Amplification-Free Detection of Circulating microRNA Biomarkers from Body Fluids Based on Fluorogenic Oligonucleotide-Templated Reaction between Engineered Peptide Nucleic Acid Probes: Application to Prostate Cancer.

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Background: MicroRNAs (or miRs) play a key role as regulators of gene expression and highly abundant in cells. A proportion of them are also detectable in biofluids making them ideal noninvasive biomarkers for pathologies in which miR levels are aberrantly expressed, such as cancer. Quantitative detection of miRs is commonly achieved using real time reverse transcription quantitative PCR (RT-qPCR). Although RT-qPCR displays high sensitivity, with minute levels of starting RNA being required, this analytical technique has many limitations: risks of contamination and error during each amplification step, high cost and lack of specificity of custom-made oligonucleotide probes, and high background fluorescence. The need for a standardized technique that is both sensitive and accurate enough to detect endogenous miRs for diagnostic purposes is clear.

Methods: Our general sensing strategy exploits the concept of oligonucleotide-templated reaction (OTR) where the miR of interest is used as a template to catalyze an otherwise highly unfavorable fluorogenic reaction between chemically engineered peptide nucleic acid (PNA) hybridization probes. PNAs are stable and uncharged oligonucleotide analogues chosen for their ability to hybridize to complementary nucleic acids with high affinity and specificity. Herein, novel PNA-based fluorogenic biosensors have been engineered that target miR biomarkers for prostate cancer (PCa). Whole blood samples of prostate cancer patients were collected at Imperial College Healthcare NHS Trust (London, UK) following written patient consent.

Results: Validated in vitro using synthetic RNAs, these biosensors were shown to be specific (responsive to point mutations) and to detect endogenous concentrations of miR in human blood samples without the need for any amplification step and with minimal sample processing. Using a cohort of 16 PCa patients, we were able to detect elevated levels of miR-141 and miR-375 in samples from patients with active disease. Results were not affected by the presence of heparin (commonly found in blood collection tubes and which confounds use of qRT-PCR), the probes operated consistently both in the presence and absence of heparin. Although tests were initially performed on extracted RNA from serum samples, similar results were also obtained when using our probes directly in serum, free from any processing steps.

Conclusions: We report a novel sensing technology for the quantitative detection of endogenous concentrations of circulating miR biomarkers in blood samples that does not require any amplification step and is isothermal and highly cost-effective. This low-cost, quantitative, and versatile sensing technology has been technically validated using gold-standard RT-qPCR. Unlike RT-qPCR however, this enzyme-free, isothermal blood test lends itself to incorporation into low-cost portable devices and could be suitable for widespread public screening (diagnostic biomarkers) as well as in predictive and prognostic tests.

Conflicts of Interest: None

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