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A homogeneous, Anti-dsDNA antibody-based assay for multicolor detection of cancer stem cell transcription factors

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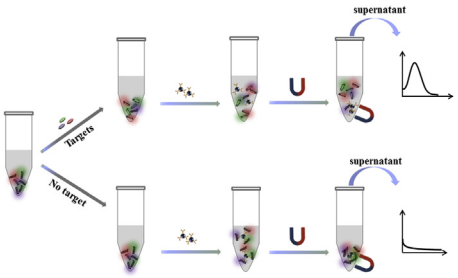
HIGHLIGHTS

- A novel method for cancer stem cell transcription factors (Oct4, Sox2 and Nanog) assay is developed.
- This assay allows one-step analysis of multiple TFs in the nuclear extracts without complex operations.
- The method can be used to analyze the polymorphism of TF binding sites, which can be further applied for exploring potential binding sites.
- The proposed method can be easily adapted to other proteins by just replacing the binding sequence.

GRAPHICAL ABSTRACT



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ABSTRACT

Cancer stem cells (CSCs) are responsible for maintaining tumor growth, metastasis and recurrence. The high expression of cancer stem cell transcription factors (Oct4, Sox2 and Nanog) is a valuable prognostic factor, suggesting a higher risk of tumor recurrence and metastasis. So, the development of a convenient and cost-effective method for multiplex assay of these transcription factors (TFs) is highly required. In this work, we have proposed a universal homogeneous assay for multicolor detection of these TFs based on anti-dsDNA antibody-decorated Fe_3O_4 magnetite nanoparticles (aadMNPs). In the presence of analytes, the dye-labeled dsDNAs are bound by specific TFs, which will inhibit the interactions between the dsDNAs and aadMNPs, generating higher fluorescence that may provide signal readout for the immunosensing process. By using the proposed method, Oct4 can be determined in a linear range from 3 to 1200 ng/mL with a detection limit of 0.035 ng/mL. Furthermore, we have presented assays for the sensitive, selective and rapid detection of Oct4, Sox2 and Nanog in cell extract, as well as the analysis of binding affinity of the mutated binding sequences. This work may provide potential applications in clinical CSCs detections, and may open new opportunity for the study of nucleotide polymorphisms in TF binding sites.

1. Introduction

The origin of cancer remains unclear; however, the “cancer stem cell theory” states that a subpopulation of cancer cells with

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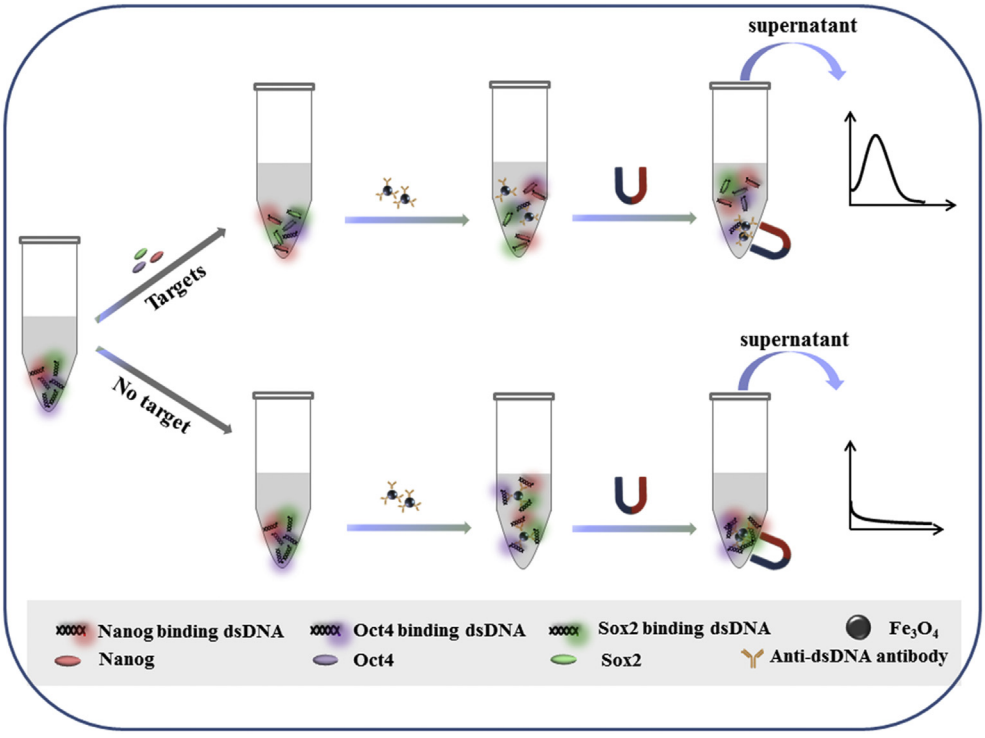
stem cell-like properties is responsible for tumor initiation, progression, cell death resistance, chemo- and radiotherapy resistance, and tumor recurrence [1]. These cancer stem cells (CSCs) are believed to be genotypically and/or phenotypically related to normal stem cells and share many of the features of embryonic stem cells such as self-renewal and a proliferative potential to generate a multi-potent cellular lineage [2–6]. Understanding CSC-specific signaling proteins and pathways is necessary to identify specific therapeutic targets that may lead to the development of more efficient therapies. The "stemness" in embryonic stem cells is controlled by some core transcription factors (TFs), such as Oct4, Sox2 and Nanog, and the combination of these factors has been shown to successfully reprogram differentiated somatic cells into pluripotent stem cells [7]. There is substantial evidence that CSCs express these specific markers. The high expression of Oct4, Sox2 and Nanog simultaneously is a valuable prognostic factor, suggesting a higher risk of tumor recurrence and metastasis. Therefore joint detection of these TFs has a potential clinical value in predicting the risk of postoperative recurrence of cancer patients [8–14]. However, the lack of rapid, sensitive, and efficient methods for multi-detection of these TFs brings enormous difficulties to related studies.

Traditional methods for detection of these TFs content (e.g., Western blotting and ELISA) are time-consuming and complicated. We once explored an alternative strategy DNA switch-based electrochemical assay for Nanog detection [15]. However, the study is only concerned with the detection of a single analyte. It is worth noting that multiple TFs are involved in the stemness of CSCs [3]. Simultaneous detection of multiple TFs will bring new possibilities for effectively improving the early diagnosis accuracy over the single-marker assay. In addition, most of the existed methods for TFs assay are only concerned with the detection of the TF concentration, few of them have attempted to identify genomic binding sites of proteins, which may lose much key information about the sequence specificity of TFs. Up to now, no method has been developed for the simultaneous analysis of Oct4, Sox2 and Nanog, especially in cancer stem cells. Thus, the development of a cost-effective and convenient method for the multiplex measurement of these TFs is highly desirable.

Herein, a simple strategy that is based on anti-dsDNA antibody-decorated Fe_3O_4 magnetite nanoparticles (aadMNPs) has been proposed in this work to attain multiple TFs quantitative information at the same time. We have further taken Sox2 as an example to screen the specific binding sequence. Anti-dsDNA antibody is known as one of the many autoantibodies in systemic lupus erythematosus (SLE). Since it was found in SLE patients sixty years ago, many efforts have been made to study it [16,17] as a biomarker for diagnosis and prognosis of cancer. It has been known that anti-dsDNA antibody has reactivity with dsDNA; however, to the best of our knowledge, nearly nobody has used it as a testing tool, thus, the application of anti-dsDNA antibody in analytical chemistry is still unexplored.

The principle of the method proposed in this work has been illustrated in Scheme 1. In this design, three favorable binding sequences of TFs (Oct4, Sox2 and Nanog) were chosen and the 3′ terminuses of the sequences were modified with three different dyes so as to give fluorescence signal. Spectral overlap is avoided when three dyes are selected (dsDNA-Oct4 labeled with FAM, dsDNA-Nanog labeled with Cy5, and dsDNA-Sox2 labeled with Cy3). To do so, anti-dsDNA antibodies were immobilized on the surface of MNPs. Meanwhile, the test sample was pre-incubated with the dsDNA. When analytes are absent, the dsDNAs were adsorbed onto the MNPs surface by immunoadsorption. After the assembly, the samples were concentrated by a magnet and the dye-labeled dsDNA fragments could be washed away, which might lead to significantly low fluorescence signal. However, in the presence of analytes, the dsDNAs were bound by specific TFs, which would block the interactions between dsDNAs and anti-dsDNA antibodies, leading to high fluorescence signal to provide quantitative measurement.



Scheme 1. Principle of the proposed assay for multicolor TFs detection.