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Biomarkers and Biosensors for Cervical Cancer Diagnosis

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ISBN 978-981-16-2585-5 ISBN 978-981-16-2586-2 (eBook)
https://doi.org/10.1007/978-981-16-2586-2

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Acknowledgments

The editors are grateful to the Ministry of Human Resource Development, Government of India for funding the project titled "Affordable Colorimetric and Electrochemical Biosensors for the Early-Stage Detection of Cervical Cancer" through Scheme for Promotion of Academic and Research Collaboration (SPARC) "SPARC/2018-2019/P402/SL." We also acknowledge the support received from the National Research Foundation (NRF) of Korea for the Basic Science Research Program funded by the Ministry of Education (NRF-2019R1A6A1A03033215). We are grateful to SASTRA Deemed University, Thanjavur, India and Sungkyunkwan University (SKKU), South Korea for providing infrastructural support. We would like to express our heartfelt gratitude to the contributing authors, who assisted us in the development of this book. We would also like to express our gratitude to Dr. Bhavik Sawhney, Editor-Biomedicine, Springer Nature for his feedback, guidance, and support in finalizing this book.

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About the Editors

John Bosco Balaguru Rayappan is a Professor and Dean-Sponsored Research at SASTRA Deemed University, Thanjavur, Tamil Nadu, India. Presently, he is heading the Nanosensors Laboratory at SASTRA through research grants obtained from DST, New Delhi, DRDO, New Delhi, DBT, New Delhi and SASTRA Deemed University. His research interests are across diverse fields of Lattice Dynamics, Nanosensors, Biosensors, Embedded System and IoT. He has published more than 310 research articles in these fields and filed 7 patents including one US patent. He is editorial board member and reviewer for several journals and has 25 years of teaching experience.

Jung Heon Lee is an Associate Professor in the School of Advanced Materials Science and Engineering at Sungkyunkwan University (SKKU). He is also affiliated with Biomedical Institute for Convergence at SKKU (BICS), the Institute of Quantum Biophysics (IQB), and the Research Center for Advanced Materials Technology at SKKU. Prof. Lee is leading the Biological and Nanoscale Materials Laboratory at SKKU, where he is focused on developing nanoscale materials or devices which can be applied to diverse levels of biological systems for diagnostics or therapeutics. He is also investigating opportunities to adapt programmable and readily modifiable properties of biomolecules in the fabrication of smart materials or advanced nanoscale devices. He has also published more than 85 research articles in peer-reviewed international journals and filed 50 patents, including 7 US patents and 5 PCT patents.

An Introduction to Cancer Biomarkers

1

Muthaiyan Lakshmanakumar, Arockia Jayalatha JBB, and Noel Nesakumar

Abstract

Cancer is the abnormal growth of cells due to the accumulation of changes in the genetic program that regulates the proliferation, growth and survival of cells. The impact of cancer on society is huge and hence, rapid, efficient and reliable detection is deemed essential in cancer management since it can lead to successful medical strategies, reduce treatment expenses and dramatically improve patient outcomes and survival rate. In this context, the barriers to cancer diagnosis and various approaches, including ultrasound, thermography, X-ray, tissue biopsy and optical techniques, pertaining to cancer detection are elucidated in this book chapter, with a special focus on how analytical techniques help clinicians detect cancers at an early stage. The merits and demerits of these analytical techniques for detecting cancers are also discussed in this book chapter. Finally, advances in

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J. B. B. Rayappan, J. H. Lee (eds.), *Biomarkers and Biosensors for Cervical Cancer Diagnosis*, https://doi.org/10.1007/978-981-16-2586-2_1

biotechnology science that have contributed to the development of biosensors for cancer detection are outlined.

Keywords

Cervical cancer · HPV · DNA · Cancer diagnosis · X-ray

1.1 Introduction

Cancer is the abnormal growth of cells due to the accumulation of mutations in the genetic program that regulates the proliferation, growth and survival of cells [1, 2]. When cancer metastasizes, the tumour starts spreading beyond the primary site and rapidly spreads throughout the body to several other organs, making it untreatable. There are more than 200 different types of cancer, including leukaemia, colon, skin, hematologic, ovarian, breast, prostate lung cancer and more. Also, a few cancers such as cervical and stomach cancer are directly linked to viral and bacterial infections, respectively [3, 4].

Cancer is considered as the second most common cause of death worldwide. The World Health Organization (WHO) report indicates that the number of cancer-related deaths in 2014 was nearly 1.3 million in the European Union and in 2015, around 8.8 million people worldwide died of cancer. In the year 2014, the average mortality rate in the European Union was almost 261.5 per 100,000 people, which was significantly lower than the mortality rate for heart diseases; however, higher than the average mortality rate for several other causes of death. It is also predicted that during the lifetime of men and women, 38.4% of men and women worldwide will be diagnosed with cancer, according to the National Institutes of Health. About 1.1 crore people are expected to develop cancer each year and it is predicted that 1.6 crore people will be diagnosed with cancer by 2020 [5, 6].

The impact of cancer on society is huge and hence rapid, efficient and reliable detection is deemed essential in cancer management since it can lead to successful medical strategies, reduce treatment expenses and dramatically improve patient outcomes and survival rate. Cancer is an extremely heterogeneous disease that presents a wide variety of genetic and morphological variations that in turn shapes its prognosis in clinical practice. In particular, cancers develop gradually over time in response to specific exogenous and/or endogenous stimuli. Cancer can indeed be described as a breakdown in the communication network of cells. The genetic changes lead to modified proteins which induce stimuli that interact with normal cellular pathways. Early diagnosis of cancer is a political and public concern and primary care is the preferred framework for this to happen. Advancements in the early diagnosis of cancer have led to a need for improved cancer screening tests, preferably those that can be used in primary care. Nevertheless, continuous and extensive work in the development of new biomarkers and other assessments has had a positive impact on the prognosis and evaluation of patients previously diagnosed with the disease. In particular, there are few advantages to enhance the accuracy and

promptness of cancer diagnosis in cancer patients who typically have symptoms. A broad variety of screening tests could be beneficial. A wide variety of stakeholders, including industry, investors, health care professionals, policymakers and customers, have recognized this great challenge to improve the accurate diagnosis of cancer [7, 8].

Cervical cancer, induced by the exponential proliferation of cells in the cervix, is one of the deadliest illnesses that requires extensive research in the field of biosensor for ultra-low detection of biomarkers specific to cervical cancer. The human papillomavirus (HPV) induces cervical cancer, which is a common sexually transmitted disease that affects the female reproductive system. Cervical cancer has become the leading cause of death after breast cancer, which has affected the socialization of the working environment of women. With operational excellence in diagnostic techniques and vaccination strategies in developed countries, the rapid recognition of early stage cervical cancer in women living in resource-limited settings with adequate medication has become a crucial requirement. At first glance, HPV may appear to be a genetically engineered cancer-causing machine, but, of the 150 HPV genotypes identified, only a few are high risk or oncogenic. Although in many HPV-infected patients the virus is rapidly removed through their own immune response, some people could be asymptomatic as the virus remains in an inactive state, and several other infected individuals may develop recurrent infections [9, 10]. Several attributes, including localization, spread, size and lesion type, determine the degree of HPV infection and assist clinicians to choose the correct treatment. Cervical cancer is usually curable if diagnosed and treated early, but in later stages, the cancer spreads throughout the body to the remaining part of the abdominal walls, rectum, bladder and uterus and eventually enters the pelvic lymphatic system, affecting other organ systems and resulting in death. Therefore, it is highly desirable to diagnose cancer at an early stage and to plan for effective drugs. Traditional histological techniques have poor accuracy and efficacy in the early detection of cancer, although they are time intensive. It is therefore essential to develop innovative tools for the rapid recognition of specific biomarkers and for monitoring therapeutic responses. Electrochemical techniques explore innovative ways to operate quickly and selectively, as cells immobilized on the surface of the electrode can generate electrochemical signals that can be beneficial in the design and fabrication of effective biosensors. The oxidation process and the ion changes that take place owing to distinct cellular functions result in the generation and transfer of electrons at the surface of living cells, and therefore, the living cell is considered to be an electrochemical system. Conversely, there are also many sensors available in the market to recognize the types of HPV. In particular, CLART®human papillomavirus 2, clinical arrays®HPV, INNO-LiPA, PapilloCheck®, linear array®, digene HC2 high-risk HPV DNA and COBAS®4800 are the main commercially exploited biosensors that provide enhanced sensitivity for ultra-low detection of pre-cancerous lesions and HPVs in human blood serum samples. Besides that, the techniques listed have shortcomings, including sophisticated equipment, the need for trained personnel, high response time and consumption of expensive reagent [11–13]. Therefore, despite the various drawbacks of established detection techniques, it

is essential to design and develop an effective advanced analytical tool for the recognition of HPV with high selectivity and improved sensitivity.

The obstacle in the diagnosis and monitoring of cancer is to identify these proteins, renegade genes as well as other biochemical compounds, including cancer-related biomarkers at a very initial stage. Healthcare professionals had recognized cancer according to its pathology before molecular detection was feasible, which is associated with its appearance under the microscope of tissue sections of the patient [14–16]. Early detection and selective drugs are the existing practical strategies that impact the lives and health of cancer sufferers. Conversely, prior to the actual onset of the disease, rapid, inexpensive and robust diagnosis of cancer markers in human blood and other such bioavailable fluids makes it possible to study the development of tumour. Conversely, significant advancements with an in-depth understanding of the growth of tumour at the cellular, molecular and genetic levels help to develop accurate diagnostic methods to identify cancer markers at the molecular level. Substantial developments have been achieved in the identification of human tumours over the past decade [17–19]. Because of the expertise gained from emerging fields including molecular imaging, metabolomics, proteomics and genomics, cancer cells can now be characterized at genetic and molecular levels via employing advanced molecular tools, including protein and gene chips, mass spectroscopy and positron emission tomography. Molecular detection can assess how well these proteins and genes communicate through patterns of activity within the cell [20–23]. Such altered expression patterns in various kinds of cancer cells or "molecular fingerprints" enhance the potential for doctors to diagnose cancer. Molecular changes in cancer cells can be analysed by examining the sequence and genetic information of deoxyribonucleic acid (DNA) and the levels of metabolic products and proteins. The gene expression patterns and the number of copies of DNA, in particular, are key characteristics for the identification of cancer cells. The advances in DNA array technology have enabled reliable and quantifiable measurements of alterations in gene expression and chromosomal gains in human tumours [24, 25]. The individual tumour cell can be characterized by unique molecular specifics in conjunction with specific gene sequencing aimed at looking for point mutations. Over the past few years, recognizing single nucleotide-polymorphism-based genotyping to identify alleles associated with a greater risk of acquiring several diseases, including cancer, diabetes or other illnesses, has also been gaining popularity and significance among doctors and the wider community. These emerging innovations and the level of knowledge of molecular signatures will improve the ability to destabilize disease progression by monitoring, controlling and catalysing progress toward the use of targeted cancer therapies. In addition to the use of specific body fluids and tissue for in vitro characterization of biomolecules as cancer biomarkers, molecular imaging approaches concentrated primarily on positron emission tomography and magnetic resonance imaging [26–28].

Usually, understanding tumour's molecular environments is imperative for guiding and offering better treatment options in clinical settings. Tissue biopsies are the popularly adopted method of obtaining specific tumour molecular information and are required to identify its nature, including screening, expression of mutations in

genes and the type of cancer. Nevertheless, it is full of issues, including the need for invasive surgical extraction, which may induce pain and discomfort in individuals. The clinical strategies associated with tissue biopsy involve many health risks and the likelihood of medical complications [29–31]. In addition, some tumours are inaccessible in certain anatomical sites, which are not suitable for a biopsy, and, in certain instances, their extraction may increase the risk of metastatic lesions. Often, the amount of tissue sample collected is insufficient for all diagnostic tests and the action must be carried out once again, which becomes necessary even otherwise if the tumour is not homogeneous [32–34]. In addition, the solid biopsy techniques are time consuming in terms of analysis, entails huge expenses and needs an operating theatre. Although biopsies from different metastatic sites can be performed at the same time, initiation of treatment may be delayed due to examination of the samples. In addition, tumour development desperately needs to be examined at different intervals for cost-effective treatment of the illness, so that solid biopsies can no longer be considered as highly invasive [35–37]. Typically, optical approaches are employed; yet, they do not render significant information about tumour. Radiology is often used routinely, but high levels of radiation can pose a health risk to the patient. Conversely, magnetic resonance imaging (MRI), a non-radiation method, is regarded as unreliable and unsuitable for the detection of minimal residual disease. In addition, safety is a concern, for example, in patients with comorbid conditions. Such approaches are also clinically impracticable and cannot cover the tumour's spatial and temporal heterogeneity [38–40].

X-ray allows clinicians to examine cancer in various parts of the body, including kidney, stomach and bones. Contrast analyses may need further planning in advance and can induce some pain and health risks based on the type of contrast materials employed. Typically, chest X-ray is the first assessment tool employed to detect lung cancer. Majority of lung tumours are present as a greyish white cloud on X-rays [41–43]. Chest X-rays, however, do not provide an appropriate diagnosis because they cannot differentiate between other conditions and cancer, including a lung abscess. Moreover, it produces harmful radiations which can pave the way for the possibility of cancer. In contrast, certain cytology assessments, including the Pap test, are being used primarily for screening, whereas others recognize cancers reliably. A diagnostic procedure is most often used when a screening test is positive [44–46].

Thermography, a non-invasive diagnostic tool, is a painless, rapid and cost-effective technique used to measure the temperature of the skin surface in a non-contact mode. Thermography is commonly used in the diagnosis of cancer and has been the focus of numerous biomedical researches in recent decades. Few thermography-based cancer diagnosis findings indicate that the venous blood entering the cancer location is always hotter than its arterial supply. Nevertheless, thermograms alone will not suffice to make an effective clinical diagnosis for healthcare professionals. The integration of statistical tools, including non-linear regression methods and artificial neural networks, is strongly recommended to accurately assess the thermogram [47, 48].

Ultrasound has played an important role in detecting cancer as a standard medical imaging method. The general characteristics that the ultrasound diagnosis of cancer