Revolutionizing CRISPR-based RNA diagnostics with single nucleotide resolution

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The global health landscape continues to be shaped by the ongoing challenges posed by RNA viruses.[1] In recent decades, RNA viruses such as HIV, influenza, Ebola and most recently SARS-CoV-2, have contributed significantly to widespread diseases and death, highlighting the urgent need for advanced diagnostic tools. RNA viruses have an extraordinary mutational rate that allows them to rapidly adapt to environmental stresses such as antiviral treatments, vaccines, and immune responses.[2,3] This rapid evolution also often leads to the emergence of new viral variants with altered transmissibility, pathogenicity, and immune evasion properties,[3,4] making early and accurate detection crucial for effective disease prevention, control, and treatment strategies. In addition, the ability to detect RNA biomarkers with high specificity and sensitivity, is of immense importance not only for disease diagnostics but also for personalized medicine.[5,6] CRISPR (clustered regularly interspaced short palindromic repeats) based technologies have proven to be a powerful tool that is revolutionizing RNA detection.[7] However, challenges remain, particularly when it comes to detecting mutations in complex, unamplified RNA samples or distinguishing between closely related single nucleotide variations (SNVs) that differ by a single base pair.[8]

In a recent issue of *eBioMedicine*, Li and colleagues have developed a one-pot, isothermal and single-nucleotide-resolvable nucleic acid assay using CRISPR/Cas13a probes and topologically constrained DNA rings (termed CasTDR$_{1pot}$) for rapid identification of SARS-CoV-2 variants at concentrations as low as 0.1 aM within 30 min.[9] This simplification is a key advantage in point-of-care testing (POCT), and the rapid results contribute to effective clinical decision making. In this study, CRISPR/Cas13a achieved specific differentiation of SNVs in the viral genome by introducing synthetic mismatches and hairpin structures of CRISPR RNA (crRNA). The CasTDR$_{1pot}$ system combines the sensitivity of Cas13a with an innovative topologically constrained DNA ring (TDR) for post-amplification. The TDR served as substrates for Cas13a trans-cleavage to enhance the amplification rate, enabling the identification of low-abundance RNA targets without pre-amplification. This feature is particularly important to avoid the biases introduced by amplification. Coupled with the coding strategy and portable device, the CasTDR$_{1pot}$ realized convenient, lab-free, and user-friendly detection of SARS-CoV-2 variants, which is of great significance for timely interventions and more accurate tracking of epidemic outbreaks.

This study introduces an innovative approach to RNA detection that combines the sensitivity and specificity of CRISPR/Cas13a with a TDR system for signal amplification. The resulting CasTDR$_{1pot}$ assay is capable of detecting SNVs in RNA with remarkable accuracy, speed, and simplicity. The advance promises to significantly improve diagnostic capabilities in viral and non-viral diseases, including the rapid identification of viral variants and RNA biomarkers. As RNA-based diagnostics become increasingly important in clinical settings, this study provides a key step towards the development of portable, low-cost and highly sensitive tests that can be deployed in resource-limited settings for epidemic surveillance and personalized medicine. Nevertheless, some issues about the implementation of this assay remains to be response, including biosafety considerations during heating, as well as repeatability and robustness at real clinical POCT settings. In addition, the storage conditions of CasTDR$_{1pot}$ assay are more demanding compared to antigen test strips, somewhat limiting its usefulness as at-home test. Future study needs to improve the multiplex capability and accurate quantification for more effective application in screening human respiratory or other diseases involving RNA biomarkers. Finally, the integration of the CasTDR$_{1pot}$ assay into existing public health surveillance systems is also critical for future investigation. The advantages of the rapid, portable design make it an attractive tool for POCT, but its widespread use in global

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health initiatives requires further validation and large-scale trials to confirm its efficacy in diverse epidemiologic settings.

Contributors

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Declaration of interests

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