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Commentary

Enzyme-Free Strategies for Biomarker Amplification in Cancer Diagnosis

Narumi Okubo*

Department of Clinical Chemistry, Kyushu University, Fukuoka, Japan

DESCRIPTION

The early and accurate detection of tumor-associated biomarkers plays a pivotal role in the diagnosis and management of cancer. Conventional methods for biomarker detection often rely on enzymatic amplification techniques, which can be complex, time-consuming, and expensive. Enzyme-free isothermal amplification strategies have emerged as innovative alternatives, offering rapid, sensitive, and cost-effective solutions for biomarker detection. Tumor-associated biomarkers are molecular indicators that reflect the presence or progression of cancer. These biomarkers can include nucleic acids (DNA, RNA), proteins, and metabolites that are specific to cancer cells or tissues.

Detecting and quantifying these biomarkers provide valuable insights into cancer diagnosis, prognosis, and treatment response. However, the low abundance of certain biomarkers in clinical samples, coupled with the need for high sensitivity and specificity, poses challenges for their accurate detection. Traditional enzymatic amplification methods, such as Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA), have been widely used for biomarker detection. While effective, these methods often require sophisticated equipment, multiple steps, and skilled personnel. Moreover, the reliance on enzymes and temperature cycling can increase the complexity and cost of the assays.

Enzyme-free isothermal amplification strategies offer a revolutionary approach to biomarker detection by eliminating the need for enzymes and complex temperature cycling. These methods operate at a constant temperature, typically around 37° C-65°C, enabling simple and rapid assays. Rolling Circle Amplification (RCA) involves the isothermal amplification of circular DNA templates through continuous strand displacement. As the DNA template is amplified, it generates long single-stranded DNA products that can be easily detected. Hybridization Chain Reaction (HCR) exploits the programmable hybridization of short DNA hairpin sequences to initiate a chain reaction. Each hybridization event leads to the opening of a new hairpin, resulting resulting in amplification. Nucleic Acid Sequence-Based Amplification (NASBA) employs target-specific primers, reverse transcriptase, and RNA polymerase to amplify RNA sequences.

The process generates multiple RNA copies that can be quantified. Cross-Circular Rolling Strand Displacement Amplification (xCRSDA) involves the simultaneous amplification of multiple target DNA sequences through cross-circular strand displacement, resulting in a significant increase in amplification efficiency. Exponential Amplification Reaction (EXPAR) uses a DNAzyme as a catalyst to facilitate the isothermal amplification of target DNA sequences. The DNAzyme cleaves its substrate to generate additional copies of the target. Enzyme-free isothermal amplification strategies have demonstrated remarkable potential in cancer detection due to their simplicity, rapidity, and sensitivity.

Enzyme-free isothermal amplification can be modified to detect specific DNA or RNA sequences associated with cancer. This includes the identification of mutations, deletions, insertions, and gene expression changes that serve as biomarkers. MicroRNAs (miRNAs) play a critical role in cancer regulation. Enzyme-free isothermal amplification techniques can accurately quantify miRNA levels, aiding in cancer diagnosis and prognosis. Amplification of DNA or RNA templates can be coupled with nucleic acid aptamers that specifically bind to tumor-associated proteins.

This approach enables the indirect detection of proteins through the amplification of nucleic acid signals. Enzyme-free isothermal amplification methods can be integrated into microfluidic devices to detect CTCs, which are shed from primary tumors and circulate in the bloodstream. Detection of Circulating Tumor Cell (CTC)-associated biomarkers has the potential to provide non-invasive cancer diagnosis and monitoring. Enzyme-free isothermal amplification eliminates the need for complex temperature cycling, enzymatic reactions and reducing assay complexity

The simplified assay procedures and reduced reliance on

Correspondence to: Narumi Okubo, Department of Clinical Chemistry, Kyushu University, Fukuoka, Japan, E-mail: narokb@gmail.com

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enzymes contribute to cost-effective detection methods, making them accessible for widespread use. Enzyme-free isothermal amplification methods can achieve high sensitivity and specificity, enabling the accurate detection of low-abundance biomarkers. The portability and simplicity of these methods make them well-suited for point-of-care diagnostics, facilitating rapid and decentralized cancer detection. However, challenges remain in optimizing enzyme-free isothermal amplification techniques for various biomarker types and clinical samples.

Ensuring hygiene, reproducibility, and minimizing false positives or negatives are essential considerations. Advancing methods that allow simultaneous detection of multiple biomarkers will enhance diagnostic accuracy and clinical utility. Miniaturization and integration of enzyme-free isothermal amplification assays into portable devices will facilitate point-of-care testing and remote monitoring. Rigorous validation of enzyme-free isothermal amplification methods in clinical settings is significant to establish their diagnostic accuracy, sensitivity, and specificity. The discovery of novel tumor-associated biomarkers that can be effectively detected using enzyme-free isothermal amplification methods will expand their clinical applications.

CONCLUSION

Enzyme-free isothermal amplification strategies represent a paradigm shift in the field of biomarker detection, offering rapid, sensitive, and cost-effective solutions for cancer diagnosis and monitoring. These methods harness the power of nucleic acid amplification without the need for enzymes and complex temperature cycling.