hematological malignancies, samples lacking *IDH1* p.R132 mutations often harbored mutations in the homologous residues p.R140 and p.R172 in the *IDH2* gene [7,9,10,13–15]. Until now, almost all of the reported mutations in the *IDH1* and *IDH2* genes were heterozygous, with sporadic exceptions in AML patients [16].

Functional assays suggested that the *IDH2* mutations p.R140 and p.R172 shared identical mechanism of carcinogenesis as that of the *IDH1* p.R132 mutations. Namely, these mutations impaired the ability of substrate affinity and cells acquired a novel function that converting alpha-ketoglutarate (α-KG) to 2-hydroxyglutarate (2-HG) in the presence of mutations; the accumulation of

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doi:10.1016/j.bbrc.2010.10.038

cancer-associated 2-HG contributed to the development of neoplasm [10,15,17].

In the present study, we analyzed a cohort of 456 Chinese samples with various types of hematological disorders for the presence of the *IDH1* and *IDH2* mutations. In addition, 270 Chinese normal controls were also recruited to detect *IDH1* mutations. There are two aims of this study: (1) to discern whether mutations in *IDH* genes are common in Chinese patients with AML and (2) to detect whether the *IDH* mutations occur in Chinese patients with other types of hematological disorders.

2. Materials and methods

2.1. Sample collection

Bone marrow or blood specimens from a total of 456 cases with hematological disorders were collected from the First Affiliated Hospital of Kunming Medical College and Zhongshan Hospital, Fudan University in China. These samples contained distinct types of hematological malignancies that were composed of non-Hodgkin lymphoma (NHL, $N = 122$), Hodgkin lymphoma (HL; $N = 10$), AML ($N = 68$), chronic myeloid leukemia (CML; $N = 24$), ALL ($N = 19$), chronic lymphoblastic leukemia (CLL; $N = 11$), acute mixed lineage leukemia (AMLL; $N = 3$), MM ($N = 62$), MDS/MPD ($N = 19$); in addition, 118 patients with non-neoplastic hematological disorders were also included (Table 1). A collection of 270 Chinese healthy individuals from Zhejiang Province, China, were also screened for the *IDH1* mutations, to test whether these mutations exist in the general Han Chinese population. The study conformed to the tenets of the Declaration of Helsinki and written informed consent was obtained from each donor prior to the study. The institutional review boards of the First Affiliated Hospital of Kunming Medical College, Zhongshan Hospital and Kunming Institute of Zoology approved this study.

2.2. DNA isolation and PCR

Genomic DNA was isolated from peripheral blood or bone marrow cells, using standard phenol–chloroform method or Takara DNA extraction kit (TaKaRa Bio, Inc., Dalian, China) according to the manufacturer's protocol. For *IDH1* gene, a PCR fragment of 269 bp spanning the codon 132 of the *IDH1* gene was amplified using the primer pair hIDH1f (5′-TGCTGCAGAAGCTATAAAGAAG-3′ [18])/IDH1r (5′-GCAAAA TCACATTATTGCCAAC-3′). For *IDH2* gene, a PCR fragment covering the entire exon 4 was amplified using the primer pair IDH2fc (5′-GCT GCA GTG GGA CCA CTA TT-3′)/IDH2rc (5′-TGT GGC CTT GTA CTG CAG AG-3′). Both PCR reactions were performed in a reaction volume of 30 μL using the following conditions: a denaturation cycle at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, and a final extension at 72 °C for 7 min.

2.3. Mutational analyses for the IDH1 and IDH2 genes

The purified PCR products were sequenced using the amplification primers and the Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA) on an ABI Prism 3730 DNA sequencer (Applied Biosystems). We optimized a SSCP method to screen the *IDH1* mutations in the samples using the mutant identified by sequencing as the positive control. PCR-SSCP was performed according to previously described protocol with minor revision [19]. Samples showing abnormal bands compared to wild type were sequenced to confirm the presence of mutations. In each run, *IDH1* PCR products with the wild type and the mutant type were used as controls. All abnormal bands detected by the PCR-SSCP method were validated to harbor mutations by direct sequencing (Fig. 1A). For *IDH2*, samples were screened by direct sequencing. Each mutation was confirmed by bidirectional sequencing (Fig. 1B).

Evolutionary conservation analysis of mutations in both *IDH1* and *IDH2* genes was performed using eight species, including *Homo sapiens* (*IDH1*, GenBank accession No. NM_005896; *IDH2*, NM_002168), *Pongo abelii* (*IDH1*, NM_001131309; *IDH2*, XM_002825816), *Rattus norvegicus* (*IDH1*, NM_031510; *IDH2*, NM_001014161), *Mus musculus* (*IDH1*, NM_001111320; *IDH2*, NM_173011), *Bos taurus* (*IDH1*, NM_181012; *IDH2*, NM_175790), *Xenopus laevis* (*IDH1*, NM_001094553; *IDH2*, NM_001086852), *Danio rerio* (*IDH1*, NM_201499; *IDH2*, NM_199564), and *Saccharomyces cerevisiae* (*IDH1*, NM_001180125; *IDH2*, NM_001182061) from GenBank.

Table 1
Distribution of the *IDH1* mutations in Chinese patients with hematological disorders and healthy control samples.

Disease	No.	p.I99M	c.315C>T	p.R132C	p.R132G	Total number of mutation	Missense mutation (%)	Wild type
Neoplastic samples								
NHL	122	0	0	1	0	1	1 (0.8%)	121
HL	10	0	0	0	0	0	0	10
AML	68	1	1	2	1	5	4 (5.9%)	63
CML	24	0	0	0	0	0	0	24
ALL	19	0	0	0	0	0	0	19
CLL	11	0	0	0	0	0	0	11
AMLL	3	0	0	0	0	0	0	3
MM	62	0	0	0	0	0	0	62
MDS/MPD	19	0	0	0	0	0	0	19
Total	338	1	1	3	1	6	5	332
Non-neoplastic samples								
Nutritional anemia	57	0	1	0	0	1	0	56
Aplastic anemia	8	0	0	0	0	0	0	8
Idiopathic thrombocytopenic purpura	45	0	0	0	0	0	0	45
Leukopenia	6	0	0	0	0	0	0	6
Castleman's disease	2	0	0	0	0	0	0	2
Total	118	0	1	0	0	1	0	117
Healthy control samples	270	0	0	0	0	0	0	270

NHL: non-Hodgkin lymphoma; HL: Hodgkin lymphoma; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; ALL: acute lymphocytic leukemia; CLL: chronic lymphocytic leukemia; AMLL: acute mixed lineage leukemia; MM: multiple myeloma; MDS/MPD: myelodysplastic/myeloproliferative diseases; Nutritional anemia: includes iron deficiency anemia and pernicious anemia.

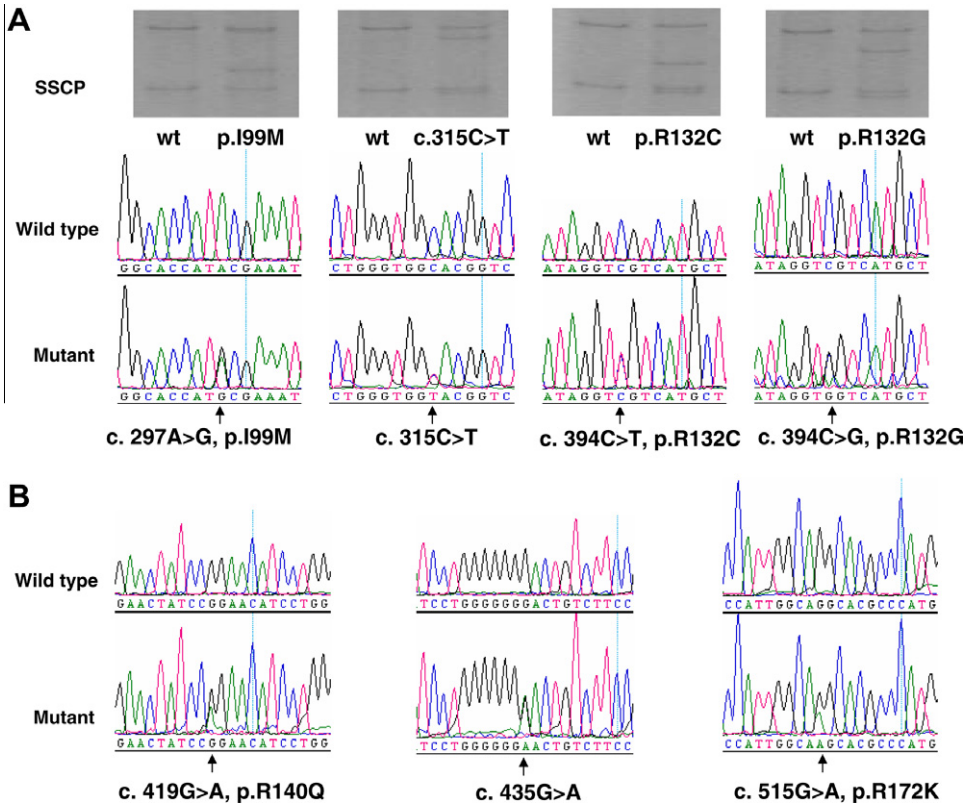

Fig. 1. Representative genotyping results for the *IDH* mutations. (A): PCR-SSCP and sequencing chromatograms of four types of *IDH1* mutations. "wt" refers to wild type *IDH1*. The arrows indicated the mutated positions. (B): Representative sequencing chromatograms of three types of *IDH2* mutations.

3. Results

3.1. IDH1 mutations in hematological disorders

A total of 456 cases with various hematological disorders and 270 normal controls were screened for the presence of *IDH1* mutations. Three non-synonymous substitutions and one synonymous substitution were identified in the *IDH1* gene in patients but not in controls. All mutations were heterozygous (Fig. 1A). The mutation type and its frequency distribution were shown in Table 1. Among the three missense mutations, p.R132C was found in two AML patients and one NHL patient. The NHL patient was a 26-year-old woman with extranodal NK/T cell lymphoma (nasal type), and she had no family history of lymphoma or other tumor type. Mutation p.R132G was found in one AML patient; the novel mutation (c.297A>G, p.I99M) was observed in one patient with AML transformed from MDS (Fig. 1A). Interestingly, four out of the five patients with the missense mutations had AML and the overall frequency of missense mutations in AML patients reached 5.9% (4/68). The synonymous substitution (c.315C>T; rs11554137) was previously described and was found in one AML patient and one patient with anemia. No *IDH1* mutation was observed in patients with other hematological disorders and in 270 control samples.

Evolutionary conservation analysis showed that the *IDH1* mutation p.I99M was highly conserved among the eight species ranging from *Homo sapiens* to *Saccharomyces cerevisiae* (Fig. 2A).

3.2. IDH2 mutations in hematological disorders

We sequenced the whole *IDH2* exon 4 in 332 patients with hematological disorders (246 neoplastic and 86 non-neoplastic samples) that were randomly selected from 456 cases. The mutation type and frequency distribution of the *IDH2* mutations were shown in Table 2. A total of three heterozygous mutations were detected, including two missense mutations (c.419G>A, p.R140Q and c.515G>A, p.R172K) and one novel silent substitution (c.435G>A) (Fig. 1B). Both mutations p.R140Q (c.419G>A) and p.R172K (c.515G>A) occurred in two AML patients. The overall frequency of missense *IDH2* mutations in Chinese patients with AML was considerably high (4/48, 8.3%). The novel synonymous substitution c.435G>A was identified in one patient with anemia. It is noteworthy to mention that the novel *IDH1* mutation p.I99M and the *IDH2* mutation p.R140Q co-occurred in a 75-year-old patient with AML that was transformed from MDS.

4. Discussion

Since the initial report for the presence of *IDH1* gene mutations in glioblastoma multiforme [4], extensive screening for mutations in this gene has been carried out for solid tumors and liquid tumors [6–16]. Acquired mutations in exon 4 of both *IDH1* and *IDH2* genes (*IDH2* p.R140, *IDH1* p.R132 and its analogous *IDH2* p.R172) were recently reported in patients with AML, ALL, MPN, and MDS/MPD, albeit with different frequency [5–7,10,11,13–16,20–27]. Subsequent studies have tried to relate the occurrence of the *IDH* mutations with the progression [18,28] or prognosis [25,29,30] of the disease and to retrieve essential information for curing the patients with potential personnel medicine. The available data have depicted the potential role of these *IDH* mutations during carcinogenesis: they impair the ability of substrate affinity of the enzymes and lead to an acquired novel function that converting α-KG to 2-HG. The resultant accumulation of 2-HG contributes to the development of cancer [10,15,17]. However, the exact mechanism of how IDH mutations can cause leukemia and brain tumors - and

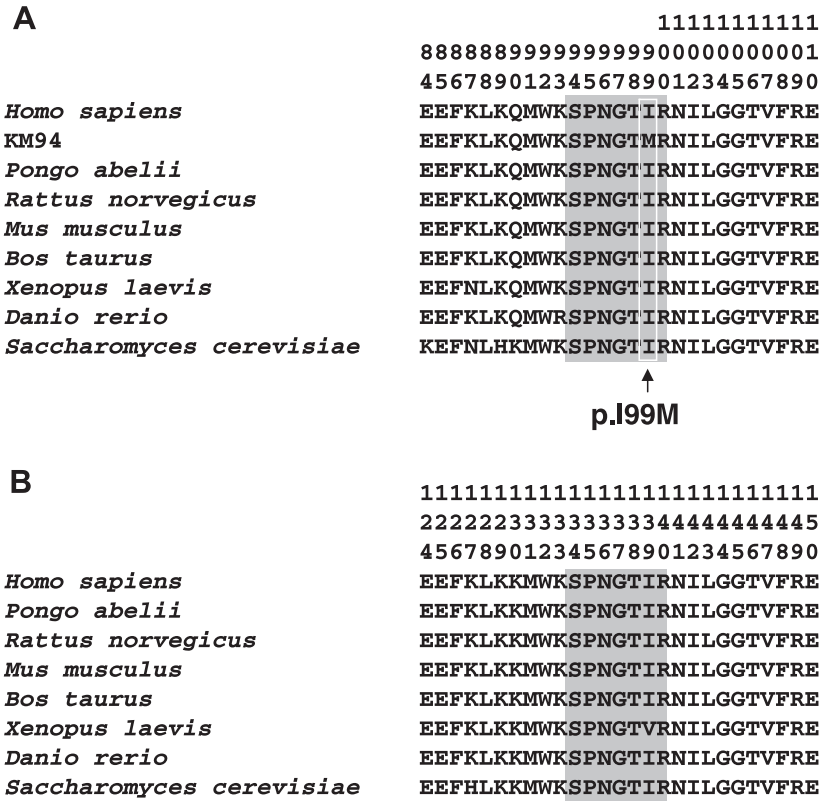

Fig. 2. Evolutionary analysis of *IDH1* mutation p.I99M and the substrate binding pockets of isocitrate in *IDH1* and *IDH2*. Patient "KM94" has *IDH1* p.I99M. The binding sites of isocitrate (marked in grey color) in *IDH1* (A) and *IDH2* (B) are located in the 94th–100th residues and the 134th–140th residues, respectively.

Table 2
Distribution of the *IDH2* mutations in Chinese patients with hematological disorders.

Disease	No.	p.R140Q	c.435G>A	p.R172K	Total number of mutation	Missense mutation (%)	Wild type
Neoplastic samples							
NHL	89	0	0	0	0	0	89
HL	7	0	0	0	0	0	7
AML	48	2	0	2	4	4 (8.3%)	44
CML	21	0	0	0	0	0	21
ALL	13	0	0	0	0	0	13
CLL	6	0	0	0	0	0	6
AMLL	3	0	0	0	0	0	3
MM	47	0	0	0	0	0	47
MDS/MPD	12	0	0	0	0	0	12
Total	246	2	0	2	4	4	242
Non-neoplastic samples							
Nutritional anemia	38	0	1	0	1	0	37
Aplastic anemia	7	0	0	0	0	0	7
Idiopathic thrombocytopenic purpura	36	0	0	0	0	0	36
Leukopenia	5	0	0	0	0	0	5
Castleman's disease	0	0	0	0	0	0	0
Total	86	0	1	0	1	0	85

NHL: non-Hodgkin lymphoma; HL: Hodgkin lymphoma; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; ALL: acute lymphocytic leukemia; CLL: chronic lymphocytic leukemia; AMLL: acute mixed lineage leukemia; MM: multiple myeloma; MDS/MPD: myelodysplastic/myeloproliferative diseases; Nutritional anemia: includes iron deficiency anemia and pernicious anemia.

whether there is a difference between these two processes - is still not fully understood.

In this study, we analyzed 456 Chinese patients with various hematological disorders for the presence of *IDH1* and *IDH2* mutations. We detected a total of five missense (3 in IDH1 and 2 in IDH2) and two silent mutations (1 in IDH1 and 1 in IDH2). Nearly all the patients with the *IDH* gene mutations had AML. Among these missense mutations, only mutation p.I99M in *IDH1* was previously unreported and it co-occurred with *IDH2* mutation p.R140Q in a patient with AML that was developed from MDS. We speculated that the novel IDH1 p.I99M might be deleterious, as this mutation was next to the IDH1 p.R100. The latter one was said to share the identical role as its analogous residue of IDH2 p.R140 in tumorigenesis according to the structural modeling for IDH1 [15,31]. Both IDH1 p.I99 and IDH1 p.R100 are located in the substrate binding pocket of isocitrate, an evolutionarily con-

served domain that is composed of the 94th–100th residues (Fig. 2A) and corresponds to the homologous region of 134th–140th in IDH2 (Fig. 2B) [31] (http://www.uniprot.org/). Of note, the IDH1 97th codon, which is also located in the binding pocket of isocitrate, was mutated (p.G97D) in two colorectal cancer cell lines which were probably derived from the same patient [12]. Therefore, we speculated that *IDH1* p.I99M mutation might promote tumorigenesis in this patient. The co-existence of mutations p.I99M in IDH1 and p.R140Q in IDH2 might account for the complex clinical syndromes observed in this patient with AML that was developed from MDS. The previously reported cancer-related *IDH* mutations involved in arginine (IDH1 p.R132, IDH2 p.R140 and p.R172), and these mutations contributed to the accumulation of 2-HG [10,15,17]; however, it remains elusive whether the IDH1 mutation p.I99M share the same mechanism as these arginine mutations. Functional assay for mutation p.I99M is ongoing in our laboratory.

The frequency of the *IDH1* mutations in patients with AML varied in previous studies, ranging from 0% to 13.1% [5,10,13,22,24–26,32–34]. One of the reasons for this difference is patient ethnicity [26]. The frequency of *IDH1* missense mutations in our AML samples was 5.9% (4/68), essentially same as that of Han Chinese from Taiwan Province, China (5.5%; 27/493) [22]. The high prevalence of *IDH1* mutations in Han Chinese from Taiwan and Mainland China indicated that *IDH1* mutations were actively participated in the pathogenesis of AML.

To date, *IDH1* mutations have been observed in several types of hematological malignancies, including AML, ALL, MPN, and MDS/MPD, but not observed in patients with other hematological disorders [5–7,10,11,13–15,20,22–27,32,33]. Most recently, Kim et al. [35] screened 131 Korean patients with NHL but failed to detect any IDH1 p.R132 mutation. In contrast, we found 1 out of 122 NHL patients with *IDH1* mutation p.R132C (1/122, 0.8%). This is, to the best of our knowledge, the first observation for *IDH1* mutation in patient with NHL. It should be noted that the *IDH* mutation frequency in our patients with NHL and HL might be underestimated because we did not analyze the malignant lymph node samples. The exact reason why the same *IDH1* mutation leads to different hematological malignancies remains unknown.

The frequency of *IDH2* mutations in AML patients varied in different studies, with a frequency ranging from 1.4% to 23.4% [7,10,15,16,25,32,33]. We combined all the published data [7,10,15,16,25,32,33] and found that the frequency of *IDH2* mutations reached 9.3% (231/2476) in the reported AML patients. In this study, we observed four patients with *IDH2* missense mutations in 48 AML patients (4/48, 8.3%). The frequency of the *IDH2* mutations in our samples was slightly lower than that of previously reported patients. Similarly, the high frequency of *IDH2* mutations in Chinese AML patients confirmed the notion that IDH2, like IDH1, played an important role in the development of AML.

Previous studies have found *IDH* mutations in patients with ALL [6] or MDS/MPD [11,13,27,32,36]. In contrast, we failed to detect any *IDH* mutation in our patients with ALL and MDS/MPD (Tables 1 and 2). This result indicated the complexity of pathogenesis in ALL and MDS/MPD, or alternatively caused by relatively small sample size analyzed in our study. The *IDH* mutation was not found in patients with MM, CML, or CLL in previous studies [6,9]. Similarly, we did not detect any *IDH* mutation in our patients with MM, CML, or CLL, as well as, in patients with other non-neoplastic hematological disorders. Taken together, our results seemed to suggest that *IDH* mutation might not be actively involved in the development of these hematological diseases. Again, this claim should be treated with caution due to the small cohort of patients.

5. Conclusion

We screened the *IDH* mutations in a large cohort of Chinese patients with different types of hematological malignancies and disorders. We found that *IDH* mutations were common in Chinese patients with AML and this result was consistent with previous observations. We identified *IDH1* p.R132C mutation in a patient with NHL and a co-occurrence of mutations p.I99M in IDH1 and p.R140Q in IDH2 in a patient with evolved hematological malignancies. Further studies with more patients and functional assay, as well as, association with clinical data are essential to decipher the enigmatic role of *IDH* mutations in the development of hematological malignancies.

Acknowledgements

We thank the sample donors who participated in this study. We are grateful to the members of Yao's lab for helpful discussion. This study was supported by the National Natural Science Foundation of China (30925021 and 81060046), Yunnan Province (2009CI119), and the Chinese Academy of Sciences.

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