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A Novel Method for Quantification of Circulating DNA in Serum by Capillary Zone Electrophoresis

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Abstract: In this paper, we first presented a novel method for quantification of circulating DNA in human serum based on capillary zone electrophoresis with laser-induced fluorescence detection (CZE-LIF). The serum was digested by proteinase to release free DNA, and then CZE-LIF system was used for the quantification of total circulating DNA. This method was successfully used to quantify the circulating DNA levels in sera from healthy individuals and certain cancer patients. We found the significantly elevated circulating DNA levels in certain prostate cancer patients. Our results demonstrated that CZE-LIF system has good linearity, excellent sensitivity (0.5 ng/mL DNA), satisfactory reproducibility (RSDs in one day and between days were both less than 5%) and reliability, and is well suitable to the quantification of the circulating DNA in human serum or plasma.

Keywords: Circulating DNA, capillary zone electrophoresis, quantification, serum.

In early 1948, Mandel and Métais[1] firstly reported the presence of cell free nucleic acids (also called circulating DNA) in the human bloodstream, unfortunately, little attention was paid to this important finding for a long time. Recently, some researchers found the significantly elevated circulating DNA levels in certain cancer patients compared to healthy controls[2-4]. It was suggested that circulating DNA in serum or plasma had potential for the molecular diagnosis and prognosis of certain cancers. So far, several technologies have been utilized to quantifying cell free DNA levels including radioimmunoassays, competitive PCR, quantitative real-time PCR, fluorimetric quantitation, spectrophotometric determination and visual comparison with commercial kits[5-7]. These methods were utilized to demonstrate the elevated circulating DNA levels in certain cancer patients, but the circulating DNA levels obtained by various methods exhibit considerable differences. Additionally, these methods are generally based on the use of commercial DNA extraction kits, which are labor-extensive and time-consuming, and not suitable to large-scale quantification of circulating DNA levels in clinical diagnosis.

In the letter, we presented a sensitive and accurate method for quantification of circulating DNA levels based on capillary zone electrophoresis with laser-induced fluorescence detection (CZE-LIF).

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Experimental

The quantification of circulating DNA was performed on a Beckman P/ACE MDQ system (Fullerton, CA, US) with a LIF system. Fused silica capillaries with 75 μm internal diameter (id) were purchased from Yongnian Optical Fiber Factory (Hebei). The digests of λ-Hind III DNA were provided by Takara Co. (JPN). Proteinase was purchased from MERCK Co. (Damstadt, Germany). SYBR Gold (Molecular Probes, Inc., US, concentration not given) was used as DNA intercalating dye.

Pretreatment of Serum

Two μL of proteinase K (10 mg/mL) was added in the mixture of 50 μL serum and 48 μL 2×TBE buffer, and then it was incubated at 50 ^{o}C for 30 min to digest the proteins in the serum. Prior to the electrophoresis, the digested serum mixture was diluted five folds with water, and then fluorescein was added as internal standard.

Capillary electrophoresis procedure

The polyacrylamide-coated capillaries [8] were used for the separation of circulating DNA in serum. Before electrophoresis, the TAPS buffer (80 mmol/L, pH=7.5) containing SYBR Gold (1/10000) was pumped into the capillary, and then the sample was introduced by pressure into the capillary. The electrophoresis was preformed in reverse polarity model. A LIF system (excitation wavelength: 488 nm, emission wavelength: 520 nm) was utilized to detect the circulating DNA.

Results and Discussion

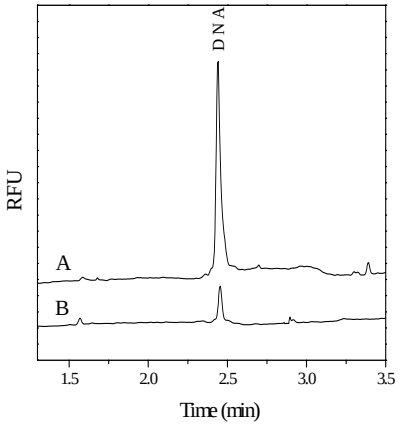
Capillary zone electrophoresis and Pretreatment of Serum

Although the circulating DNA fragments possess different sizes, they have the same electrophoretic mobility in CZE due to their similar charge densities. We found that in CZE model, λ DNA containing 8 fragments from 125 bp to 23130 bp showed only one peak in the electropherogram. And thus, the total DNA amount can be measured by CZE. The circulating DNA in serum is associated with various proteins, and thus, proteinase digestion to release free DNA is necessary before electrophoresis. As showed in **Figure 1**, after the serum was digested by proteinase K for 30 minutes, a peak would emerge at the migration time of free DNA. In order to identify the peak of nucleic acids, we added λ-Hind III DNA into the digested serum and run electrophoresis. The increased peak in **Figure 1** implied that the peak in serum was from circulating DNA.

Linearity, Reproducibility and Reliability

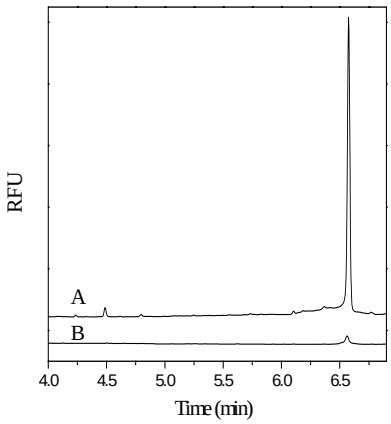
According to the capillary electrophoresis procedure, we investigated the linearity, the reproducibility and reliability of this method. We found that calibration curve of the peak area ratios to DNA concentrations had a good linearity (R=0.9992) in the low range of DNA concentrations (4 to 40 ng/mL), and detection limit was 0.5 ng/mL for DNA

Figure 1 Electropherograms of circulation DNA and its mixture with λDNA



The coated capillary (20 cm effective length, 75 μm id) was used, filling with 80 mmol/L TAPS containing SYBR Gold (1/10000). Electrophoresis buffer was 80 mmol/L TAPS (pH 7.5), the temperature was 25 °C, and the applied voltage was –400 V/cm. Samples were introduced by pressure (5 s at 0.5 psi). A, serum and λDNA; B, serum.

Figure 2 Comparison of circulating DNA levels between a prostate cancer patient and a normal individual



The coated capillary (30 cm effective length, 75 μm id) was used, filling with 80 mmol/L TAPS containing SYBR Gold (1/10000). Electrophoresis buffer was 80 mmol/L TAPS (pH 7.5), the temperature was 25 °C, and the applied voltage was –200 V/cm. Samples were introduced by pressure (5 s at 0.5 psi). A, prostate cancer patient serum (957 ng/mL DNA); B, healthy serum (79 ng/mL DNA).

(S/N=3). The relative standard derivations (RSDs) in one day and between days were both less than 5%, and the recovery 89% was achieved. Moreover, the circulating DNA level was estimated using QIAamp DNA Blood Mini Kit. The results of two methods only had a little difference (CZE: 62.9 ng/mL; Kit: 65.5 ng/mL). Our data demonstrated that CZE–LIF method was practical and reliable for quantification of the circulating DNA.

Assay of samples

Serum samples of 20 persons (from Shanghai Jiaotong University) as healthy controls were assayed. We found that the average of circulating DNA concentrations was 82 ng/mL and individual concentration varied from 40 to 140 ng/mL. In addition, several samples of patients with tumor burden were analyzed, and the significantly elevated circulating DNA levels were examined. **Figure 2** showed the significant difference of circulating DNA levels between a control serum and a prostate cancer patient serum (provided by Anhui Provincial Hospital). Our preliminary results indicated that the circulating DNA quantification likely was useful for diagnosis of certain cancers. The possibility of utilizing this method into diagnosis and prognosis for some cancers and the relations

between circulating DNA level and other clinical factors were under investigation in our group.

Conclusion

We presented a novel method to quantify the circulating DNA in human serum based on CZE-LIF. Under the CZE model, all of the DNA fragments have similar electrophoretic mobility, and thus the total DNA in serum can be quantified by CZE. This method was high sensitivity, excellent reproducibility and short analysis time, and well suitable to quantification of the circulating DNA in human serum or plasma. Furthermore, we also found the significantly elevated circulating DNA levels in certain cancers.

Acknowledgments

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