РАЗДЕЛ II

БИОТЕХНОЛОГИИ

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ULTRASENSITIVE-SELECTIVE DNA MACHINE FOR TESTICULAR GERM CELL TUMORS DETECTION *

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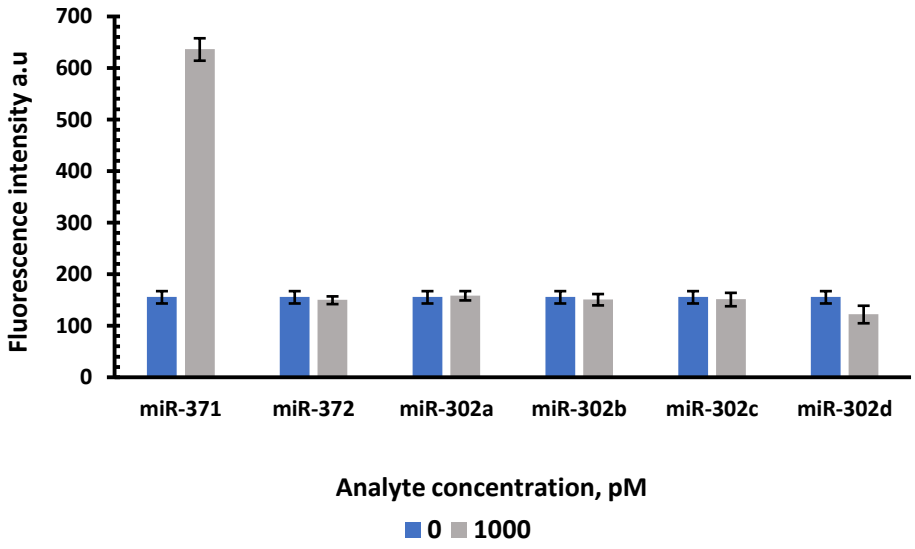
Abstract

Although advancements in cancer biomarkers detection have improved, current techniques for detecting Testicular Germ Cell Tumors (TGCTs) struggle with specificity. This study introduces a novel DNA machine (DNM) that ensures high selectivity in detecting miR371, a critical biomarker for TGCTs, overcoming the challenges posed by the presence of similar miRNAs.

TGCTs are the leading solid malignancy in young men, originating from germ cells in the testicles. Early and precise detection is crucial for effective treatment and better patient outcomes. miR371, a microRNA elevated in the early stages of TGCT, can be considered as a TGCT related oncomarker [1], but its high sequence similarity with five other miRNAs (miR372, miR203a, miR302b, miR302c, miR302d) complicates detection using conventional PCR methods, reducing diagnostic specificity.



Testing the DNM design to detect miR371 compared to five other similar miRNAs

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DNA machines based on binary deoxyribozyme (Dz) offer a cost-effective, sequence-specific, and sensitive alternative for DNA/RNA analysis. The catalytic core formation of binary Dz is pivotal for accurate analyte recognition. A binary Dz consists of two separate strands that hybridize to the target miRNA, forming a catalytic core. This core subsequently binds and cleaves a fluorogenic reporter substrate, amplifying the detection signal [2]. Recent developments, including the hook-equipped DNA machine (HDNM), have enhanced detection sensitivity [3].

Our DNM design incorporates miR371-binding arms complementary to miR371 but with deliberate mismatches for the five similar miRNAs. These mismatches increase free energy and cause unstable hybridization, preventing stable Dz catalytic core formation with non-target miRNAs. This instability ensures high selectivity, specifically targeting miR371. Initial experiments with synthetic miRNAs confirmed the DNM's superior selectivity and sensitivity (see Figure), achieving a limit of detection of 28.9 pM. Further tests with clinical samples from TGCT patients and healthy donors demonstrated a 68.2 % accuracy rate in identifying TGCT subtypes from 22 samples.

The innovative design of our DNM enhances selectivity, addressing key issues with current methods. Although PCR is still needed for sensitivity, DNM's high selectivity for miR371 reduces false positives, improving diagnostic accuracy. This makes DNM a valuable tool for early and precise TGCT diagnosis.

References

1. Nestler T., Schoch J., Belge G., Dieckmann K. MicroRNA-371a-3p — The Novel Serum Biomarker in Testicular Germ Cell Tumors // Cancers. 2022. Vol. 15 (15). P. 3944.

2. Kolpashchikov D. M. A binary deoxyribozyme for nucleic acid analysis // Chembiochem: A Eur. J. Chem. Biol. 2007. Vol. 8, 17. P. 2039–2042.

3. Hussein Z., Nour M. A. Y., Kozlova A. V. et al. DNAzyme Nanomachine with Fluorogenic Substrate Delivery Function: Advancing Sensitivity in Nucleic Acid Detection // Anal. Chem. 2023 Vol. 95 (51). P. 18667–18672.