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LETTER TO THE EDITOR Open Access

Comment on "A single-cell digital PCR method tailored for quantification of HBV DNA positive cells in liver biopsy tissues"

YuWei Chen[1], Guodong Zhao[3*] and JiaLi Song[2*]

Letter to the Editor

I would like to commend the authors for their outstanding work presented in the article titled "A single-cell digital PCR method tailored for quantification of HBV DNA positive cells in liver biopsy tissues" [1]. The study represents a significant methodological breakthrough in the field of hepatitis B virus (HBV) detection by addressing a crucial gap in single-cell resolution quantification. The development of single-cell infection detection PCR (scID-PCR), which combines single-cell encapsulation with dual-target detection (β-actin/cccDNA), enables precise quantification of HBV-positive cells in liver biopsy samples for the first time. This method offers superior sensitivity compared to traditional serum-based HBV detection, providing an important tool for personalized treatment in chronic hepatitis B (CHB) patients.

While the study demonstrates notable improvements in method optimization, there are a few areas that could be further explored to enhance the performance of scID-PCR. Firstly, the method is limited to detecting HBV genotypes A-E, excluding genotypes F, G, and H [1]. This may lead to potential false-negative results in some clinical cases. A simple improvement could involve designing reverse primers for genotypes F, G, and H, and combining them with the existing R3 primer mix. This approach has already been applied clinically for detecting multiple genotypes simultaneously [2].

Secondly, while the DNase I digestion approach optimized the detection of single HBV-positive droplets, a small number of HBV single-positive droplets remain [1]. This may result from differences in amplification efficiency between cccDNA and ACTB. Specifically, ACTB amplification may be less efficient than cccDNA, causing small number of droplets with single cells to appear cccDNA-positive but ACTB-negative. Additionally, inhibitors in the cellular environment may affect ACTB amplification more than cccDNA, leading to inconsistent results. The study did not explore the separate detection limits of cccDNA and ACTB, nor did it evaluate potential interference in dual-target detection. Addressing these aspects in future experiments could help clarify the cause of these residual droplets. Such optimizations would further enhance the sensitivity and accuracy of scID-PCR, ultimately improving its overall performance.

Lastly, while the authors rightly acknowledge the limitation of using liver biopsy samples, which are difficult to obtain regularly [1], but we believe that scID-PCR can be extended to other sample types such as bronchoalveolar lavage fluid [3] and urine [4]. These non-invasive samples could provide valuable insights into pulmonary and urinary cell exfoliation, allowing scID-PCR to be used for

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*Correspondence:
Guodong Zhao
gddn89429@hotmail.com

JiaLi Song
calliesong@163.com
[1]Department of Orthopedics, Suzhou Ninth Hospital Affiliated to Soochow University, Suzhou, Jiangsu 215200, China
[2]Department of Geriatrics, Suzhou Ninth Hospital Affiliated to Soochow University, Suzhou, China
[3]China Jiliang University, Hangzhou 310018, China

personalized and high-sensitivity detection in lung cancer and urological cancers.

In conclusion, this study marks a significant step forward in HBV detection, and I believe with these proposed refinements, scID-PCR has the potential to further advance clinical applications, particularly in personalized medicine for chronic hepatitis B and other diseases.

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Data availability
Not applicable.

Declarations

Ethical approval
Not applicable.

Consent for publication
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Competing interests
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