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Nanopore Detection of Cancer Biomarkers: A Challenge to Science

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Abstract

Cancer is the most complex and leading cause of fatality worldwide. Despite meritorious research in the field of cancer, it is still a substantial threat to human life. In this article, we address a question on the present strategies and manifest the importance of critical biomarkers for cancer screening and early diagnosis before the symptoms appear. However, this goal can only be achieved if scientists will focus on ultra-sensitive detection techniques such as "Nanopore." Nanopore sensing is a simple and rapid single-molecule detection technique that can detect multiple cancer biomarkers in femto-Molar concentrations in real time. Last but not least, we propose a systematic policy to win the war against cancer that is a big challenge to science.

Keywords

nanopore, early detection, cancer, cancer biomarkers, screening, diagnosis

Abbreviations

DNA, deoxyribonucleic acid; RNA, ribonucleic acid; α-Hemolysin, Alpha Hemolysin; MspA, *Mycobacterium Smegmatis* porin A; Si_3N_4, silicon nitride; miRNA, MicroRNA; GSH, glutathione; CD, circular dichroism; FRET: fluorescence resonance energy transfer

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Cancer, a term that is self-explanatory in context of complexities and deadliness, is one of the greatest causes of death worldwide and is responsible for almost 10 million deaths in 2020.[1] Despite rigorous research on cancer, it is still a matter of great concern because of the drawbacks in the present detection techniques and hence treatment strategies. We want to draw the attention of scientists towards a noteworthy question that "Why cancer cannot be cured completely, however science is making progress day by day?" The answer is because almost all types of cancer penetrate their roots into the body before the symptoms appear. This simple answer points towards a significant area where the science is lacking somehow that is *"Sensitive Detection Techniques"* and emphasizing the gist that "early detection of cancer is as crucial as treating the cancer." Therefore, we believe that there is an urgent need to review our diagnostic methods to overcome cancer-related financial burden and mortality.

In this regard, the role of early and accurate detection of cancer biomarkers is of paramount importance in cancer screening, diagnosis, and monitoring treatment efficacy. Cancer biomarkers are found in tumor tissues and body fluids (such as blood and urine) and can be classified into 3 main types depending upon its application, namely: (1) prognostic, (2) diagnostic, and (3) predictive biomarkers. Cancer biomarkers hold a wide range of molecules that includes DNA, RNA, proteins, enzymes, and other small molecules, therefore, cancer biomarkers are further classified on the type of molecule.[2] Hence, the best way to reduce cancer prevalence and mortality is to implement cancer screening/early diagnosis of cancer by assessing cancer biomarkers and that demands an extremely sensitive, accurate, and noninvasive detection technique.

"Nanopore sensing" is a single-molecule technique that fits well to the definition of ultra-sensitive detection techniques owing to high throughput,[3] label-free,[4] rapid,[5] ultra-sensitive,[6] specific,[7] and a small concentration of target molecule is enough to be detected.[8] Nanopores are classified into 2 main types: (1) Biological/Protein nanopore embedded in lipid bilayer such as α-hemolysin[9] and *Mycobacterium Smegmatis*

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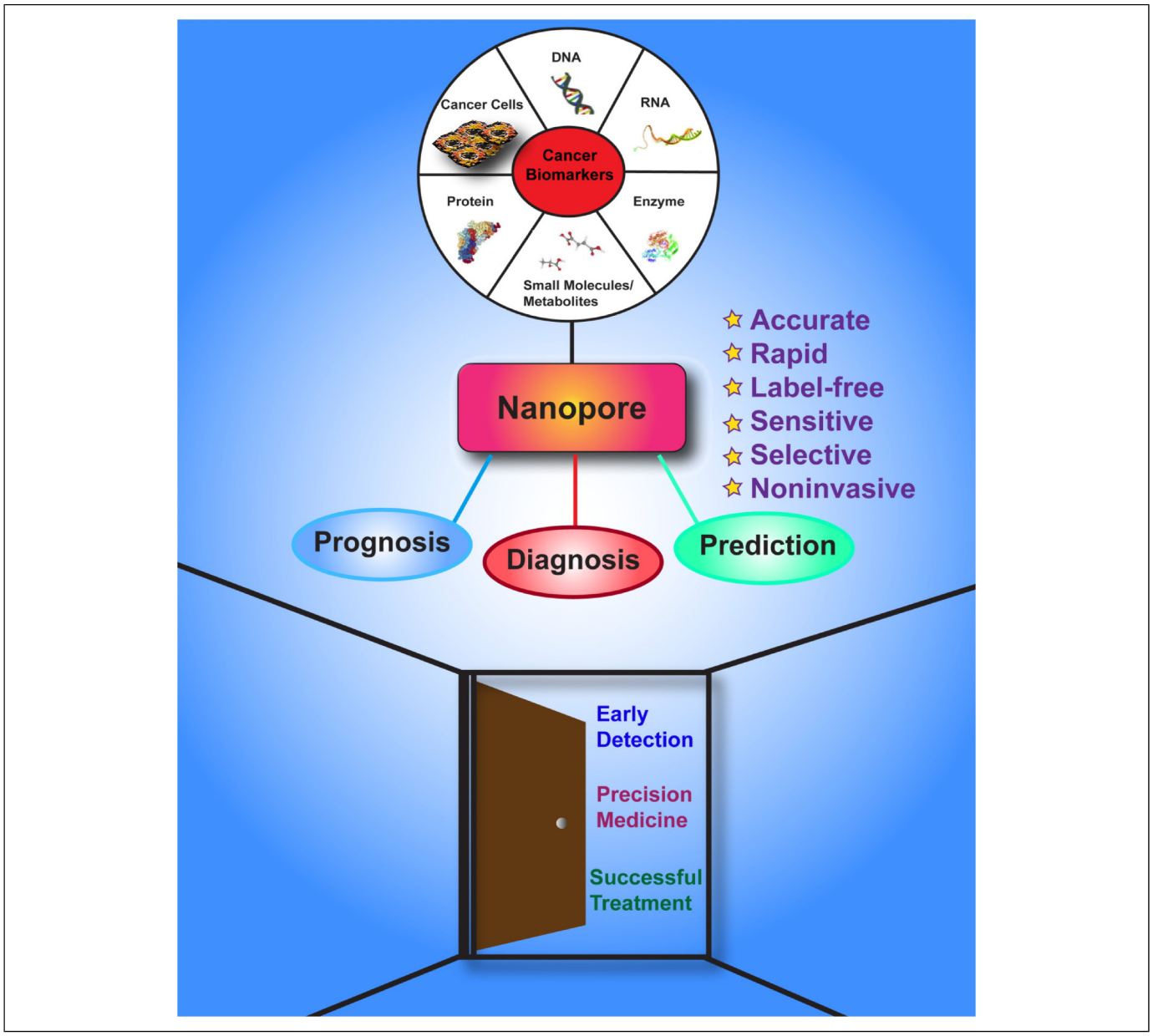

Figure 1. Nanopore detection of cancer biomarkers.

porin A (MspA) nanopore;[10] (2) Solid-state/Synthetic nanopore fabricated in synthetic membrane like Si_3N_4[11] and graphene.[12] Biological nanopores are more convenient because of their natural tendency towards analyte biomolecule and also the smaller size of the constriction zone favors high signal-to-noise ratio as compared to solid-state nanopore.[13,14] However, the stability and adjustable size of solid-state nanopore are beneficial in sensing some nontrivial macromolecules that cannot accommodate in biological nanopore.[15]

In addition to DNA, RNA, and protein sequencing,[16-18] both types of nanopore have shown tremendous applications in detecting a variety of critical molecules including cancer biomarkers;[19,20] for example, DNA methylation,[21,22] lesion,[23,24] circulating tumor DNA,[25] and point mutations.[26,27] Moreover, nanopore has shown remarkable merits in identifying other significant cancer biomarkers including microRNA,[28,29] protein,[30] small molecules,[31] enzymes,[32] and cancer cells itself.[33,34] Recently, glutathione (GSH) is in the limelight as a potent cancer biomarker, but their assessment is challenging by conventional methods.[35] Interestingly, nanopore sensing eradicated the analytical mishandling of GSH by direct insertion of glass nanopore into single cancer cell.[36] Moreover, exosomes and their miRNA have also been receiving attention as cancer biomarkers and detected by nanopore in real time from cancer cells.[37,38] Research shows that cancer can be screened 4 years earlier before the symptoms of cancer arise.[39] There are various routine tests that are common to screen all kinds of cancer but further confirmation is made by some invasive

detection methods such as tissue biopsy, lumbar puncture or radiological assays. These examinations are painful and risky and need follow ups for several months; therefore, cancer is challenging not only at diagnostic and treatment stages but in prognostic stage also. Nanopore sensing has been surpassing these challenges too by its noninvasive detection strategy.[40]

Despite commendable research in nanopore detection of cancer biomarkers (Figure 1), some momentous shortcomings must be addressed before the implementation of technique in clinical practice. Firstly, the translocation speed of an analyte is fast to reliably read the ionic current of an individual nucleotide in a biomolecule[41] and secondly, the homopolymer sequence in a DNA stretch is prone to error.[42] However, it can be overcome by employing some methods for example, lowering temperature of nanofluidic cell, increasing viscosity of solution, surface modification, by introducing some adaptors or by utilizing genetic engineering in case of biological nanopore. Additionally, machine learning algorithm and other assisted technologies such as circular dichroism, fluorescence resonance energy transfer (FRET), and so on have been facilitating nanopore in this regard.

Thus, we believe that nanopore sensing is the rapidly growing field owing to its simple, rapid, noninvasive, and ultrasensitive detection skills; therefore it can overcome various flaws of the present techniques in not only diagnosing cancer at early stages but in monitoring anticancer drug resistance also.[36] We anticipate that nanopore sensing can upgrade the cancer screening and diagnosis to the next level if the strategy given below is followed: (1) selection of an appropriate set of biomarkers, (2) accurate nanopore detection of biomarkers, and then (3) a meticulous treatment plan should be set on individual patient's need. This manoeuver will dramatically ameliorate the advancement in personalized medicine and hence goal to defeat cancer can be accomplished.

Declaration of Conflicting Interests

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