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On-site microRNA detection with 'off-the-shelf' glucose meter empowered by chimeric probe connecting CRISPR/Cas13a activation to kinases-driven glucose phosphorylation

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Abstract

MicroRNAs (miRNAs) are promising biomarkers for cancer diagnosis due to their stability in body fluids and disease-specific expression profiles. However, current detection methods suffer from limitations including cumbersome workflows, heavy instrumentation for signal readout, or vulnerability in minimizing instrumentation. To address these challenges, we describe a novel point-of-care miRNA detection platform executable with "off-the-shelf", personal glucose meter (PGM), termed 'KEY-FACT (Kinases Ensemble-driven glucose phosphorYlation upon Fuel-Aided CRISPR acTivation)'. Upon recognition of target miRNA, a fuel-assisted toehold-mediated strand displacement reactions liberate guide RNAs (gRNAs) to activate Cas13a to cleave a chimeric reporter probe, producing 2',3'-cyclic adenosine monophosphates (cAMP). Subsequent dephosphorylation and kinases ensemble-mediated phosphorylation/dephosphorylation cycles lead cAMP to consume a large amount of glucose. A user can immediately measure resulting glucose level change with PGM on the spot. This strategy allows sensitive, prompt detection of miR-135b, a gastric cancer (GC) biomarker, with a limit of detection (LOD) of 1.4 pM within 2 h. KEY-FACT is specific to the target miRNA and is applicable to body fluids such as human serum with dilution (95.2% < recovery rates < 104.3%, coefficients of variation ≤ 13%). Owing to its simple probe design, KEY-FACT was readily expanded to detect another GC biomarker, miR-21, with comparable sensitivity (LOD = 1.5 pM). The proposed platform fulfills minimal instrumentation and thus enables cost-effective, field-deployable analysis, paving the way for practical, on-demand miRNA diagnostics.


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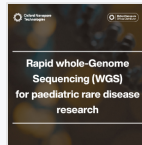
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