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Chemiluminescence resonance energy transfer imaging on magnetic particles for single-nucleotide polymorphism detection based on ligation chain reaction

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ABSTRACT

A novel ligation chain reaction (LCR) methodology for single-nucleotide polymorphism (SNP) detection was developed based on luminol–H_2O_2–horseradish peroxidase (HRP)-mimicking DNAzyme–fluorescein chemiluminescence resonance energy transfer (CRET) imaging on magnetic particles. For LCR, four unique target-complement probes (X and X*, YG and Y*) for the amplification of K-ras (G12C) were designed by modifying G-quadruplex sequence at 3′-end of YG and fluorescein at 5′-end of Y*. After the LCR, the resulting products of XYG/X*Y* with biotin-labeled X* were captured onto streptavidin-coated magnetic particles (SA-MPs) via specific biotin-SA interaction, which stimulated the CRET reaction from hemin/G-quadruplex-catalyzed luminol–H_2O_2 CL system to fluorescein. By collecting signals by a cooled low-light CCD, a CRET imaging method was proposed for visual detection and quantitative analysis of SNP. As low as 0.86 fM mutant DNA was detected by this assay, and positive mutation detection was achieved with a wild-type to mutant ratio of 10,000:1. This high sensitivity and specificity could be attributed to not only the exponential amplification and excellent discrimination of LCR but also the employment of SA-MPs. SA-MPs ensured the feasibility of the proposed strategy, which also simplified the operations through magnetic separation and separated the reaction and detection procedures to improve sensitivity. The proposed LCR-CRET imaging strategy extends the application of signal amplification techniques to SNP detection, providing a promising platform for effective and high-throughput genetic diagnosis.

1. Introduction

Single nucleotide polymorphism (SNP) is the most common variations in human genomes, which associates closely with many human diseases, such as cancer and mitochondrial diseases (Kwok, 2003). For example, K-ras (G12C), a common oncogenic mutant, has been found to be the most expressed mutation in lung cancer (Ostrem et al., 2013). In addition, different types of K-ras mutations could affect tumor behavior and drug sensitivity (Garassino et al., 2011). Thus, SNP analysis is of great significance in determining the genetic predisposition for inherited diseases, early diagnosis and risk assessment of malignancy, and drug response. The development of effective assays for SNP screening is in urgent demand.

So far, many methods have been developed for SNP detection, especially amplification techniques. For example, enzyme conjugates (Patolsky et al., 2001; Ermini et al., 2014) and nanoparticles (Abbaspour and Noori, 2012; Wang et al., 2013) have been used as amplifying labels for SNP genotyping. In addition, as the standard method for amplification of nucleic acids, polymerase chain reaction (PCR) is used to detect trace amount of samples from genomic DNA, which however could introduce errors into SNP detection during the exponential amplification process (Mhlanga and Malmberg, 2001). Alternatively, ligase chain reaction (LCR) has become a powerful and robust technique for SNP detection due to its simplicity and rapidity, high amplification efficiency, and good specificity (Shen et al., 2012; Zhang et al., 2013). A typical LCR system is consisted of two pairs of oligonucleotide probes. Upon the introduction of target DNA, the complementary adjacent probes can be ligated by thermophilic DNA ligase, such as

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ampligase. The ligated products in turn serve as templates to ligate their correspondingly adjacent probes, resulting in an exponential amplification process by thermal cycles of annealing, ligation and melting. Due to the excellent discrimination capacity of ligation, LCR holds better specificity than primer extension reaction for SNP (Shen et al., 2013; Chen et al., 2012; Zhou et al., 2013).

In general, the LCR products were detected by gel electrophoresis (Barany, 1991; Yan et al., 2010) or fluorescence techniques (Cheng et al., 2012). Recently, chemiluminescence (CL) has become an attractive tool for biochemical research (Créton and Jaffe, 2001). Due to the emission of light caused by a chemical reaction without an external excitation source, CL has virtually nonspecific signals and is extremely sensitive with simple instrumental set-up. Chemiluminescence resonance energy transfer (CRET) occurs via nonradiative dipole–dipole energy transfer between a CL donor and a suitable acceptor molecule that are in close proximity without the need of a light source (Lei et al., 2014; Chen and Li, 2014; Mun et al., 2014). The CRET signal can be easily readout by photometers or luminometers, especially by sensitive imaging photon detector of cooled low-light charge-coupled device (CCD) for chemiluminescence imaging assay (Bi et al., 2013; Zong et al., 2012).

Herein, a LCR-CRET imaging strategy is proposed for SNP detection with high sensitivity and specificity. This method is based on the specific ligation of thermostable ligase to discriminate single-base variation and the exponential amplification of LCR to improve sensitivity. In addition, the probes that are designed with G-quadruplex sequence and fluorescein enable the ligated LCR products to perform the horseradish peroxidase (HRP)-mimicking DNAzyme-catalyzed luminol–H_2O_2 CL reaction in the presence of hemin, leading to the CRET to fluorescein. Moreover, streptavidin-coated magnetic particles (SA-MPs) are employed to capture the biotin-tagged LCR products, which can significantly improve the sensitivity by removing the unintercalated hemin that could induce high background. This platform provides a rapid, specific, sensitive, and high-throughput technique for SNP detection.

2. Experimental section

2.1. Materials

Oligonucleotides used in this study were synthesized by Shanghai Sangon Biotech Co., Ltd. (China) (sequences see Table S1). Ampligase® enzyme and buffer were purchased from Epicenter Biotechnologies (Madison, WI). Streptavidin-coated magnetic particles (SA-MPs) (1 μm) were ordered from Zhengzhou Innosep Biosciences Co., Ltd. (China). Hemin, luminol, hydrogen peroxide, and 4-(2-hydroxyethyl)piperazine-1 ethanesulfonic acid sodium salt (HEPES) were obtained from Aladdin Chemistry Co. Ltd. (China). Magnetic separation rack for 96-well plate was purchased from Tiajin BaseLine Chromtech Research Centre (China). The chemicals were of analytical grade and used as received without further purification. Double-distilled, deionized ultrapure water was used in all of the experiments.

2.2. LCR process

A 15 μL of reaction solution contains 2.5 μL of TE buffer (10 mM Tris–HCl, 1 mM EDTA, 12.5 mM $MgCl_2$, pH 8.0), 1.5 μL of 10 × ampligase reaction buffer, probes X, X*, YG, and Y* with the final concentration of each component of 0.1 μM, and the various amounts of target DNA. After heating the mixture to 95 °C for 3 min, 1 μL of ampligase (5U) was added at 75 °C. 30 Thermal cycles for LCR were carried out on a Little Genius thermal cycler (Hangzhou Bioer, China) with each cycle consisting of a 1 min at 95 °C for denaturation and 1 min at 50 °C for annealing/ligation. After the thermal cycling, the mixture was further reaction at 37 °C for 30 min.

2.3. CRET imaging

A 20 μL of SA-MPs was added to capture the biotin-labeled ligated products at room temperature for 30 min. After magnetic separation, 5.0×10^{-7} M hemin in HEPES was added and incubated at 25 °C for 20 min to form the HRP-mimicking DNAzymes. Subsequently, the SA-MPs were washed with HEPES buffer twice, followed by adding 0.02 M luminol and 0.2 M H_2O_2 to initiate the CRET reaction. The CRET images were recorded using an EC3 imaging system with a thermoelectrically cooled CCD camera (UVP, USA). The CRET intensity of each spot was calculated as the mean pixel within a circle of a given diameter around spot center. In specific experiments, the mixture of mutDNA and wtDNA at different ratios with a total concentration of 1.0×10^{-11} M was used as a target sample to carry out the LCR-CRET reaction as mentioned above.

3. Results and dicussion

3.1. Assay principle

The principle of the proposed ligase chain reaction (LCR)-based chemiluminescence resonance energy transfer (CRET) imaging of single-nucleotide polymorphism (SNP) by using magnetic particles (MPs) to reduce background is illustrated in Scheme 1. The system is consisted of four short single-stranded DNA probes (X and X*, YG and Y*; G represents the G-quadruplex nucleic acid structure), target DNA (mutDNA or wt DNA), and ampligase. The targets DNA are fragments of the common human oncogene K-ras, in which the wtDNA and mutDNA are the wide-type and mutant genes, respectively. The wtDNA is different from mutDNA with a single base of C-A transversion that causes G12C mutation in the K-ras gene. X and Y are designed the same as one half of the mutDNA target, while X* and Y* are complementary to one half of the target. In the presence of mutDNA, probes X* and Y* are hybridized to adjacent positions on the target template at 50 °C, which are subsequently covalently joined by thermostable ampligase to form the ligated product of X*Y*, whereas the duplexes are simultaneously formed between X and X*, Y and Y*. Upon heated to 95 °C, all duplexes are denatured, releasing target DNA, the ligated product X*Y*, and partial probes. In this way, when the temperature is reduced to 50 °C, the DNA target is recycled to perform the target-recycled ligation, while the ligated X*Y* serves as the new template for the probes X and YG to form the new ligation product of XYG that also serves as the secondary target for the subsequent annealing/ligation with probes X* and Y* to form the ligation product of X*Y*. It should be noted that excess probes over target DNA play an important role in minimizing the re-hybridization of the target to the ligated products. Thus, from the second thermal cycle, the amount of the ligated products will be doubled theoretically after each cycle, resulting in an exponential amplification of mutDNA to generate a large number of ligation probes (X*Y* and XYG) through repeating the thermal cycling of annealing/ligation at 50 °C and denaturation at 95 °C. This process is named as ligation chain reaction (LCR).

After LCR, the mixture is further reacted at 37 °C for 30 min to make the resulting duplex products stable with four types of mutDNA/X*Y*, XYG/X*Y*, X/X*, and YG/Y*. Here probe Y* is designed with fluorescein label at its 5′-end, and probe X* is modified with biotin at its 3′-end. Thus, the duplex products with X* can be easily captured on streptavidin-coated magnetic particles