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A CRISPR-based method for detecting mRNA in extracellular vesicles

A CRISPR-based assay both recognizes and amplifies target mRNA, achieving sub-attomolar sensitivity with single-nucleotide resolution. This method enables the detection of low-abundance mRNA in extracellular vesicles, providing clinically relevant information for precision oncology.

The problem

Liquid biopsy-based detection of actionable cancer mutations or minimal residual disease continues to gain traction[1]. However, its broader adoption and clinical impact are constrained by the focus on using this method to detect circulating tumor DNA, which is present in low concentrations and primarily represents dying cells. In contrast, the detection of messenger RNA (mRNA) offers potential advantages as it is more abundant than circulating tumor DNA and could provide insights into the functional states of tumors, potentially benefiting preclinical and clinical investigations.

Extracellular vesicles (EVs) have emerged as promising targets for mRNA analysis[2]. These nanoparticles protect mRNA from degradation and might serve as cellular surrogates, reflecting the properties of the parent cell. Despite these advantages, detecting EV-mRNA poses substantial technical challenges. EVs typically contain low mRNA copy numbers[3], and tumor-derived EVs constitute only a minor fraction of total EVs in blood. Thus, current EV-mRNA analyses require large sample volumes (>2 mL plasma) and sophisticated resources, impeding the integration of EV-mRNA tests into routine clinical assays. These limitations underscore the need for more sensitive and efficient analytical methods in EV-based liquid biopsy.

The solution

We drew inspiration from the CRISPR (clustered regularly interspaced short palindromic repeats) approach to biosensing. CRISPR technologies are increasingly adopted for molecular diagnostics because of their sequence-specific nuclease activity[4]. CRISPR-associated (Cas) proteins become active nucleases upon recognizing target nucleic acids. This property is used to cut quenched fluorescent reporter probes; these Cas-generated signals can be detected and usually increase linearly over time. Most assays amplify target nucleic acids before Cas recognition, but any errors during this amplification could lead to false positives.

We repurposed Cas activity to amplify both the target mRNA and the detection signal (Fig. 1a). The approach employs a signal template, a hybrid design that integrates a fluorescent reporter probe with a template for mRNA amplification. Unlike other approaches, the assay requires Cas13a to recognize its specific mRNA target before RNA replication and the exponential amplification of fluorescence signals is triggered. This distinct mechanism enables the assay to discriminate between single-nucleotide polymorphisms (SNPs) expressed in EVs, a capability attributed to the high selectivity of Cas13a. Moreover, the dual amplification of mRNA and fluorescence signals enables mRNA detection down to the sub-attomolar range.

We dubbed this assay SCOPE (self-amplified and CRISPR-aided operation to profile EVs) and validated its performance through systematic preclinical and clinical studies. In a notable example, SCOPE effectively differentiated *KRAS* wild-type mRNA in EVs from the SNPs G12C, G12D, G12S and G12V, surpassing the performance of a commercial assay for detecting these mutations. This validation of SCOPE catalyzed the design of our EV assays for monitoring tumor mutational burden in patients with colorectal cancer undergoing standard care (Fig. 1b), detecting early-stage lung cancer in mouse models, and molecularly stratifying cases of glioma.

The implications

SCOPE should enhance our understanding of EV characteristics as we explore the impact of its ability to detect highly sensitive and specific tumor mutations in preclinical and clinical research. We demonstrate that SCOPE detects circulating EVs harboring genetic mutations that correspond to those found in tumor tissues, further substantiating EVs as reliable liquid biopsy biomarkers. SCOPE has the potential to facilitate biomarker-driven opportunities for elucidating the mechanisms underlying cancer growth and the emergence of resistance, as well as for evaluating treatment response and identifying minimal residual disease. These outcomes could expedite clinical decision-making and the use of EVs in liquid biopsy applications.

Future assay development could incorporate protein detection into SCOPE's workflow, capitalizing on the intrinsic advantage of EVs as multi-cargo carriers. Detecting proteins would complement mRNA analyses, bridging tumor genotypes with phenotypes. For example, protein markers might provide additional insights into a tumor's tissue of origin[5], information that is challenging to ascertain from mRNA assays alone. The extension of SCOPE into other fields, such as infectious diseases and agriculture, where timely, sensitive and specific readouts are paramount, could also enhance its reach.

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EXPERT OPINION

"The SCOPE assay holds the potential to advance the analysis of scarce amounts of mRNAs in biospecimens, including mRNA encapsulated in EVs. The authors have characterized the EV samples in compliance with MISEV guidelines, enzymatic treatments have been used to confirm that the analyzed mRNA is encapsulated in EVs, and diverse preparation methods have been implemented to explore their compatibility with the SCOPE assay." **An Hendrix, Ghent University, Ghent, Belgium.**

FIGURE

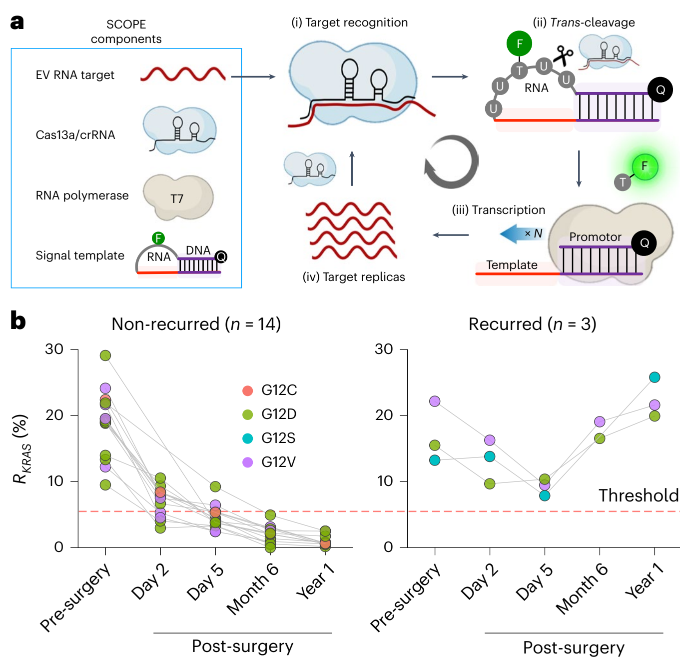

Fig. 1 | SCOPE assay. a, Target recognition by Cas13a (i), guided by CRISPR RNA (crRNA), triggers RNA *trans*-cleavage (ii), which turns on fluorescence signals by releasing fluorophores (F), which are conjugated to thymine (T), from quenchers (Q). This *trans*-cleavage also exposes the DNA template (iii), allowing polymerase (T7) to replicate RNA targets (iv). The signal template is a key component in coupling Cas13a and polymerase reactions. **b**, Longitudinal EV monitoring of patients with colorectal cancer. All patients exhibited a decreased *KRAS* SNP signal from their pre-surgical values following surgical intervention. In the cohort of patients with non-recurrent colorectal cancer, the SNP signal continued to decrease. Conversely, in patients experiencing cancer recurrence, the *KRAS* SNP burden gradually increased. R_{KRAS} indicates the ratio between *KRAS* mutation and wild type. © 2024, Song, J. et al.

BEHIND THE PAPER

We've held a long-term interest in EVs, advancing both hardware (sensors) and software (assays) for analyzing them. For years, detecting EV mRNA — an important but technically difficult task — had been on our minds. A catalytic moment occurred with the arrival of research fellow Jayeon Song. Her idea for a rapid COVID-19 test using CRISPR, based on the fact that the SARS-CoV-2 RNA particles look like EVs containing mRNA, gave us a fresh perspective.

A team came together to develop this concept into a tangible outcome. Engineers built a compact device and clinical partners helped validate the assay. The National Cancer Institute, through its Alliance for Nanotechnology and Liquid Biopsy Consortium, facilitated collaboration and resource sharing. We are indebted to Lynn Sorbara, the former program director of the Consortium, for her wholehearted support and enthusiasm. May this work serve as a fitting testament to her legacy. **C.M.C. & H.L.**

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FROM THE EDITOR

"In this manuscript, Song and colleagues introduce and evaluate the SCOPE assay, a novel integrated platform for the detection of EV-associated mRNA in biospecimens utilizing CRISPR-mediated target recognition, replication and signal amplification. The SCOPE assay employs a sophisticated Cas13/T7 polymerase-mediated dual target amplification loop, alongside Cas13-driven release of fluorescent dye from DNA/RNA signal sequences." **Editorial Team, *Nature Biotechnology*.**