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Original Article

An Ultrasensitive FRET-based DNA Sensor via the Accumulated QD System Derivatized in the Nano-beads

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Abstract Förster resonance energy transfer (FRET) is extremely sensitive to the separation distance between the donor and the acceptor which is ideal for probing such biological phenomena. Also, FRET-based probes have been developing for detecting an unamplified, low-abundance of target DNA. Here we describe the development of FRET based DNA sensor based on an accumulated QD system for detecting KRAS G12D mutation which is the most common mutation in cancer. The accumulated QD system consists of the polystyrene beads which surface is modified with carboxyl modified QDs. The QDs are sandwich-hybridized with DNA of a capture probe, a reporter probe with Texas-red, and a target DNA by EDC-NHS coupling. Because the carboxyl modified QDs are located closely to each other in the accumulated QDs, these neighboring QDs are enough to transfer the energy to the acceptor dyes. Therefore the FRET factor in the bead system is enhancing by the additional increase of 29.2% as compared to that in a single QD system. These results suggest that the accumulated nanobead probe with conjugated QDs can be used as ultrasensitive DNA nanosensors detecting the mutation in the various cancers.

Keywords: FRET, QD, DNA sensor, Bead

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Introduction

RAS (Renin Angiotension System Protein) is the most commonly mutated oncogene in cancer, with distinct RAS isoforms detected in various cancers[1-4]. Therefore, RAS inhibitors are being studied as a treatment for cancer with RAS overexpression. Especially, KRAS (Kirsten rat sarcoma viral oncogene homolog) G12D mutations are the most common in cancers of the pancreas, colon, biliary tract, and lung.

FRET-based system is a widely used probing method for understanding the interactions between biomolecules. FRET-based nanosensors with QDs as donor and organic dyes as acceptors have long been used in the detection as proteins, DNA, and RNA[5-25], because quantum dots (QDs) have excellent optical properties such as a broad absorption, narrow emission wavelength, and high quantum yield[26-28]. Although it is an ultrasensitive probing system, many studies reported that it needs to overcome the difficulty in enhancing the detection limit. For instance, Chun-Yang Zhang *et al*. (2007) controlled the velocity of the micro flows in the capillary chip to break the FRET limit for enhancing the FRET signal[29,30]. Lan-Hee Yang *et al*. (2016) developed an ultrasensitive FRET-based DNA sensor using PNA/DNA hybridization to enhance the FRET signal[31].

In this study, we describes a DNA nanosensor based on the FRET system consisting of the nanobeads surface-derivatized with accumulated quantum dots (QDs) in order to enhance the FRET signal and the detection limit of the target DNA. The accumulated QD system consists of the polystyrene beads which surface is modified with carboxyl modified QDs. The QDs are sandwich-hybridized with DNA complex of a