J Biosens Bioelectron 2017, 8:2(Suppl)
DOI: 10.4172/2155-6210-C1-034

High sensitive and selective EGFR colorimetric detection based on gold nanoparticles and target catalytic hairpin assembly amplification

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For diagnosis of cancer patients, mutational analysis is necessary. Especially, status of epidermal growth factor receptor (EGFR) mutations is very important factor of non-small cell lung cancer (NSCLC) diagnosis. Circulating cell-free tumor DNA (ctDNA) is a novel target material as a tool for liquid biopsy that monitors cancer status. In this paper, we detect EGFR mutations of ctDNAs using target-catalytic hairpin assembly (CHA) that is hybridized on gold nanoparticles (AuNPs). The detection is based on colorimetric method that occurs by the aggregation of AuNPs. In detail, three thiolated hairpin DNAs (H1, H2, and H3), catalyst DNA (C), and catalyst complementary DNA (c-C) are introduced to perform the CHA mechanism. Because the EGFR mutation DNA (target) contains very long nucleotides to detect directly, we devise catalyst and catalyst complementary DNAs. Firstly, we attached three hairpin DNAs to the AuNPs (d=20 nm) using thiol binding. We prepared C and c-C DNAs complex solution. When the target DNA is added to the solution containing C and c-C DNAs complex, target DNA displaces the C DNA from the complex. Then H1, H2, and H3 DNAs are activated in the presence of C DNAs and the hairpin DNAs are hybridized. As a consequence, AuNPs are aggregated corresponding to a red-to-blue color change. The result can be measured by naked eyes.

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Rapid bio-optical sensors for molecular diagnostic in human diseases

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Rapid, early, and accurate diagnosis of human diseases including human cancers, infectious diseases is essential for effective disease management and surveillance, and can reduce morbidity and mortality associated with the disease. Although significant advances have been achieved for the diagnosis of human diseases, these technologies are still far from ideal, being time consuming, complex and poorly sensitive and specific for clinical use as well as requiring separate assays for sample processing and detection. Recently, my team reports an isothermal, label-free, one-step DNA or RNA amplification and detection system, termed as ISAD for DNA and iROAD for RNA, for the diagnosis of human diseases. It couples one-step isothermal nucleic acid amplification method and bio-optical sensor based on silicon micro-ring resonator for simultaneous DNA or RNA amplification/detection in a label-free and real-time manner. The bio-optical sensor assay offers a one-step amplification/detection example to rapid analysis (< 20 min). The detection limit of the bio-optical sensor assay was found to be 10-times more sensitive than that of conventional methods including PCR and real-time PCR. We confirmed the clinical utility of the bio-optical sensor by detecting several targets (DNA or RNA) obtained from several human disease samples, such as tuberculosis, respiratory virus, malaria, and human cancers. We envision that the bio-optical sensor assay will be useful and potentially adaptable for better diagnosis of diverse human diseases including emerging infectious diseases, cancer, and neurological disorders.

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