Site-selective Cleavage of RNA at Two Sites by Tandem DNAzyme and its Detection by Mass Spectrometry for Genotyping of SNP

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Accurate and low-cost methods of genotyping of single nucleotide polymorphism (SNP) caused by single base-pair substitution are crucially important for prediction of hereditary diseases, design of individualized medicine, and prognosis of SNP-related diseases. For example, drug resistance in leukemia is often caused by several point mutations occurred in the leukemogenic gene. Such point mutations that constitute drug-resistant SNPs in chronic myelogenous leukemia (CML) include the T315I mutant, in which threonine (T) at position 315 is replaced with isoleucine (I) due to a single base change (C to U) in the ABL gene.

A number of methods to precisely detect SNPs have been developed to date, including allele-specific PCR and extended DNA primers analysis by mass spectrometry (MS). Previously, we developed methods to quantitatively detect SNP in RNA containing a single-base mutation by using PNA-directed clamping PCR, or RNA nick-joining or nick-generation method. In the nick-generation method for SNP detection, we used RNA-cleaving DNAzyme to make a site-selective scission at the mutant base in RNA. DNAzyme is a novel oligoDNA that can bind to specific sequences of RNA through Watson-Crick base-pairing and cleave target RNA at a phosphodiester bond located between an unpaired purine and pyrimidine. The DNAzyme can be designed to target different RNA substrates by changing the substrate recognition sequences of DNAzyme.

Here we present a new method for genotyping of SNP utilizing MS analysis of RNA fragments, in which short RNA fragments of interest that are generated by clipping out of the RNA substrate with the tandem DNAzyme. It is based on our recent finding of site-selective cleavage of RNA strand at two sites with the tandem DNAzyme that combines two separate DNAzymes with oligo dT spacers. By using the tandem DNAzyme with two separate binding sequences, two distant phosphodiester linkages in RNA were selectively hydrolyzed. Thus, two-site RNA scission should be possible with the use of tandem DNAzyme designed for clipping out RNA fragments.

Our previously designed DNAzyme (T315IDz) was engineered as a tandem DNAzyme to generate RNA fragments, in which the GU-T315I tandem DNAzyme cleaves ABL-U RNA substrate at the point mutation site (T315I) as well as at the upstream control site (Scheme 1).

Scheme 1. Sequences of DNAzymes and generation of RNA fragments for MS analysis. (a) The RNA oligonucleotides of wild type ABL and T315I mutant ABL. Two DNAzymes are shown to cleave at two different sites in ABL-U RNA. (b) Tandem DNAzyme is shown with two cleavage sites in ABL-U RNA. Shown are RNA fragments of expected size, which are produced by tandem DNAzyme.
between tandem DNAzyme to cleave the observed. be generated by a mixture of GU Dz and T315 I Dz was not 45-mer RNA substrate, the 13-mer RNA fragment that would be generated by a mixture of GU Dz and T315 I Dz was not observed. The two DNAzymes were connected together to make the tandem DNAzyme to cleave the ABL-U RNA at two separate sites (Scheme 1(b)). Both of the target sites were efficiently cleaved by the tandem DNAzyme, generating RNA fragments of expected size (Fig. 1(b)). Of importance, the 13-mer RNA fragment was not produced with the 45-mer RNA substrate (100 nM) was mixed and incubated at room temperature for 2 h, and the RNA cleavage products were separated by 10% denaturing PAGE. (b) The GU-T315I tandem DNAzyme was used to cleave at two distant sites (shown in red arrows) in ABL-U RNA substrate that is internally labeled at each phosphate with 32P (i.e. Body-labeling). Resulting RNA fragments were resolved by 10% denaturing PAGE. Figure 1. RNA-cleavage with DNAzymes. (a) GU DNAzyme and/or T315 DNAzyme (1.0 µM) and 5^-32P labeled RNA substrate (100 nM) was mixed and incubated at room temperature for 2 h, and the RNA cleavage products were separated by 10% denaturing PAGE. (b) The GU-T315 tandem DNAzyme was used to cleave at two distant sites (shown in red arrows) in ABL-U RNA substrate that is internally labeled at each phosphate with 32P (i.e. Body-labeling). Resulting RNA fragments were resolved by 10% denaturing PAGE. "Figure 2. The mass spectra of T315I mutant ABL-U RNA (45-mer) (a), and after incubation with the GU-T315I tandem Dz (b)."5 were clearly detected for a purpose of SNP genotyping in RNA. In summary, we have a new method to clip out RNA substrate containing the point mutation by using the bidentate RNA-cleaving tandem DNAzyme. The clipped out RNA fragments are readily analyzed for their exact sizes by MALDI-TOF MS. Our strategy can be applicable for precise genotyping of SNPs in RNA samples derived from human serum or cells. Such applications, as well as studies to improve sensitivity and RNA cleavage efficiencies are currently underway in our group. Acknowledgments. This work was supported by the Konkuk University research grant in 2011. References and Notes 1. (a) Roses, A. D. Nature 2000, 405, 857-865. (b) Syvanen, A. C. Nat. Genet. 2005, 37, Suppl, S5-10. 2. (a) Druker, B. J.; Talpaz, M.; Resta, D. J.; Peng, B.; Buchdunger, E.; Ford, J. M.; Lydon, N. B.; Kantarjian, H.; Capdeville, R.; Ohno-Jones, S.; Sawyers, C. L. N Engl. J. Med. 2001, 344, 1031-1037. (b) Steinberg, M. Clin. Ther. 2007, 29, 2289-2308. 3. Wu, D. Y.; Ugozzoli, L.; Pal, B. K.; Wallace, R. B., Proc. Natl. Acad. Sci. USA 1989, 86, 2757-2760. 4. (a) Lechner, D.; Lathrop, G. M.; Gut, I. G. Curr. Opin. Chem. Biol. 2002, 6, 31-38. (b) Ross, P.; Hall, L.; Smirnov, I.; Haff, L. Nat. Biotechnol. 1998, 16, 1347-1351. 5. Park, K.; Jung, W.; Choi, B. R.; Park, H.; Kim, D. E. Bull. Korean Chem. Soc. 2010, 31, 2077-2080. 6. Park, K.; Choi, B. R.; Kim, Y. S.; Shin, S.; Hah, S. S.; Jung, W.; Oh, S.; Kim, D. E. Anal. Biochem. 2011, 414, 303-305. 7. Santoro, S. W.; Joyce, G. F. Proc. Natl. Acad. Sci. USA 1997, 94, 4262-4266. 8. (a) Kim, J. E.; Yoon, S.; Choi, B. R.; Kim, K. P.; Cho, Y. H.; Jung, W.; Kim, D. W.; Oh, S.; Kim, D. E. Leukemia 2013, 27, 1650-1658. (b) Kim, J. E.; Yoon, S.; Mok, H.; Jung, W.; Kim, D. E. FEBS Lett. 2012, 586, 3865-3869. 9. Mass analysis of RNAs was performed using an Autoflex III MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) equipped with a smart beam laser as an ionization source in linear positive mode. All spectra were acquired with 19 kV accelerating voltage, 50 Hz repetition rate, and an average of ~2000 shots. Equal volume mixture of 3-hydroxypicolinic acid (20 mg/mL in acetonitrile/water = 1:1) and diammonium citrate (50 mg/mL in water) was used as a matrix.