To the editor:

A single-tube, sensitive multiplex method for screening of isocitrate dehydrogenase 1 (IDH1) mutations

In the 8 April 2010 issue of Blood, we reported characterization of IDH1 mutation in a large cohort of acute myeloid leukemia (AML) patients.1 Very recently, a series of reports about this mutation in AML have been published in a cluster of manuscripts.1-7 As IDH1 mutation brings prognostic information in glioma and possibly AML,4,8-9 identification of IDH1 R132 mutations will bring increasing clinical relevance. Moreover, the mutation seems quite stable and may serve as a marker for monitoring minimal residual disease.1 Hence, a sensitive and simple method for detecting this mutation will be highly desirable. We here report a very sensitive, single-tube, multiplex polymerase chain reaction (PCR)–based method, which has been verified by direct sequencing method. With this method, we then determined the stability of the R132 mutation in sequential samples of AML and measured the incidence of this mutation in a cohort of patients with myelodysplastic syndrome (MDS). This study has been approved by the Institutional Review Board of the National Taiwan University Hospital.

We designed 6 forward mutation-specific primers to cover all possible types of R132 mutations (Figure 1A), although only 5 of them had been reported until now.1-7 Another upstream forward primer was used to generate a product as an internal control. Combination of these 7 forward primers and a common reverse primer would generate a shorter mutant and a longer control product in any genomic DNA containing any type of R132 mutation, whereas only the longer product would be seen in samples without this mutation (Figure 1B). PCR was performed by heating at 95°C for 10 minutes, followed by 45 cycles of 95°C, 62°C, and 72°C for 30 seconds each in a total of 25 μL containing 200nM each dNTP, 1.5mM MgSO4, 50 ng of genomic DNA, 100nM upstream forward primer, 500nM each of the other 7 primers, and 1.5 units of PlatinumTaq polymerase (Invitrogen). A single tube combining 8 primers would be easy, economic, and fast in screening any possible type of IDH1 R132 mutation.

We verified the utility of this multiplex method in 103 AML marrow samples previously studied by PCR and direct sequencing.1 Such selection is based solely on availability of samples, without consideration of any other factors. Comparison of this multiplex PCR with direct sequencing revealed perfect concordance except for 1 patient whose IDH1 mutation was detected only by multiplex PCR but not by direct sequencing (FL91 in Figure 1C). Furthermore, in 9 patients with IDH1 mutation at diagnosis the same mutation could be detected by multiplex method in all 11 samples obtained at disease relapse, including the 4 in which mutant signals were no longer seen by sequencing (Figure 1D and data not shown). The sensitivity of this method was approximately 0.5%, obviously higher than sequencing (Figure 1E). We also

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**Figure 1.** A multiplex, allele-specific PCR for rapid and sensitive screening of IDH1 R132 mutations. (A) The sequences of the primers. The 3rd last nucleotide was intentionally mutated to avoid background signals (italic and underlined). The last nucleotide matches only to mutant but not wild-type sequences (italic). Wild-type sequence was shown for reference. (B) Schematic representation of our multiplex, allele-specific PCR strategy. The red vertical bar represents the site of R132 mutation. (C) Multiplex PCR on genomic DNA from bone marrow cell of AML patients. The top band (arrow) indicated an internal control band. The bottom band (arrowhead) represented the mutant signal. Samples w291 and FL94 were positive for IDH1 mutation in both sequencing and multiplex methods. FL91 was negative by sequencing but yielded a mutant band in multiplex PCR. 293T represented a complex genomic DNA from this cell line without IDH1 mutation and served as a negative control. Results from other samples were not shown. (D) Mutant signals were evident in patients’ DNA diluted with 293T genomic DNA up to 200-fold (top panel). On the other hand, the mutant signal was barely seen in direct sequencing at 20-fold dilution (bottom panel). In our previous report, only 5 types of IDH1 mutation were seen (no R132P).1 (Top panel) One, 10, and 200 mean dilution fold of mutant genomic DNA with 293T DNA. WT indicates 293T genomic DNA alone.
screened 113 patients with MDS (22 refractory anemia [RA], 9 RA with ring sideroblasts, 19 RA with excess blasts-1 [RAEB-1], 11 RAEB-2, 26 RAEB with transformation, and 26 chronic myelomonocytic leukemia) by this method and found 3 (2.7%; 2 RAEB with transformation and 1 RAEB-2) bearing this mutation, which was then confirmed by direct sequencing. Thus, we provide a quick, economic, and sensitive method for screening and monitoring minimal residual disease of IDH1 R132 mutations, and conclude that this mutation is quite stable during disease evolution in AML and is rare in MDS.

Wen-Chien Chou
Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan

Yen-Ning Huang
Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan

Chi-Fei Huang
Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan

Mei-Hsuan Tseng
Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan

Hwei-Fang Tien
Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan

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Correspondence: Hwei-Fang Tien, Division of Hematology, Department of Internal Medicine, National Taiwan University Hospital, No. 7, Chung-Shan S Rd, Taipei, Taiwan, 100; e-mail: hftien@ntu.edu.tw; or Wen-Chien Chou, Department of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, No. 7, Chung-Shan S Rd, Taipei, Taiwan, 100; e-mail: wchou@ntu.edu.tw.

References


To the editor:

**Wnt3a nonredundantly controls hematopoietic stem cell function and its deficiency results in complete absence of canonical Wnt signaling**

Hematopoietic stem cells (HSCs) reside in specialized niches that provide signals regulating stem cell function and fate decisions. The canonical Wnt signaling pathway has been implicated in this process, but the role of specific Wnt proteins and possible functional redundancy has remained elusive.1

We recently investigated hematopoiesis in Wnt3a-deficient mice.2 Due to early embryonic lethality,3 this analysis was performed in fetal liver (FL) at embryonic day 12.5. Remarkably, Wnt3a deficiency leads to reduced numbers of long-term HSC and multipotent progenitors, which are severely and irreversibly impaired in long-term reconstitution capacity as observed in serial transplantation assays.2 This severe phenotype suggested that Wnt3a is the most prominent Wnt for FL HSC function. However, it is still unknown to what extent Wnt signaling was affected in these HSC, whether other Wnt genes could take over the role of Wnt3a, and whether its action was autocrine or paracrine.

Therefore, we first determined the expression profile in FL of several Wnt genes previously shown to regulate hematopoiesis1 and whether Wnt3a deficiency affects the expression of those Wnt genes. From the panel of Wnt genes analyzed, Wnt4, Wnt5a, Wnt5b, and Wnt10b were expressed at high levels. Wnt3a was expressed at relatively low levels, and, interestingly, Wnt5a deficiency did not significantly influence the expression of the other Wnt genes (Figure 1A), indicating that the lack of self-renewal by the Wnt3a−/− HSCs was not due to an effect on the expression of other Wnt genes.

To determine the effect of Wnt3a deficiency on the activation of canonical Wnt signaling, we used an established Wnt reporter mouse (Bat-Gal) in which the LacZ gene (encoding β-galactosidase) is under control of 3 Wnt responsive T-cell factor/lymphoid-enhancer factor (Tcf/Lef)–binding sites.4 Analysis of reporter activity in E12.5 FL LSKs (Lin− c-Kit+Sca1+) showed that approximately 7% of these cells undergo active signaling. Analysis of Wnt3a-deficient embryos carrying the reporter transgene showed a profound reduction in the frequency of LacZ-positive LSKs (Figure 1B-C), which was then confirmed by direct sequencing. Thus, Wnt3a−/− LSKs show a complete abolishment of canonical Wnt signaling in comparison with littermate wild-type embryos.

Activation of the Wnt signaling pathway has been used to expand and enhance HSC function. Interestingly, Wnt3A was shown to preserve HSCs with an immature phenotype in vitro or to induce true stem cell characteristics in hematopoietic progenitors.5,6

**Correspondence:** Mei-Hsuan Tseng, Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan; Yen-Ning Huang, Department of Laboratory Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan; Chi-Fei Huang, Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan; Mei-Hsuan Tseng, Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan; Hwei-Fang Tien, Department of Internal Medicine, National Taiwan University Hospital, National Taiwan University, Taipei, Taiwan.
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