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Communication

Development of a rapid and sensitivity magnetic chemiluminescence immunoassay for DNA methyltransferase 1 in human serum

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ABSTRACT

DNA methyltransferase 1 (DNMT1) is a useful biomarker for lung cancer in early clinical diagnosis. A rapid magnetic chemiluminescence immunoassay (MCLIA) for DNMT1 in human serum has been developed. Horseradish peroxidase (HRP)-second-Ab was used to labeled polyclonal antibodies of anti-DNMT1. DNMT1 in sample integrates with specific immunomagnetic beads and can constitute a supersandwiched immunoreaction. In magnetic field, nonspecific materials can be separated. After luminescent substrate luminol-H_2O_2-BIP was added, the relative light unit (RLU) of HRP was detected and was discovered to be directly proportional to the content of DNMT1 in sample. The correlative variables involved in the MCLIA value were optimized and the methodological evaluation was carried out. After optimization, in the range of 0.5–128 ng/mL, the linear regression equation was $y = 0.5014x + 1.769$ (x was $\log C_{DNMT1}$, y was relative luminescence units (RLU)/RLU_0), and the limit of detection was 0.01 ng/mL. The RSD of intra- and inter-assays were 15.8%–16.9% and 14.3%–18.1%, respectively. The recovery was from 70.0% to 106.2%. Furthermore, paralleled with purchasable enzyme-linked immunosorbent assay (ELISA) kits, MCLEIA had lower detection limit, wider linear range and shorter detection time. Therefore, the MCLEIA established in this study could be used for the sensitive detection of DNMT1 in serum sample.

Epigenetic regulation of tumor is the advancing spot in the present international biochemistry research [1]. Recent studies have indicated that epigenetic alterations play an essential role in this type of cancer [2]. DNA methyltransferases (DNMTs) are the important representations of epigenetic alterations. There are numerous of reports have shown that the aberrant expressions of DNMTs in breast cancer [3–5], liver cancer [6], lung cancer [7] and gastric cancer [8]. DNA methylation is mainly formed by the addition of 1-methyl at the 5′-position of cytosine to 5-methylcytosine catalyzed by methyltransferase [9]. One of 5-methylcytosine synthesis pathways is DNA methyltransferase 1 (DNMT1) catalyzes complete methylation of the unmethylated chain in the semi-methylated DNA and maintains methylation consistent with the protocell. In other words, the activity and concentration of DNMT1 directly affect the degree of DNA methylation. Overexpression of DNMT1 may cause aberrant methylation of tumor suppressor gene or proto-oncogene promoter, further leading to tumorigenesis.

Many methods for detecting DNMT1 activity were well established which include radioactive labeling strategy [10], high performance liquid chromatography [11], gel electrophoresis [12], and immune-based assay [13–15]. However, they are usually used for the DNMT1 activity detection, hardly applied for the quantitative analysis. The detection of enzyme activity must be based on the measurement of enzyme content, so the determination of enzyme concentration is of great concern.

Common enzyme quantitative methods include enzyme-linked immunosorbent assay [16,17] (ELISA), chemiluminescence (CLIA) [18–20] and immunochromatography [21]. The advantages of ELISA are cost reduction and automation, but non-specific reaction caused by HOOK effect, false positive caused by cross-contamination, low sensitivity, low detection rate of window phase is its inherent defect. As a new detection method in recent years, CLIA method [22,23] has good specificity for immunological reaction and high sensitivity for the detection of luminescence method.

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Compared with ELISA, CLIA is a heterogeneous immunoassay, so that antigen and antibody can bind efficiently without the shortcomings of traditional one-step method. At the same time, CLIA results are more stable, and the detection time is shorter. However, few reports have been found on the clinical application of DNMT1 using the CLIA without the participation of enzyme.

In this study, we developed a novel magnetic enhanced chemiluminescence enzyme immunoassay (MCLEIA) to achieve the simple, economical and easily commercialized DNMT1 detection in serum samples, and this method was compared with the purchasable ELISA kits. The experimental conditions, including the reaction time of DNMT1 and MBs@PcAb$_{DNMT1}$, the dilution ratio of PcAb$_{DNMT1}$, and the dilution ratio of HRP-second-Ab were examined and optimized. The methodology parameters including precision, accuracy and specificity were also evaluated. By comparing the established two methods, the results indicated that MCLEIA has shorter total reaction time, fewer capture antibodies, and lower detection limit. Therefore, the proposed MCLEIA could be used for the high-throughput detection of DNMT1 in clinical (Scheme 1).

The experimental section, including materials and instruments, buffers and solutions, immobilization of antibody on magnetic beads, characterization of the MBs@McAb$_{DNMT1}$, theory and program of MCLEIA immunoassay and theory and program of ELISA immunoassay can be found in Supporting information.

We first conducted characterization of MBs@McAb$_{DNMT1}$. The coupling rate determined by coomassie brilliant blue staining was more than 75%. As shown in Figs. 1 A and B, the MBs@McAb$_{DNMT1}$ surface morphology has changed; the surface is rough and covered with a layer of white substance, presumed to be McAb$_{DNMT1}$. Therefore, the results show that the coupling is successful. The Fig. 1C showed that the 20 times dilution of immunomagnetic beads has the highest absorbance compared with the other two dilutions and there is a gradient between the three groups. It was shown that the carboxylate magnetic particle surface was successfully modified with McAb$_{DNMT1}$ and maintained the immune activity of the antibody, which can be used in subsequent experiments. The magnetism characterization of MBs@McAb$_{DNMT1}$ were presented in Fig. 1D, from which we could see in the left figure, the magnetic bead solution was brown, the solution was well dispersed, and no precipitation occurred within a few hours without the effect of external magnetic field. In the right figure, the immune magnetic beads were rapidly enriched with good magnetic response, and the solution was partially clarified in magnetic field. Therefore, the prepared MBs@McAb$_{DNMT1}$ still had good magnetic response.

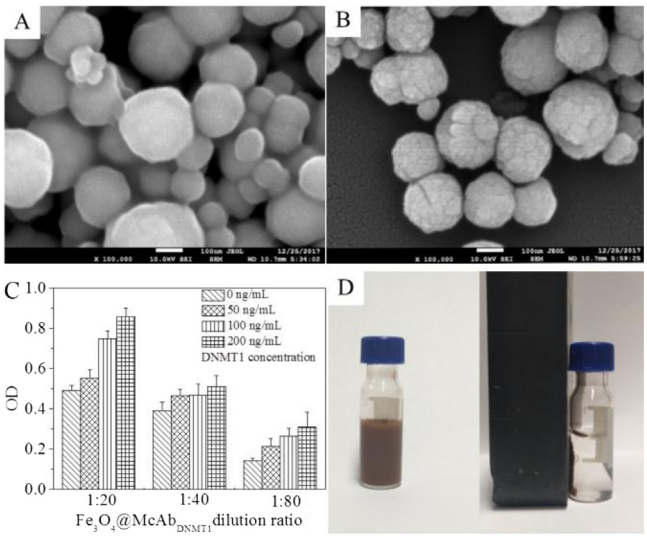
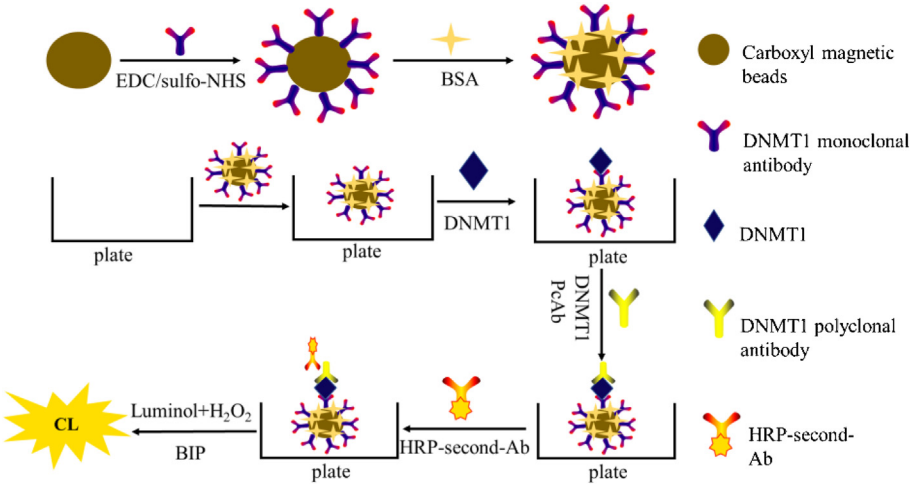


Fig. 1. (A) The SEM images of the carboxyl magnetic beads (MBs), Scale bar: 100 nm. (B) The SEM images of MBs@McAb$_{DNMT1}$, Scale bar: 100 nm. (C) Biological activity verification of MBs@McAb$_{DNMT1}$. (D) Magnetism constancy of MBs@McAb$_{DNMT1}$.

Next, we optimized MCLEIA method. MBs@McAb$_{DNMT1}$ with different dilution ratios was optimized (Fig. 2A). The concentration of DNMT 1 was 100 ng/mL. When the dilution ratio of MBs@McAb$_{DNMT1}$ was 1:40, the RLU intensity reached the maximum, and the difference between the experimental group and the control group was the largest. Therefore, 1:40 was chosen as the optimal dilution ratio of MBs@McAb$_{DNMT1}$. Results as shown in Fig. 2B, the RLU value was relatively high when the reaction time between MBs@McAb$_{DNMT1}$ and DNMT1 was 90 min and 120 min, but the RLU value did not change significantly after 90 min, indicating that the balance of antigen antibody complexes has reached stabilization. Thus, the optimal reaction time was 90 min for further experiments. After different dilutions of PcAb$_{DNMT1}$, the chemiluminescence values of DNMT1 (100 ng/mL) were determined. RLU was determined with 0 ng/mL as blank control, and the difference value ΔRLU ($RLU_{100} - RLU_0$) was used to dependent variable (Fig. 2C). When the dilution ratio of PcAb$_{DNMT1}$ was 1: 2000, the ΔRLU intensity was the strongest. The result indicated the best dilution ratio of PcAb$_{DNMT1}$



Scheme 1. The principle of MCLEIA.