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Highly sensitive ECL-PCR method for detection of K-*ras* point mutation

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Abstract

A highly sensitive electrochemiluminescence-polymerase chain reaction (ECL-PCR) method for K-*ras* point mutation detection is developed. Briefly, K-*ras* oncogene was amplified by a $Ru(bpy)_3^{2+}$ (TBR)-labeled forward and a biotin-labeled reverse primer, and followed by digestion with *Mva*I restriction enzyme, which only cut the wild-type amplicon containing its cutting site. The digested product was then adsorbed to the streptavidin-coated microbead through the biotin label and detected by ECL assay. The experiment results showed that the different genotypes can be clearly discriminated by ECL-PCR method. It is useful in point mutation detection, due to its sensitivity, safety, and simplicity.

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Keywords: Electrochemiluminescence-polymerase chain reaction; K-*ras* oncogene; Point mutation detection

The ability to detect point mutation is important in molecular genetics. However, conventional electrophoresis-based methods often require long time with multi-step, using radioactive isotopes or other hazardous materials [1]. Recently, electrochemiluminescence (ECL) technique has been developed. It employs a chemiluminescence (CL) reaction of $Ru(bpy)_3^{2+}$ (TBR) and tripropylamine (TPA) that takes place on an electrode surface, to emit photons with high efficiency [2–4]. The method has been applied in nucleic acid analysis due to its sensitivity, safety, and simplicity [5–10].

In this study, an electrochemiluminescence-polymerase chain reaction (PCR-ECL) method, combining PCR and restriction endonuclease digestion with ECL assay, has been developed for point mutation detection. The method was applied to detect the codon 12-point mutation of K-*ras* oncogene, which is often found in human malignancy [1].

1. Experimental

Human embryo kidney (HEK) cell line 293 was purchased from Center of Experimental Animal of Sun Yat-sen University. Human colorectal adenocarcinoma cell line SW480, which harbors a homozygous K-*ras* codon 12 mutation (G12V), was purchased from China Center for Type Culture Collection. Primers were synthesized by Shanghai Sangon Biological Engineering & Technology services Co., Ltd. (SSBE). The forward primer was 5′-TBR-gac tga ata taa act tgt ggt agt tgg acc t-3′. The reverse primer was 5′-biotin-cta ttg ttg gat cat att cgt cc-3′. The forward

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primer contained a mismatch near the 3′ ends (underlined base) to generate an *Mva*I site right upstream of codon 12 in cases of wild-type, but not in K-*ras* mutants.

Genome DNA was extracted according to the protocol of UNIQ-10 column genome DNA extraction kit (SSBE). PCR was conducted in a PTC-100 thermocycler (MJ Research). The amplification protocol consisted of 94 °C for 5 min for initial denaturation, 35 cycles of 1 min at 94 °C for denaturation, 1 min at 55 °C for primer annealing, and 1 min at 72 °C for extension. After amplification, the sample was split into two aliquots. One aliquot was digested with *Mva*I restriction enzyme, which only cutted the wild-type amplicon, containing its cutting site (5′-CC*A(T)GG-3′), and thus removed the TBR-labeled 5′ part of the amplicon from the biotin-labeled 3′ part. The other aliquot remained unaltered. The two aliquots were then adsorbed to the streptavidin- coated microbead through the biotin label, other components without biotin label were washed away. The amount of uncutted amplicon with both TBR and biotin labels was determined by measuring the ECL signal generated from the reaction of TBR with TPA in the electrochemical reaction cell of ECL analyzer (built in our lab) [7].

For determination if the sample contains mutant K-*ras* oncogene, a cutoff value is defined as that, when the measured ECL intensity is above it, the sample is considered to be positive for K-*ras* point mutation. The cutoff value can be derived as the average + three times the standard deviation of the background signal, which is obtained by measuring the ECL signal from the blank control (PCR without template) [8].

2. Results and discussion

Fig. 1 shows the ECL detection result of wild-type homozygous K-*ras* oncogene using HEK293 DNA as template. The result showed that the average ECL intensity of the undigested aliquot was 212.9 cps, while that of the digested aliquot was 11.9 cps, which was close to the average ECL intensity of the blank control (11.4 cps).

In the wild-type homozygous K-*ras* model, HEK293 DNA was used as template. On: potentiostat on; Off: potentiostat off.

K-*ras* genotyping using HEK293 DNA (wild-type), SW480 DNA (GGT mutant), and 1:1 DNA mixture of SW480 and HEK293 (heterozygote) as template. Each bar represents an average value based on 30 1-s measurements from three parallel samples, respectively, with the error bar (standard deviation) reflecting the measurement variation of the system. The dash line represents the cutoff value for K-*ras* point mutation positive results.

Fig. 2 shows the ECL-PCR detection result of K-*ras* point mutation. In the wild-type case, the ECL intensity of the undigested aliquot was 213 ± 18 cps, while the ECL intensity of the digested aliquot was 11.2 ± 0.8 cps, which was under the cutoff value (14.1 cps). The difference in ECL intensities between undigested and digested aliquot was statistically significant ($P < 0.05$). In the mutant case, the ECL intensities of the undigested and the digested aliquot were 215 ± 18.6 and 213 ± 17.5 cps, respectively. Both of them were over 10 times higher than the cutoff value. The difference in ECL intensities between undigested and digested aliquot was negligible ($P > 0.05$). In the heterozygote case, the ECL intensities of the undigested and the digested aliquot were 207 ± 16.5 cps and 96 ± 8.4 cps, respectively. Both of them were over the cutoff value. The difference in ECL intensities between undigested and digested aliquot was statistically significant ($P < 0.05$).

In this study, an ECL-PCR method for discrimination of different genotypes of K-*ras* oncogene was described. The different genotypes were discriminated by comparison of the ECL intensities between the digested and the undigested

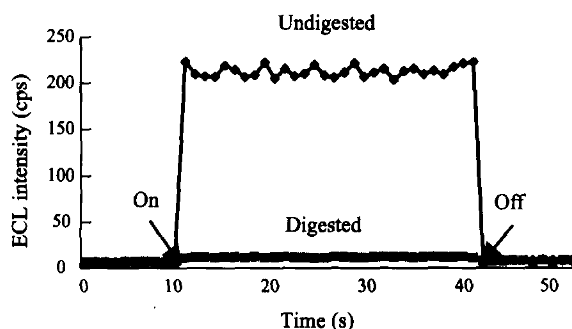

Fig. 1. ECL detection result of wild-type homozygous K-*ras*.

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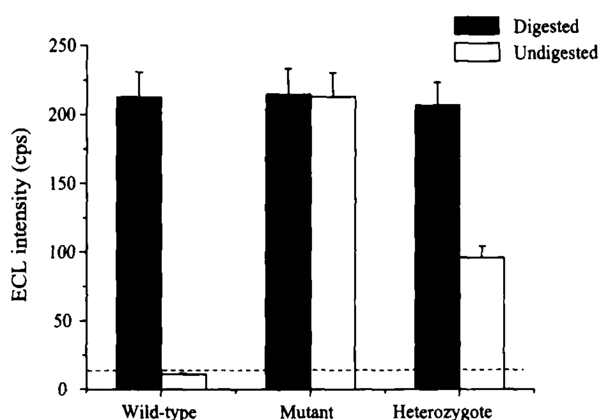

Fig. 2. ECL-PCR detection of K-*ras* point mutation.

aliquots. This assay is safety and accurate, does not need specialized ethidium bromide-stained gels. The results can be interpreted by computer. This method does not include electrophoresis, therefore it is highly specific as it is unaffected by the formation of spurious PCR amplification products.

In conclusion, we have developed a sensitive ECL-PCR method for point mutation detection. This approach will have an enormous potential for reliable point mutation analysis, due to its sensitivity, safety and simplicity.

Acknowledgments

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