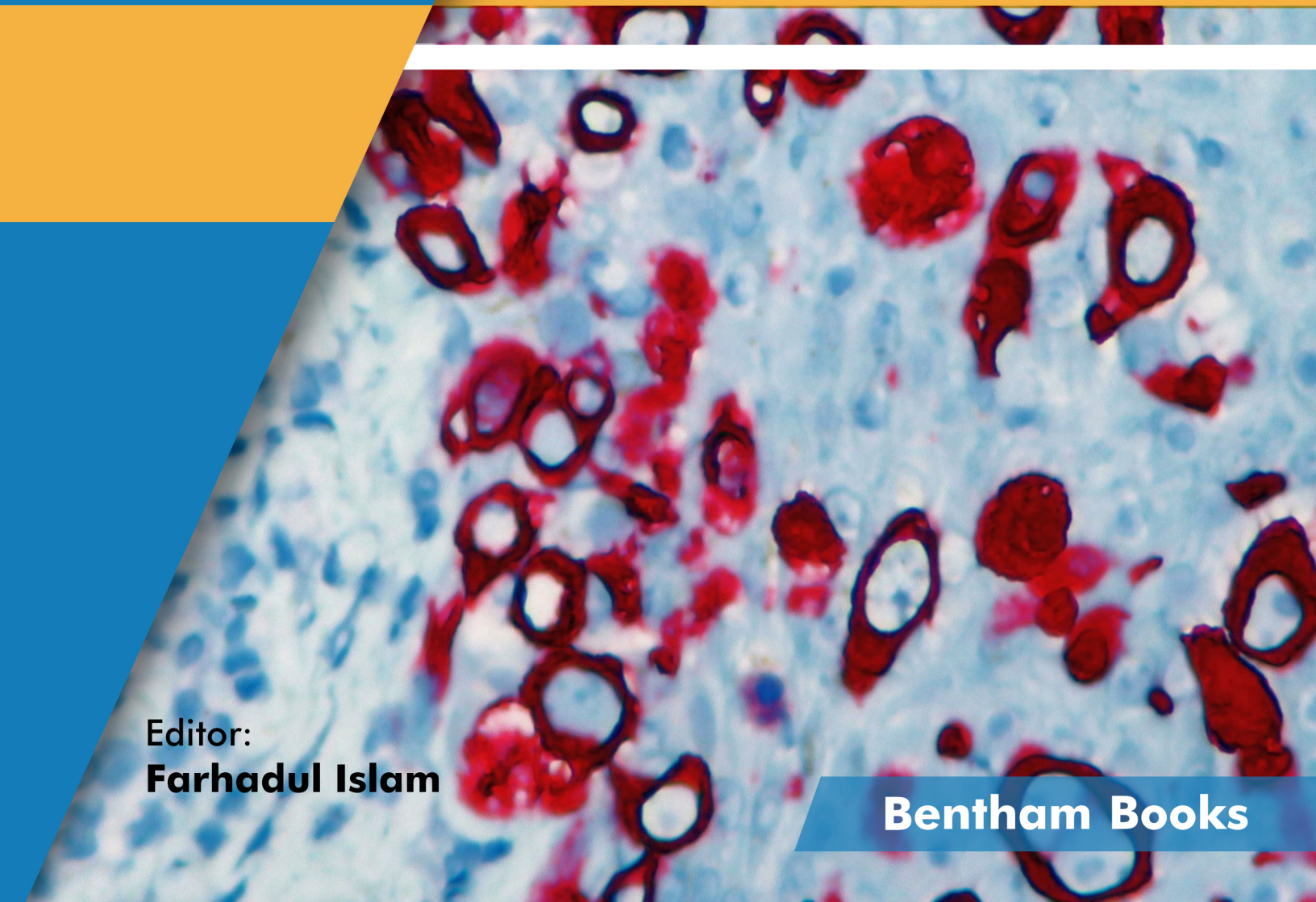
CURRENT CANCER BIOMARKERS



Editor:
Farhadul Islam

Bentham Books

Current Cancer Biomarkers

Edited by

Farhadul Islam
Department of Biochemistry and Molecular Biology,
University of Rajshahi, Rajshahi, Bangladesh

Current Cancer Biomarkers

Editor: Farhadul Islam

ISBN (Online): 978-981-5079-36-4

ISBN (Print): 978-981-5079-37-1

ISBN (Paperback): 978-981-5079-38-8

First published in 2023.

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

FOREWORD i

PREFACE ii

LIST OF CONTRIBUTORS iii

PART 1 INTRODUCTION AND CLINICALLY USED BIOMARKER

CHAPTER 1 INTRODUCTION: CURRENT STATUS AND FUTURE ADVANCES IN CANCER BIOMARKERS 1

Farhadul Islam

INTRODUCTION 1

Classical Cancer Biomarker 3

Diagnostic Cancer Biomarkers 3

Prognostic Cancer Biomarkers 4

Predictive Cancer Biomarkers 5

Future Cancer Biomarkers 5

CONCLUDING REMARKS 8

CONSENT FOR PUBLICATION 8

CONFLICT OF INTEREST 8

ACKNKOWLEDGEMENT 8

REFERENCES 8

CHAPTER 2 TUMOUR MARKERS IN CLINICAL USE 11

Sujani M. K. Gamage, Chamath D. Ranaweera, Tracie T. Cheng, Sharmin Aktar, Vinod Gopalan and *Farhadul Islam*

INTRODUCTION 11

Markers for Colorectal Cancer 12

Diagnosis 13

Prediction of Prognosis 13

Follow up 13

Markers for Breast Cancer 14

Markers for Thyroid Cancer 15

Importance of Thyroglobulin in Differentiated Thyroid Cancer (DTC) 15

Surveillance after Thyroidectomy 15

Determination of the Requirement of Radioactive Iodine Therapy 16

Importance of Calcitonin in Medullary Thyroid Carcinoma (MTC) 16

Marker for Hepatocellular Carcinoma 17

Screening 17

Staging and Further Assessment 18

Surveillance in HCC 18

Markers for Gall Bladder and Cholangiocarcinoma 18

Screening 18

Diagnosis 19

Follow up 19

Markers for Pancreatic Carcinoma 19

Screening 19

Diagnosis and Workup 19

Follow up 20

Markers for Ovarian Carcinoma 20

Screening 21

Diagnosis 21

Follow up 21
Markers for Testicular Carcinoma 22
Beta-hCG (β-hCG) 22
Lactate Dehydrogenase (LDH) 23
Alpha-Fetoprotein (AFP) 23
Markers in Neuroendocrine Tumours 23
Markers in Prostate Cancer 24
Screening 24
Diagnosis 25
Treatment 25
Follow up 25
CONCLUSION 27
CONSENT FOR PUBLICATION 27
CONFLICT OF INTEREST 27
ACKNOWLEDGEMENTS 27
REFERENCES 27

PART 2 DNA/RNA BIOMARKERS

CHAPTER 3 DNA METHYLATION LANDSCAPES IN CANCER AND NON-CANCER CELLS 33
Shaun Stangl and *Vinod Gopalan*
INTRODUCTION 33
Normal DNA Methylation Location 35
Aberrant DNA Methylation in Cancer Genomes 36
Methylation-Based Biomarkers 39
Bladder Cancer 40
Breast Cancer 40
Colorectal Cancer 41
Lung Cancer 41
Ovarian Cancer 42
Prostate Cancer 42
CONCLUDING REMARKS 43
CONSENT OF PUBLICATION: 43
CONFLICT OF INTEREST 43
ACKNOWLEDGMENTS 43
REFERENCES 43

CHAPTER 4 KARYOTYPING AND CHROMOSOMAL ABERRATIONS IN CANCER: MOLECULAR AND DIAGNOSTIC BIOMARKERS 50
Tracie T. Cheng, Sujani M. K. Gamage, Sharmin Aktar, Vinod Gopalan and *Farhadul Islam*
INTRODUCTION 51
CHROMOSOMAL ABERRATIONS AND CANCER 52
The Cell Cycle Condensed 52
Structural Aberrations and Associated Cancer Markers 52
Genetic Biomarkers for Structural Chromosome Aberrations 58
DNA Damage Checkpoint 59
Replication Fork 60
DNA Repair 61
Characteristic Aneuploidy as a Cancer Marker and Its Associated Genes 62
The Centrosome and the Centromere 68

The Kinetochore-Microtubule Complex 70
The Spindle Assembly Checkpoint 70
CONCLUDING REMARKS 72
CONSENT FOR PUBLICATION 72
CONFLICT OF INTEREST 72
ACKNOWLEDGEMENT 73
REFERENCES 73

CHAPTER 5 TUMOUR DNA SEQUENCING 81
Farhadul Islam
INTRODUCTION 81
Genetic Alterations in Cancer 83
Genetic Tests for Cancer 84
Breast Cancer 85
Ovarian Cancer 85
Colorectal Cancer 86
Thyroid Cancer 87
Prostate Cancer 88
Pancreatic Cancer 89
Lung Cancer 89
Skin Cancer 90
Cancer Genome Sequencing: The Future of Precision Medicine 92
CONCLUSION 94
CONSENT FOR PUBLICATION 94
CONFLICT OF INTEREST 94
ACKNOWLEDGEMENT 94
REFERENCES 94

CHAPTER 6 CIRCULATING TUMOUR DNA: A PROMISING CANCER BIOMARKER 100
Sharmin Aktar, Plabon Kumar Das, Vinod Gopalan, Alfred King-yin Lam and *Farhadul Islam*
INTRODUCTION 101
CIRCULATING TUMOR DNA (CTDNA) 102
Biology of ctDNA 102
The Mechanism of ctDNA Entry into the Bloodstream 103
Detection of ctDNA 104
ctDNA as a Promising Biomarker in Cancer Diagnosis and Prognosis 106
ctDNA as Diagnostic Biomarker 106
ctDNA as Prognostic Biomarker 107
Challenges 108
CONCLUSION 109
CONSENT FOR PUBLICATION 110
CONFLICT OF INTEREST 110
ACKNOWLEDGEMENT 110
REFERENCES 110

CHAPTER 7 CIRCULATING TUMOUR CELLS IN SOLID CANCER 115
Sharmin Aktar, Tracie T. Cheng, Sujani M. K. Gamage, Vinod Gopalan and *Farhadul Islam*
INTRODUCTION 115
Circulating Tumour Cells (CTCs): Cytomorphology, Biology and Isolation Techniques 117
CTCs in Solid Cancers 120

Breast Cancer 121
Lung Cancer 121
Gastrointestinal Tract Cancers 122
Head and Neck Cancer 123
Prostate Cancer 123
Renal Carcinoma 124
Other Cancers 124
CTCs as Surrogate Biomarker in Clinical Application 125
CTCs as Diagnostic Biomarkers 125
CTCs as Prognostic Biomarkers for Survival Analysis 126
CTCS AS A PREDICTIVE BIOMARKER 129
In Treatment Monitoring 129
In Risk of Disease Relapse 131
Current Challenges in CTC Clinical Research 132
CONCLUSION 132
CONSENT FOR PUBLICATION 132
CONFLICT OF INTEREST 132
ACKNOWLEDGEMENT 132
REFERENCES 133

PART 3 PROTEIN/ENZYME BIOMARKERS

CHAPTER 8 PROTEIN CANCER BIOMARKERS 148
Sarath S. Joseph, Dan H. V. Tran, Farhadul Islam and *Vinod Gopalan*
INTRODUCTION 148
PROTEIN BIOMARKERS 150
α-Smooth Muscle Actin (α-SMA) 150
BRAF 151
Breast Cancer Gene 1 and 2 (BRCA 1 and 2) 152
Calretinin 153
CD117 153
CD20 154
CD30 154
Chromogranin A 155
Cytokeratin (TPA, TPS & CYFRA 21.1) 155
Desmin 156
Epidermal Growth Factor Receptor (EGFR) 156
Echinoderm Microtubule-Associated Protein-Like 4 (EML4) and Anaplastic Lymphoma Kinase (ALK) Fusion (EML4-ALK Fusion) 157
Estrogen Receptor/Progesterone Receptor (ER/PR) 157
FIP1-like-1/Platelet-derived Growth Factor Alpha (FIP1L1-PDGFRα) 158
Friend Leukaemia Integration-1 Protein (FLI-1) 159
Glial Fibrillary Acidic Protein (GFAP) 159
Gross Cystic Disease Fluid Protein-15 (GCDFP-15) 159
Human Epidermal Growth Factor Receptor 2 (HER2)/neu 160
hPG80 160
Human Melanoma Black 45 (HMB-45) 161
Inhibin 161
Keratin 17 and 19 162
KRAS 162
Melanoma Antigen Recognized by T cells 1 (MART-1) 163
Myogenic Differentiation 1 (MyoD1) 163

Muscle-Specific Actin (MSA) 163
Neurofilament 164
Platelet-Derived Growth Factor Receptor (PDGFR) 164
Promyelocytic Leukemia Protein–Retinoic Acid Receptor alpha (PML/RARα) 165
S100 165
Synaptophysin 166
Thyroid Transcription Factor 1 (TTF-1) 166
Vimentin 167
CONCLUDING REMARKS 167
CONSENT FOR PUBLICATION 168
CONFLICT OF INTEREST 168
ACKNOWLEDGEMENTS 168
REFERENCES 168

CHAPTER 9 ENZYMES: TUMOUR ASSOCIATED BIOMARKER 180
Farhadul Islam
INTRODUCTION 181
Enzyme Biomarkers in Cancer 182
Ghrelin O-Acyl Transferase (GOAT) 183
Lactate Dehydrogenase (LDH) 184
Neuron Specific Enolase (NSE) 184
Alkaline Phosphatases (ALPs) 185
Thymidine Kinase 1 (TK1) 185
Tumour M2-PK 186
Urokinase-Type Plasminogen Activator (uPA) 186
Carbonic Anhydrase XII (CAXII) 187
Aldehyde Dehydrogenase 1 (ALDH1) 187
Matrix Metalloproteinases (MMPs) 188
Hexokinase (HK) 188
Glocuse-6-Phosphate Dehydrogenase (G6PD) 189
CONCLUDING REMARKS 189
CONSENT FOR PUBLICATION 190
CONFLICT OF INTEREST 190
ACKNOWLEDGEMENT 190
REFERENCES 190

CHAPTER 10 GLYCOPROTEINS AND CANCER BIOMARKERS 195
Md Abedul Haque
INTRODUCTION 195
Protein Glycosylation in Cancer 197
Glycoproteins as Cancer Biomarkers 198
Glycoproteins in Liver Cancer 199
α-fetoprotein (AFP) 201
Sialyl Lewis A antigen (CA19-9) 202
Glycoproteins in Prostate Cancer 202
Prostate-Specific Antigen (PSA) 202
Glycoproteins in Ovarian Cancer 203
CA125 (Cancer Antigen 125) 204
Human Epidermis Protein 4 (WFDC2) 204
Glycoproteins in Breast Cancer 205
Mucin 1 (MUC1) 205
Human Epidermal Growth Factor Receptor 2 (HER2) 206

Carcinoembryonic Antigen (CEA) 206
Glycoproteins in Colon Cancer 207
CEA 207
Carbohydrate Antigen 19-9 (CA19-9) 207
Glycoproteins in Lung Cancer 208
Mucins 208
Epidermal Growth Factor Receptor (EGFR) 209
CEA 209
Glycoproteins in Pancreatic Cancer 210
Cancer Antigen 19-9 (CA 19-9) 210
CEA 211
CONCLUSION 211
CONSENT FOR PUBLICATION 212
CONFLICT OF INTEREST 212
ACKNOWLEDGEMENT 212
REFERENCES 212

PART 4 HORMONE & SMALL MOLECULAR BIOMARKERS

CHAPTER 11 HORMONES AS CANCER BIOMARKERS 228
Plabon Kumar Das and *Farhadul Islam*
INTRODUCTION 228
HORMONES AS BIOMARKERS IN VARIOUS CANCERS 230
Peptide Hormones as Cancer Biomarkers 230
Calcitonin 230
Human Chorionic Gonadotropin (hCG) 231
Insulin 232
Gastrin 232
Glucagon 233
Prolactin 233
Thyroid Stimulating Hormone (TSH) 233
Steroid Hormones as Biomarker for Various Cancers 233
Estrogen 234
Progesterone 234
Testosterone 235
CONCLUSION AND PERSPECTIVE 235
CONSENT FOR PUBLICATION 236
CONFLICT OF INTEREST 236
ACKNOWLEDGEMENT 236
REFERENCES 236

CHAPTER 12 MIRNAS AS EPIGENETIC CANCER BIOMARKER 241
Afraa Mamoori
INTRODUCTION 241
MiRNAs and Epigenetics 242
Mode of Action of MicroRNAs 243
Targets Selection by MicroRNAs 243
MiRNAs and Clinical Facts 244
Over 60% of Human Protein-coding Genes are Controlled by miRNA 244
Tissue-specific miRNAs 244
miRNAs Expressed in Different types of Body Fluid, and They are Highly Stable in Human Samples 245

miRNAs are Relevant Biomarkers in Cancer Diagnosis and Prognosis 247
Commercialized miRNAs, which are Currently in Clinical Practice as Diagnostic Markers 254
miRview Meso Test 254
miRview Squamous 254
miRview Lung 255
miRview Mets 256
miRview Kidney 256
Mirnas as Crucial Regulatory Marker in Epithelial-Mesenchymal Transition Process 258
CONCLUSION 259
CONSENT FOR PUBLICATION 259
CONFLICT OF INTEREST 259
ACKNOWLEDGEMENT 259
REFERENCES 260

PART 5 NOVEL METHODS FOR CANCER BIOMARKER DETECTION

CHAPTER 13 ELECTROCHEMICAL AND OPTICAL DETECTION OF MICRORNAS AS BIOMARKERS FOR CANCER DIAGNOSIS 272
Riham Zayani, Amira Ben Hassine, Amal Rabti, Amal Raouafi and *Noureddine Raouafi*
INTRODUCTION 272
What are miRNAs? 274
Biogenesis of miRNAs 275
Biological Role of miRNAs 276
miRNAs and Cancer 276
OVERVIEW OF MOLECULAR BIOLOGY DETECTION METHODS 281
Reverse Transcription Polymerase Chain Reaction 281
Loop-mediated Isothermal Amplification 282
Catalytic Hairpin Assembly 283
Rolling Circle Amplification 284
Hybridization Chain Reaction 285
ELECTROCHEMICAL DETECTION METHODS 286
Amperometry 287
Potentiometry 290
Classical Potentiometric Biosensors 291
FET-Based Biosensors 292
Cyclic Voltammetry 293
Differential Pulse Voltammetry 294
Square Wave Voltammetry 300
Electrochemical Impedance Spectroscopy 305
OPTICAL DETECTION METHODS 309
FLUORESCENCE 309
Signal ON Biosensors 309
Signal OFF Biosensors 311
FRET Biosensors 312
UV-Visible 315
Surface Plasmon Resonance 319
Surface-Enhanced Raman Scattering 325
CONCLUDING REMARKS 328
CONSENT FOR PUBLICATION 328
CONFLICT OF INTEREST 329
ACKNOWLEDGEMENTS 329

ABBREVIATIONS 329
REFERENCES 331

CHAPTER 14 ELECTROCHEMICAL BIOSENSOR FOR CANCER BIOMARKERS DETECTION 349
Md Arifuzzaman, Mostafa Kamal Masud, Asif Ahmed, Md Morsaline Billah and *Md Nazmul Islam*
INTRODUCTION 350
SOME KEY TERMS IN BIOSENSOR-BASED ASSAYS 351
TYPES OF BIOSENSORS 352
ELECTROCHEMICAL READOUT METHODS 354
ELECTROCHEMICAL SENSOR FOR NUCLEIC ACID BIOMARKERS 356
Electrochemical DNA Sensors 357
Electrochemical RNA Sensors 361
ELECTROCHEMICAL SENSORS FOR PROTEIN BIOMARKERS 363
ELECTROCHEMICAL SENSORS FOR CIRCULATING TUMOUR CELLS 364
ELECTROCHEMICAL SENSORS FOR EXTRACELLULAR VESICLES 366
CONCLUSION 366
CONSENT FOR PUBLICATION 367
CONFLICT OF INTEREST 367
ACKNOWLEDGEMENT 367
REFERENCES 367

SUBJECT INDEX 372

FOREWORD

CANCER is the leading cause of the world's death toll and has the greatest adverse economic impact from premature death and disability, ultimately causes of death worldwide. Its economic burden is 1.5 percent of the world's gross domestic product (GDP), around $900 billion excluding direct medical costs, which is 19 percent higher than heart disease. Thus, alleviating cancer will save millions of lives and billions of dollars.

The burden of cancer and economic impacts can be improved, if the disease is diagnosed with precancerous lesions or even early-stage of cancer. Also, the quality of life of patients could be improved by providing personalized treatment and counselling based on the prognosis of the disease. However, robust and effective markers for screening, diagnosis, prognosis and prediction are yet to be established.

The book 'Current Cancer Biomarkers' provides a comprehensive review, and details the clinical implications of currently useful and potential biomarkers for screening, diagnosis and management of patients with cancer. This book could help clinicians, medical students and researchers, at least to some extent, to collect scientific and clinical information on cancer biomarkers.

M. Khalilur Rahman Khan
Professor of Physics & Dean Faculty of Science
University of Rajshahi
Rajshahi-6205
Bangladesh

PREFACE

Cancer is the second leading cause of human mortality worldwide, and researches in the field are enormous to fight against the deadly disease. The huge research on cancer includes cancer biomarker development, diagnosis and detection method development, cellular & molecular characterization of cancers, target identification, therapy development, clinical management, and so on. Since the clinical outcome of patients with cancer largely depends on early and proper diagnosis of the disease, thus, significant efforts on cancer biomarker research are going based on cancer biomarkers for detection, diagnosis, prognosis, therapy response, molecular typing, classification and stratification of cancers. Therefore, this book, Current Cancer Biomarkers, provides a comprehensive review based on the current status of biomarkers for various types of cancer, which could give great potential in disseminating the knowledge and information to a broad range of readers.

This book starts with an introduction to the basic characteristics of cancer biomarkers, which are used for various cancer treatments, and the biomarkers are under development. The next major portion of the book highlights the potential and effective biomarkers such as genomic, epigenomic, transcriptomic, and proteomics, cellular and morphologic factors associated with cancer, indicating the occurrence of cancers. The other part of the book discusses novel technologies to detect and analyse potential cancer biomarkers in point-of-care applications.

This book provided an all-inclusive review and the most updated information based on different aspects of cancer markers in the clinical setting. We hope that the topics covered in this book are useful and enrich our understanding of the disease, which could help better manage patients with cancers.

I am thankful to all the Authors and the editorial staff from Bentham Science for their contribution and support.

Farhadul Islam
Department of Biochemistry and Molecular Biology
University of Rajshahi
Rajshahi
Bangladesh

List of Contributors

Afraa Mamoori	Department of Pathology and Forensic Medicine, School of Medicine, University of Babylon, Hillah, Iraq
Alfred King-yin Lam	Pathology Queensland, Gold Coast University Hospital, Gold Coast, Australia School of Medicine and Dentistry, Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia
Amal Rabti	Sensors and Biosensors Group, Analytical Chemistry & Electrochemistry Lab. (LR99ES15), Faculty of Science, University of Tunis El Manar, Tunis El Manar, Tunisia
Amal Raouafi	Sensors and Biosensors Group, Analytical Chemistry & Electrochemistry Lab. (LR99ES15), Faculty of Science, University of Tunis El Manar, Tunis El Manar, Tunisia
Amira Ben Hassine	Sensors and Biosensors Group, Analytical Chemistry & Electrochemistry Lab. (LR99ES15), Faculty of Science, University of Tunis El Manar, Tunis El Manar, Tunisia
Asif Ahmed	Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna 9208, Bangladesh
Chamath D. Ranaweera	National Cancer Institute of Sri Lanka, Maharagama, Sri Lanka Sunshine Coast Hospital and Health Service, QLD, Australia,
Dan H. V. Tran	Molecular and Cellular Pathology, School of Medicine and Dentistry, Griffith University, Gold Coast, Australia
Farhadul Islam	Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh
Md Abedul Haque	The University of Texas MD Anderson Cancer Center, Houston, Texas-77033, USA
Md Arifuzzaman	Institute of Tissue Banking and Biomaterial Research, Atomic Energy Research Establishment, Savar, Dhaka-1349, Bangladesh
Md Morsaline Billah	Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna 9208, Bangladesh
Md Nazmul Islam	School of Health & Life Sciences, Teesside University, Middlesbrough TS1 3BA, United Kingdom National Horizons Centre, Teesside University, Darlington DH1 1HG, United Kingdom
Mostafa Kamal Masud	Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Brisbane, QLD, 4072 Australia International Center for Materials Nanoarchitectonics (WPI-MANA), National Institute for Materials Science, Tsukuba, Ibaraki, 305-0044 Japan
Noureddine Raouafi	Sensors and Biosensors Group, Analytical Chemistry & Electrochemistry Lab. (LR99ES15), Faculty of Science, University of Tunis El Manar, Tunis El Manar, Tunisia
Plabon Kumar Das	Institute for Glycomics, Griffith University, Gold Coast, Australia

Riham Zayani	Sensors and Biosensors Group, Analytical Chemistry & Electrochemistry Lab. (LR99ES15), Faculty of Science, University of Tunis El Manar, Tunis El Manar, Tunisia
Sarath S. Joseph	Molecular and Cellular Pathology, School of Medicine and Dentistry, Griffith University, Gold Coast, Australia
Sharmin Aktar	Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh School of Medicine and Dentistry, Griffith University, Gold Coast, QLD, Australia
Sujani M.K. Gamage	School of Medicine and Dentistry, Griffith University, Gold Coast, QLD, Australia, Faculty of Medicine, University of Peradeniya, Galaha Rd, 20400, Sri Lanka
Tracie T. Cheng	School of Medicine, Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia
Vinod Gopalan	Molecular and Cellular Pathology, School of Medicine and Dentistry, Griffith University, Gold Coast, Australia

Part 1: Introduction and Clinically Used Biomarker

CHAPTER 1

Introduction: Current Status and Future Advances in Cancer Biomarkers

Farhadul Islam[1,*]

[1] *Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

Abstract: Cancer is a major health problem and a leading cause of morbidity and mortality worldwide. The cancer burden can be reduced significantly using reliable, robust, sensitive, accurate, validated and specific biomarkers for early diagnosis, better prognosis and prediction. Traditionally, a number of biomolecules exhibit the potential to be used as diagnostic, prognostic and predictive biomarkers roles, however, they failed to be used in point-of-care settings for routine analysis. Recent advancements in sequencing techniques and analytical methods facilitate the development of novel and effective cancer biomarkers (liquid biopsies) with the fidelity of clinical application. These biomarkers provide personalized "omics" based information on the pathological state, molecular nature and biological aggressiveness of individual patients. Nevertheless, standardized platforms and/or methods for these biomarkers are yet to be established. Thus, adopting a combination of classical and new cancer biomarkers would offer a better understanding of the disease, resulting in improved clinical outcomes for patients with cancer.

Keywords: Biomarkers, Cancer markers, Cancer management, Cancer burden, Cell-free DNA, Circulating tumour DNA, Circulating tumour cells, Classical cancer markers, Clinical application, Diagnostic markers, Drug toxicity, Liquid biopsy, Non-coding RNAs, Predictive markers, Prognostic markers, Personalized treatment, Precise medication, Tumour-derived exosomes, Tumor-derived extracellular vesicles.

INTRODUCTION

Cancer, a group of diseases, can affect any part of the body, involving uncontrolled growth and proliferation of cells caused by different factors, resulting in extreme molecular and cellular heterogeneity in different and even in a single cancer [1]. However, one defining feature of cancer is the rapid generation of abnormal cells that can grow beyond their usual boundaries and

* **Corresponding author Farhadul Islam:** Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh; E-mail: farhad_bio83@ru.ac.bd

have the potential of local invasion and distance metastasis- migrating to the other parts of the body. The latter, metastases, is the primary reason for cancer-related mortality in patients with cancers.

Cancer is a major health burden and a leading cause of death worldwide. In 2020, cancer accounted for about ten million deaths, with 19.3 million cases globally [2]. According to GLOBOCAN 2020, 1 in 5 people develop cancer during their lifetime, while 1 in 8 men and 1 in 11 women die from the disease globally [3]. The burden of cancer can be reduced (30 to 50%) by avoiding risk factors (*e.g.* tobacco and alcohol use, unhealthy diet, physical inactivity, air pollution, other non-communicable diseases *etc*.) and by using existing evidence-based prevention strategies. In addition, the cancer burden can also be reduced by early detection, proper care and appropriate treatment modalities [4]. Many cancers have a high chance of cure if diagnosed early in the disease.

Early detection and diagnosis of cancers, especially asymptomatic cases, are challenging and effective markers or methods yet to be established for point-o--care applications. Thus, there is an urgent need to develop effective biomarkers or panel of biomarkers (cancer biomarkers), which can diagnose patients with cancer at early stage with high specificity and sensitivity. Also, sensitive and specific biomarkers for stratification of stages, disease progression and therapy response could improve the clinical outcome of patients with various cancers. A biomarker or biological marker is a measurable indicator of biological condition and/or state using blood, urine, stool or soft tissues [5]. Measurement of a biomarker provides information on normal biological/cellular processes, pathogenic processes or pharmacologic responses to therapy. Thus, in principle, cancer or tumour biomarker is a biomolecule or part of biomolecules found in blood, urine, stool or body tissues, which elevated in the presence of cancer in biological samples. These biomarkers can be generated directly by a cancer cell or by a non-cancer cell in presence of cancer. Most of the cancer biomarkers can be categorized as tumour-specific antigens or tumour-associated antigens. They could be either (i) products of mutated oncogenes, (ii) products of tumour suppressor genes, (iii) products of other mutated genes, including (a) overexpressed or abnormally expressed cellular proteins or protein fragments, (b) tumour antigens produced by oncogenic viruses, (c) oncofetal antigens, (d) altered cell surface glycoproteins and glycolipids and (e) cell-type specific differentiation antigens, and (iv) altered or aberrant genetic and epigenetic make-up in cancer cells. Thus, a biomarker can be genetic materials, such as DNA, RNAs, including non-coding RNAs, or their products, *i.e.* proteins or peptides or even epigenetic changes, such as DNA-methylation. However, an ideal cancer biomarker should have the following criteria for effective use in clinical applications [6].

A. Produced in the presence of cancer (significantly elevated levels).

B. Associated with the cancer burden and provide sufficient lead time (length of time between the disease detection and its usual clinical presentation and diagnosis).

C. Significantly higher levels in blood, urine, stools or other biological samples in patients with cancer than in healthy individuals, especially at early or preclinical stages of patients.

D. Highly sensitive and specific for cancer types, preferably one type of cancer.

E. Easy, cost-effective, less labour intensive and able to measure in small quantities in point-of-care settings.

Classical Cancer Biomarker

A cancer biomarker can be used for screening, diagnosis, monitoring disease progression, therapy response and disease recurrence/relapse [7], therefore, giving the information of the disease status in particular patients, which in turn can facilitate the personalized cancer management in patients. Thus, cancer biomarkers can be classified into three broad categories, (i) diagnostic, (ii) prognostic and (iii) predictive biomarkers. However, a biomarker can be used for more than one clinical applications, thereby can fall into more than one of these groups.

Diagnostic Cancer Biomarkers

As mentioned earlier that early detection of cancer can significantly reduce the cancer burden, thereby alleviating economic and social costs associated with cancer. Early detection of cancer allows better response to the treatment, resulting in higher survival rates and less morbidity, along with the lower cost of management [8]. Therefore, a significant improvement can be made in patients with cancers by detecting them at earlier stages, especially at the asymptomatic stages and avoiding delays in proper care.

Early cancer detection can be carried out by screening of mass population using appropriate biomarker tests with the aim to identify individuals with findings indicative of specific cancer or pre-cancer at the asymptomatic phase. Identification of abnormalities during screening suggests further tests to diagnose the disease. The screening test must be inexpensive and safe enough to be used by mass populations and should be very highly sensitive and specific to avoid too many false positives in tested populations [9]. The screening programs for early detection are effective for some cancer types but not all cancer types. Also, the

CHAPTER 2

Tumour Markers in Clinical Use

Sujani M. K. Gamage[1,2,*], Chamath D. Ranaweera[3,4], Tracie T. Cheng[1], Sharmin Aktar[1,5], Vinod Gopalan[1] and **Farhadul Islam[6]**

[1] *School of Medicine, Griffith University, Gold Coast, QLD, Australia*

[2] *Faculty of Medicine, University of Peradeniya, Galaha Rd, 20400, Sri Lanka*

[3] *National Cancer Institute of Sri Lanka, Maharagama, Sri Lanka*

[4] *Sunshine Coast Hospital and Health Service, QLD, Australia*

[5] *Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh*

[6] *Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

Abstract: Despite ever-growing experimental evidence for the utility of a wide range of tumour markers, only a handful are understood to be useful in clinical applications. Tumour markers are useful for screening and diagnosis of cancers, prognostication, guiding treatment pathways and post-treatment surveillance studies. The tumour makers play a significant role in cancer care and the markers included in the current treatment guidelines will be discussed in detail in this chapter. The utility of the tumour markers in the management of colorectal, breast, thyroid, hepatobiliary, pancreatic, ovarian, testicular, neuroendocrine and prostate cancer are detailed herein to provide an update on the current use of tumour markers in the clinical settings.

Keywords: Alpha-fetoprotein (AFP), Breast cancer, Calcitonin cancer, Cancer treatment, Carcinoembryonic antigen (CEA), Chromogranin A, Colorectal cancer, Current guidelines in cancer care, CA 125, CA 19.9, Follow-up in cancer care, Hepatobiliary cancer, Neuroendocrine tumours, Ovarian cancer, Pancreatic cancer, Prostate cancer, Prostate-specific antigen (PSA), Screening, Surveillance, Testicular cancer, Thyroglobulin (Tg), Thyroid cancer, Tumour markers.

INTRODUCTION

Tumour marker is a substance (commonly a protein, enzyme or hormone) that is present or produced either by tumour cell or by other cells in the body due to the effects of cancer, which can be detectable in body fluids or tissue and provides

* **Corresponding author Sujani M. K. Gamage:** School of Medicine, Griffith University, Gold Coast, QLD, Australia; E-mail: s.kodagodagamage@griffith.edu.au

Farhadul Islam (Ed.)

valuable information about the aggressiveness of cancer, potential treatment modalities as well as disease and treatment outcomes. Also, they can either be produced within the cancer cells or by the non-cancerous cells as a result of the metabolic alterations caused by cancer [1]. Genetic alterations are also being considered tumour markers, especially for screening purposes. Theoretically, an ideal tumour marker should have the following characteristics: (i) having a high degree of specificity to the particular tumour; (ii) possessing high sensitivity to rule out possible false positives; (iii) allowing a sufficient time frame (lead time) to alter the natural course of the disease; (iv) detectable levels being reliably correlated with the tumour burden, while closely reflecting tumour progression; (v) having a short half-life, permitting serial measurements and (vi) being affordable for patients. Unfortunately, such an ideal tumour marker does not exist, and the available markers have their advantages and limitations [2].

Tumour markers are particularly important in assessing treatment response and residual disease, surveillance and follow-up. Following treatment, normalization of tumour markers usually denotes adequate cancer resection/ treatment. Normalized tumour marker levels with radiological evidence of persistent disease may indicate that the tumour is non-viable. There may be a transient increase in tumour marker levels following effective treatment, secondary to cell lysis. Therefore, an increase in tumour marker levels on its own does not signify treatment failure. However, increasing tumour markers in a clinically deteriorating patient may indicate treatment failure or recurrence, warranting further work-up [3].

Though there are experimental evidence for the utility of a wide range of tumour markers for each cancer, only a handful are used in the actual clinical setting (Table **1**). Selected tumour markers, which are routinely used in the clinical setup and are important in the diagnosis and management of cancers, will be discussed in detail in this chapter.

Markers for Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer worldwide, affecting mostly the population in developed countries [4]. However, the prognosis of patients with CRC depends mainly on the cancer staging at the time of presentation. Tumour markers have particular importance in the diagnosis of disease and determination of prognosis of patients with CRC. Carcinoembryonic antigen (CEA) is the most used tumour marker in clinical practice for CRC. In addition, tissue polypeptide-specific antigen (TPS), carbohydrate antigen (CA 19.9), hematopoietic growth factors (HGF-s) and tumour-associated glycoprotein-72 (TAG-72) are promising as potential tumour markers in CRC [5, 6].

Macrophage-colony stimulating factor (M-CSF), granulocyte-macrophage-colony stimulating factor (GM-CSF), Cathepsin D, interleukin-3, interleukin-6 and enzymes, such as Alcohol Dehydrogenase and Lysosomal Exoglycosidases, are also recognised as potential CRC related tumour markers [7 - 10]. However, these are still not widely used in clinical practice. Since most cancer-related deaths are due to metastasis, investigations on circulating tumour cells (CTCs) will be promising with respect to gaining information on the prognosis and treatment response of patients with CRC [11 - 13]. Nevertheless, currently, only CEA is considered a reliable marker for CRC in clinical practice.

Diagnosis

CEA is an oncofetal antigen present in most epithelial tumours and is the most versatile and frequently used tumour marker in clinical settings [6]. Relative cost-effectiveness has warranted its use in routine clinical practice. However, serum CEA levels are elevated in only 17-47% of patients with CRC [14 - 16]. CEA can be elevated in many benign conditions, including benign liver and kidney disease, pancreatitis, inflammatory bowel disease, obstructive pulmonary disease and other malignancies including gastric, oesophageal, pancreatic, lung, breast and mesotheliomas. Levels may also be higher than the reference range in chronic smokers, and the cut-off point for the upper normal value is twice as for a non-smoker [17, 18]. Therefore, CEA is not used in isolation to diagnose CRC due to its lack of sensitivity and specificity. However, the presence of a high concentration of CEA can be useful in circumstances in which there is a high clinical probability of cancer, and the patient is not fit enough to undergo invasive investigations. Also, markedly raised serum CEA levels (>40μg/L) are usually indicative of metastatic disease [18].

Prediction of Prognosis

Although pathological staging of CRC is the most reliable prognostic predictor, serum CEA levels are routinely performed to reinforce decision-making. High CEA level during the preoperative period is correlated with poor prognosis [15, 18].

Follow up

Several studies have confirmed that intense post-treatment surveillance improves overall survival by detecting treatable metastasis [19, 20]. Along with imaging and endoscopic surveillance, serum CEA is a reliable tumour marker for the detection of recurrences in CRC patients. Up to 50% of patients who undergo curative resection for CRC can develop liver metastasis within 5 years. Resection of resectable liver metastasis is the only curative treatment apart from a liver

Part 2: DNA/RNA Biomarkers

CHAPTER 3

DNA Methylation Landscapes in Cancer and Non-Cancer Cells

Shaun Stangl[1,*] and **Vinod Gopalan**[1,*]

[1] *Cancer Molecular Pathology, School of Medicine and Dentistry, Griffith University, Gold Coast, Queensland 4222, Australia*

Abstract: Epigenetic modifications are heritable changes to gene expression without physical changes to the actual DNA sequence. The most widely studied epigenetic modification is DNA methylation, as it is influenced by aging, diet, diseases and the environment. DNA methylation involves direct chemical modification to the DNA and plays an important role in gene regulation by preventing proteins from binding to certain regions of the DNA, which causes these regions to be repressed. It is essential for normal development, cell differentiation and regulation of cellular biology. The DNA methylation landscape of each unique cell type helps to determine which genes are expressed and silenced. It is well known today that the accumulation of both genetic and epigenetic abnormalities contributes to the development of cancers. Aberrant DNA methylation is a hallmark of cancer. During cancer development and progression, the methylation landscape undergoes aberrant remodelling. Recently within cancer research, the advancements in DNA methylation mapping technologies have enabled methylation landscapes to be studied in greater detail, sparking new interest in how the methylation landscape undergoes a change in cancer and possible applications of DNA methylation. This chapter focuses on reviewing DNA methylation landscapes in normal cells and then how they are altered in cancer. It also discusses the applications of DNA methylation as cancer biomarkers.

Keywords: Biomarkers, Cancer, Demethylation, DNA, Epigenetics, Gene-bodies, Gene expression, Hypermethylation, Hypomethylation, Methylation, Promoter, Transcription.

INTRODUCTION

DNA methylation is a heritable epigenetic modification that plays an important role in regulating gene expression. It allows cells that are genetically identical to establish distinct cellular phenotypes without changing the actual DNA sequence.

[*] **Corresponding authors Vinod Gopalan:** Cancer Molecular Pathology, School of Medicine and Dentistry, Griffith University, Gold Coast, Queensland 4222, Australia; Email: v.gopalan@griffith.edu.au;
Shaun Stangel: Cancer Molecular Pathology, School of Medicine and Dentistry, Griffith University, Gold Coast, Queensland 4222, Australia; Email: shaun.stangl@alumni.griffithuni.edu.au

Farhadul Islam (Ed.)

Among all the different types of epigenetic mechanisms, DNA methylation is the most widely studied, as it is influenced by aging, diet, diseases and the environment. In mammalian genomes, DNA methylation is essential for normal development as well as several other biological processes, including cell differentiation, tissue-specific gene expression, maintaining genome stability, chromatin status, genomic imprinting, and silencing of potentially harmful genetic elements [1] and X-chromosome inactivation in females [2]. DNA methylation refers to the transfer of a methyl group (-CH3) from the S-adenosylmethionine (SAMe) molecule to the carbon-5 position of cytosine bases, to form 5-methylcytosine (5mC). The process is catalysed by three DNA methyltransferase enzymes (DNMTs) DNMT1, DNMT3A and DNMT3B. Maintenance DNA methyltransferase DNMT1, is responsible for maintaining DNA methylation patterns during cell division. DNMT1 copies the DNA methylation patterns of the parent strand onto the newly synthesised daughter strand of hemi-methylated DNA. The de novo DNA methyltransferases DNMT3A and DNMT3B, are mainly active during early development and help to establish new DNA methylation patterns on unmethylated DNA [3, 4].

DNA demethylation refers to the removal of methyl groups from 5mC. Recent studies have discovered that the ten-eleven translocation (TET) enzymes can oxidise 5mC, to produce 5-hydroxymethylcytosine (5hmC); 5hmC has been recognised as an important intermediate of DNA demethylation processes [5]. This recent discovery has generated new interest into the dynamics of how DNA methylation landscapes are remodelled with the contribution of DNA demethylation during early development, normal cell biology and cancer. Initially, oxidation of 5mC was hypothesised as a potential way of reactivating genes that had been silenced by DNA methylation [6, 7], however, this has since been ruled out [8 - 10]. DNA demethylation is achieved by both a passive and active process. Passive DNA demethylation occurs during DNA replication as a result of reduced DNMT1, causing each successful replication cycle to further dilute the content of 5mC in the genome as methylation patterns are not being copied onto newly replicated DNA [11]. Pathways of active DNA demethylation can occur independently of DNA replication. Active DNA demethylation uses three different enzyme families: (i) TET enzymes further oxidise 5hmC to form in order 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [12]. (ii) The activation-induced cytidine deaminase/ apolipoprotein B editing complex (AID/APOBEC) family enzymes deaminate 5hmC to form 5-hydroxymethyluracil (5hmU) and 5mC to form thymine. (iii) Base excise repair (BER) glycosylase enzymes such as thymine DNA glycosylase (TDG) recognise and excise the damaged or incorrect base (*i.e.*, 5fC, 5caC, 5hmU and thymine), creating an abasic site (apurinic/apyrimidinic site; AP site). The AP site is then repaired with

unmethylated cytosine by the BER pathway, concluding active DNA demethylation [13, 14].

Although there are still a lot of outstanding questions regarding cancer development, it is well known that age, diet, disease and the environment are capable of influencing changes to the epigenetic landscape, causing an increased risk of cancer development. It is well known that DNA methylation landscapes undergo aberrant changes during cancer development and progression. Patterns of DNA hypomethylation genome-wide accompanied by focal hypermethylation have been observed in all cancers ever studied [15]. With the recent advancements in DNA methylation mapping technologies combined with our growing knowledge of other epigenetic modifications, we are continuously improving our understanding of the role that DNA methylation plays in normal and disease states.

Normal DNA Methylation Location

The DNA methylation landscape of each cell type is different and plays an essential role in regulating patterns of gene expression. In mammals, DNA methylation predominantly occurs at cytosine-phosphate-guanine dinucleotides (CpG sites) [16]. In the human genome, CpG sites only make up approximately 1% of all dinucleotides, and this is because DNA methylation is mutagenic. CpG sites are under-represented in the human genome due to spontaneous deamination of 5mC to thymine [17]. CpG sites are not evenly distributed in the genome, and sequences that contain a high concentration of CpG sites are referred to as CpG islands (CGIs) [18]. CGIs are defined as short DNA sequences that are typically 500 to 1000 base pairs (bp) in length with a CpG content $\geq$ 50% and an observed to expected CpG ratio $\geq$ 60%. The majority of CGIs, approximately 50-70%, are located at gene promoters, especially the promoters of housekeeping genes. The methylation of promoter CGIs typically correlates with gene activity [19, 20].

In normal somatic cells, the majority of CpG sites are methylated, while CpG sites within promoter CGIs typically remain unmethylated and in a transcriptionally permissive chromatin state. The high frequency of unmethylated CpG sites in promoter CGIs increases the potential that transcription factors will bind, whereas DNA methylation of promoter CGIs along with chromatin folding leads to stable transcriptional silencing by physically blocking the binding of transcription factors to DNA. In addition, DNA methylation further indirectly inhibits transcription by the presence of methyl-CpG binding proteins (MBPs) that recruits chromatin-modifying proteins. Heterochromatin has a tightly packaged chromatin structure that is generally heavily methylated and transcriptionally silenced. In contrast, euchromatin has a lightly packaged chromatin structure that

CHAPTER 4

Karyotyping and Chromosomal Aberrations in Cancer: Molecular and Diagnostic Biomarkers

Tracie T. Cheng[1,*], Sujani M. K. Gamage[1,2], Sharmin Aktar[1], Vinod Gopalan[1] and **Farhadul Islam[3,4]**

[1] School of Medicine, Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia

[2] Department of Anatomy, Faculty of Medicine, University of Peradeniya, Galaha Rd, 20400, Sri Lanka

[3] Griffith University, Institute for Glycomics, Gold Coast, Australia

[4] Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh

Abstract: Chromosomal abnormalities induce genomic instability and are associated with cancer hallmarks. Chromosomal abnormalities can be categorised into structural and numerical aberrations and are seen under a light microscope. Given the ease of detecting and observing such changes using karyotyping, chromosomal aberrations may be a useful diagnostic tool. For example, the discovery of the Philadelphia chromosome was a cytogenetic hallmark of chronic myeloid leukaemia and acute lymphoblastic leukaemia. Thus, this chapter explores potential aberrations which have the potential to be used as cancer markers in a clinical setting. Recurrent structural aberrations with known genetic mutations are observed in cancers of the bones, lungs, salivary glands, soft tissue, stomach, thyroid, and uterus. The association of these genetic alterations with various cancers suggests a causative role of structural aberrations in carcinogenesis and is characteristic of some cancers. Additionally, mono- and tri-somies, known as aneuploidy, are common to all cancer types, however, their roles as a cause or consequence are difficult to establish due to the sheer loss or gain of genetic material, respectively. Cancers with the most frequent trisomies, include Ewing's sarcoma of the bone, astrocytoma of the brain, and renal adenocarcinoma. Common cancer monosomies include meningioma of the brain and ovarian adenocarcinoma. These chromosomal aberrations forge the path to a better understanding of cancer genetics. Though there are potential chromosome markers in cancer, the heterogeneity of cancer genetics makes this a challenging tool to incorporate into current oncological diagnostic guidelines.

Keywords: Aneuploidy, Biomarker, Cancer, Chromosomal aberrations, Clinical oncology, Cytogenetics, Diagnostic, Karyotype, Structural aberration.

[*] **Corresponding author Tracie T. Cheng:** School of Medicine, Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia; Email: tracie.cheng@griffith.edu.au

INTRODUCTION

Cancer is a common and deadly disease that continues to be diagnosed and treated at late stages. Early detection and accurate diagnoses are paramount to bettering prognoses and decreasing cancer-related morbidity. Although cancer cells are heterogeneous in nature and continually evolve and mutate, they are still a distinct population which exhibit targetable traits. These hallmarks of cancer (sustained proliferative signalling, evasion of growth suppressors, resistant to apoptosis, replicative immortality, promotion of angiogenesis, and metastasis) are underpinned by genomic instability, giving rise to aggressive and abnormal cellular behaviour [1]. Genomic instability refers to the rate of errors generated in a genome, and encompasses everything from a single point mutation to major chromosomal aberrations [2]. Genomic instability can arise from two distinct pathways; microsatellite instability (recessive trait) and chromosomal instability (dominant trait) [3]. This chapter focuses on chromosomal aberrations and biomarkers in clinical diagnostics.

Chromosomal aberrations describe any defect that compromises an organism's normal karyotype, and, therefore, can be detected using cytogenetic methods [4]. Karyotyping describes methods of organising and examining the chromosomal makeup of a cell to provide a holistic view of an organism's genetic material. Chromosomal instability (CIN) is a product of chromosomal aberrations, which can largely be grouped into structural or numerical abnormalities, describing the alteration of part of a chromosome and whole chromosomes, respectively [5]. Currently, cytogenetics plays an increasingly important role in cancer diagnostics, with physicians routinely using cytogenetic studies for cancer patients [6]. Whilst both benign and malignant tumours can express abnormal karyotypes, malignant tumours tend to show more numerous aberrations. Additionally, karyotypic complexity and numerous chromosome aberrations have been associated with aggressive clinical and poor histological features [7].

The Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer comprehensively collates literature regarding cancer cases and their associated cytogenetic changes [8]. Currently, over 70,000 cases involving more than 14,000 genes have been recorded, however, many aberrations are limited in the number of reported cases [8]. Due to the inherent heterogeneity of a tumour population, an almost infinite combination of chromosomal aberrations could exist. However, recurrent chromosomal aberrations are often found in certain cancers, and may be useful as a diagnostic marker, which can be easily identified using karyotyping and light microscopy [9].

CHROMOSOMAL ABERRATIONS AND CANCER

The Cell Cycle Condensed

Cancer cells and tumours are the consequence of inappropriate proliferation due to improper regulation of the cell cycle and the development of survival mechanisms. At any given time, many cells in the body remain in a quiescent state and only re-enter the cell cycle in response to appropriate stimuli [10]. Thus, inappropriate signalling to reactivate cell cycle progression is a major driver of tumorigenesis. In contrast, if a cell fails to re-enter quiescence and continuously progresses through the cell cycle, the result is a mass of uncontrolled proliferative cells [11]. Within the cell cycle itself, the cell must accurately replicate its DNA, and segregate and divide its genetic material accordingly amongst the two daughter cells with high fidelity. Progression through the cell cycle is dependent on levels of key molecules, which are altered through processes such as de/phosphorylation, SUMOylation, ubiquitin-dependent degradation, or acetylation [12]. Due to the sheer amount of information the cell must replicate and organise, a multitude of mechanisms exist to protect the cell from improper cell cycle progression. Cell cycle checkpoints are regulatory proteins, which exist at specific stages of the process and ensure the appropriate completion of previous stages before allowing the cell to continue through its replicative process [13].

Exiting the quiescent state (G0), a cell enters the first gap (G1) phase by passing through the restriction point, thus committing to the cell division process. Shortly after this, during the synthesis (S) phase of the cell cycle, chromosomes and some cellular components from the mother cell are replicated once to create two identical sets of genetic material. The cell then enters a longer gap phase (G2) before beginning the division process known as mitosis (M phase) [11, 14]. Dysfunctional replication of genetic material, particularly in S phase, may result in chromosomal translocations, inversions, and deletions (structural aberrations) [15].

Structural Aberrations and Associated Cancer Markers

Structural aberrations are the consequence of chromosome breakage and improper reunion or fusion [9]. Structural aberrations arise from errors in DNA replication in the synthesis phase of the cell cycle or failure to repair DNA/halt cell cycle progression at cell cycle checkpoints [16]. These aberrations depicted in Fig. (**1**) can be categorised into balanced and unbalanced translocations, which involve equal and unequal chromatin rearrangement across chromosomes, respectively; deletions which are a loss of a chromosome segment; inversions which result when two broken ends of the same chromosome are incorrectly re-joined; and isochromosomes which is the formation of a mirror image of a chromosome arm

CHAPTER 5

Tumour DNA Sequencing

Farhadul Islam[1,*]

[1] *Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

Abstract: Cancer pathogenesis is a multistep process involving the accumulation of complex genetic and epigenetic alterations. The disease can be sporadic or familial in nature. The genes associated with much familial cancer or inherited cancer susceptible syndrome have already been identified. Thus, genetic testing for pathogenic variants of these genes could predict whether an individual has a high risk of developing cancer in their lifetime. Also, tumour DNA sequencing in patients with cancer can be used for therapy selection and to predict treatment outcomes. The recent development of high throughput sequencing enables the exploration of whole genome profiling, including mutations, structural variations, transcriptomes, splicing events, *etc*., in patients with cancer, thereby providing guidelines for personalized precision medicine in clinical practice. However, the translation of cancer genome sequencing information into the clinical treatment plan is highly complicated, needs multidisciplinary expert panels and is not cost-effective for mass application. Further development in sequencing analysis and data interpretation are imperative for point-of-care settings applications. This chapter outlines the clinical significance of tumour DNA testing and genomic sequencing in various cancers.

Keywords: Tumour DNA, Genetic testing, Genomic sequencing, Cancer genome sequencing, High-throughput sequencing, Sporadic cancer, Familial cancer, Cancer predisposition, Structural variation, Somatic mutation, Hereditary cancer, Cancer syndrome, Breast cancer, Ovarian cancer, Colorectal cancer, Skin cancer, Prostate cancer, Lung cancer, Thyroid cancer, Pancreatic cancer, Next-generation sequencing.

INTRODUCTION

Cancer is a genetic and cellular disease caused by the abnormal growth and proliferation of cells. The process of carcinogenesis is multi-facet and involves a series of complex genetic and epigenetic alterations that lead to the transformation of a normal cell into a cancerous cell [1 - 4]. The changes can be identified by tu-

* **Corresponding author Farhadul Islam:** Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh; E-mail: farhad_bio83@ru.ac.bd

mour profiling- a laboratory test used to detect specific genes or gene mutations and /or alterations, proteins or other biomarkers in a patient with cancer [5]. This molecular profiling of tumour could help in guiding personalised treatment decisions and can predict the metastasis and recurrence of cancer.

The presence of extensive genetic heterogeneity (both intertumoural and intratumoural) in cancer has significant implications for the selection of cancer biomarkers to guide decision-making in cancer treatment [6]. Therefore, cancer genome sequencing or tumour DNA sequencing permits oncologists and clinicians to identify the specific and unique alterations a patient has undergone during the development of their cancer [7]. This information could guide the personalized therapeutic options for the patients.

Numerous mutations underlie the development of each cancer, and it is reported that mutations in more than 1% of human genes (*i.e.,* cancer genes) contribute to cancer pathogenesis [8]. Also, 90% of the cancer genes showed somatic mutations, 20% showed germline, while 10% cancer genes showed both somatic and germline mutations [8]. The germline alterations (*i. e.,* mutations) associated with various cancer, especially familial cancer, have already been identified (Table **1**). For example, mutations in *BRCA1* and *BRCA2* genes cause HBOC syndrome, a known risk factor for the development of breast and ovarian cancer [9]. Patients with HBOC syndrome have a chance (50 to 85%) to develop breast cancer in their lifetime [9]. Thus, direct sequencing of *BRCA1* and *BRCA2* has been done to screen women suspicious for hereditary cancers. In addition, mutations in many other genes, such as *ATM, BRCA1, BRCA2, CDH1, CHEK2, NBN, NF1, PALB2, PTEN, STK11*, and *TP53,* are associated with the pathogenesis of breast cancer. Most importantly, somatic mutations in genes involved in cell proliferation, survival and death, such as *p53*, *RAS* family *etc*., are associated with the development of various cancer [8]. For instance, more than 50% cancers had shown *p53* somatic or acquired mutations [10]. Also, mutations in RAS have been identified in different types of cancers, including pancreas (90%), colorectal (50%), thyroid (50%), lung (30%), ovarian (15%), bladder (6%), breast, liver, skin, kidney and leukaemia [11]. Hence, tumour DNA sequencing could provide insightful, personalized information for molecular characterization of cancer along with susceptibility screening.

Additionally, in the context of personalised-precision medicine, discovery, development and validation of clinically useful biomarkers are prerequisites for better management of patients with cancer. Cancer biomarkers for susceptibility assessment, screening (early detection), stratification of cancer, the prognosis of the disease, the decision of appropriate therapeutics and duration of treatment, monitoring of therapy response and cancer recurrence are needed. Tumour DNA

sequencing, especially cancer genome sequencing, could be useful in therapy selection, response monitoring and cancer risk prediction. Therefore, the clinical implication (such as early detection, therapy selection, and response monitoring and cancer risk assessment) of tumour DNA sequencing is described in this chapter.

Table 1. Mutations associated with hereditary cancers.

Cancer	Genes
Breast cancer in women	ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, CDH1, NF1, NBN, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53
Breast cancer in men	BRCA1, BRCA2, CHEK2, PALB2
Colorectal cancer	APC, EPCAM, MLH1, MSH2, MSH6, PMS2, CHEK2, PTEN, STK11, TP53, MUTYH
Endometrial cancer	EPCAM, MLH1, MSH2, MSH6, PMS2, PTEN, STK11
Fallopian tube, ovarian, primary peritoneal cancer	ATM, BRCA1, BRCA2, BRIP1, EPCAM, MLH1, MSH2, MSH6, NB, PALB2, RAD51C, RAD51D, STK11
Gastric cancer	CDH1, STK11, EPCAM, MLH1, MSH2, MSH6, PMS2
Melanoma	BAP1, BRCA2 CDK4, CDKN2A, PTEN, TP53
Pancreatic cancer	ATM, BRCA1, BRCA2, CDKN2A, EPCAM, MLH1, MSH2, MSH6, PALB2, STK11, TP53
Prostate cancer	ATM, BRCA1, BRCA2, CHEK2,HOXB13, PALB2, EPCAM, MLH1, MSH2, MSH6, PMS2

Genetic Alterations in Cancer

Cancer genomes encompass a number of genetic changes, including chromosomal structural alterations, nucleotide changes, transcriptome changes, *etc*. Gross chromosomal structural changes in cancer can be accomplished by amplification, deletion, translocation and/or inversion of chromosomal segments or entire chromosomes. These structural changes, of course, duly alter genes in a number of ways, which in turn could be critical to the onset or progression of the disease [12]. Therefore, the identification and characterization of chromosomal structural variations associated with cancer could provide valuable information with increased clinical significance.

Alterations in certain nucleotides in tumour DNA have also been reported in various cancer pathogenesis [13, 14]. Targeted sequencing followed by amplification of tumour DNA is a powerful approach to successfully identify the key somatic mutations in cancer genomes. The targeted tumour DNA sequencing can characterize hundreds of genes, even the entire exome (all the protein-coding

CHAPTER 6

Circulating Tumour DNA: A Promising Cancer Biomarker

Sharmin Aktar[1,2], Plabon Kumar Das[3], Vinod Gopalan[1], Alfred King-yin Lam[1, 4] and **Farhadul Islam[5,*]**

[1] *School of Medicine, Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia*

[2] *Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh*

[3] *Institute for Glycomics, Griffith University, Gold Coast, Australia*

[4] *Pathology Queensland, Gold Coast University Hospital, Gold Coast, Australia*

[5] *Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

Abstract: Liquid biopsies, such as tumor-relevant proteins, miRNAs, circulating tumour cells (CTC) and cell-free DNA (cfDNA), have all been shown to have promising potential to be used as cancer biomarkers. However, the sensitivity and specificity of these biomarkers are currently insufficient, prohibiting their widespread application in clinical practice. Circulating tumour DNA (ctDNA) has received a lot of attention in recent years as a potential diagnostic and prognostic tool. Since tumours release genetic material, (*i. e.* ctDNA) into the bloodstream before they are apparent on imaging or cause symptoms, thus, ctDNA is one of the most promising liquid biopsy biomarkers for early diagnosis, prognosis, and treatment monitoring of patients with cancer. Accordingly, extensive preclinical and clinical research support that ctDNA has the potential to be considered a novel tool in early cancer diagnosis and prognosis. Also, ctDNA analysis can reliably predict tumour growth and treatment efficacy, as well as can aid in targeted therapy. Herein, this chapter will discuss the clinical significance of ctDNA in the management of patients with cancer as a potential liquid biopsy biomarker.

Keywords: Cancer biomarkers, Circulating tumour DNA (ctDNA), Early cancer detection, Liquid biopsy, Prognosis, Risk of relapse, Treatment efficacy.

[*] **Corresponding author Farhadul Islam:** Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh; Email: farhad_bio83@ru.ac.bd

INTRODUCTION

A number of traditional pathological examinations, such as tissue biopsy and imaging technologies, are used to diagnose cancers in clinical settings presently. Though tissue biopsy and imaging-based tumour diagnosis are considered the gold standard, they have numerous limitations, such as being ineffective for early-stage tumours or residual lesions detection. Also, limited application in assessing the treatment efficacy and prognosis of the disease [1]. As a result, a new branch of oncology research has emerged in recent years that focuses on cancer-derived components that circulate in the bloodstream [2].

Hence, over the last few years, a novel diagnostic approach known as "liquid biopsy"— the analysis of tumours using biomarkers circulating in peripheral blood such as circulating tumour cells (CTCs) and circulating cell-free tumour DNA (ctDNA) as well as exosomes, Tumour-relevant protein molecules and miRNAs- has gotten a lot of attention [2 - 9]. Liquid biopsy currently has a high specificity, allowing for the collection of consistent and reliable data from a blood sample in a simple and non-invasive manner [10]. Liquid biopsies are more convenient than traditional biopsies, and they pose no risk to the patient [4, 11]. Therefore, it is becoming increasingly popular as emerging tumour-specific markers for diagnosis and prognosis. Tumour-relevant protein molecules and miRNAs as well as circulating tumour cells (CTC), have all been demonstrated to be acceptable tumour biomarkers as a liquid biopsy in different cancers [5 - 9]. For example, large-scale clinical investigations have focused on the use of CTC counts as prognosis and monitoring therapy response in various cancer patients in recent years [3, 4]. However, the sensitivity and specificity of these biomarkers are currently inadequate, preventing their broader use in clinical practise. As a result, the development of new, highly sensitive, and specific tumour biomarkers is needed for better management of patients with cancer.

At the early stage of cancer, the tumours are small, less complex, and non-metastatic; milder therapy is more likely to be effective and could improve the survival of patients along with their quality of life [12]. Thus, there is a great need to develop accurate tests for the early detection of cancer. During tumour apoptosis or necrosis, tumour cells and CTCs may release their genetic materials into the bloodstream which is called ctDNA [13]. Since ctDNA is released into the circulation before tumours are visible on imaging or produce symptoms, *i. e.,* at early stages, hence ctDNA is considered one of the most promising biomarkers among liquid biopsy for early cancer diagnosis [14]. As the mutations of the original tumour are preserved, researchers have discovered that screening genetic lesions using ctDNA is very sensitive and specific, implying that employing ctDNA as a liquid biopsy could greatly improve current tumour diagnosis and

prognosis outcome [1]. For example, ctDNA levels are higher in patients at advanced stages of breast, colorectal, pancreatic and gastro-esophageal cancer than in early-stage patients [1, 15]. ctDNA analysis can also reliably predict tumour growth and prognosis, as well as aid in targeted therapy [16 - 18]. Furthermore, as a result of recent breakthroughs in ctDNA analysis, a number of studies have recently described the potential efficacy of their application in cancer management [4, 19]. Herein, the focus of this chapter is on the clinical implications of ctDNA as a critical component of liquid biopsies in cancer patients.

CIRCULATING TUMOR DNA (CTDNA)

Biology of ctDNA

Tumor DNA can be discharged into the bloodstream of cancer patients by primary tumours, CTCs, micrometastasis, or overt metastases during disease progression [1, 4, 13]. The majority of this ctDNA comes from tumour cells that have died or necrotized and released their fragmented DNA into the bloodstream [13]. ctDNA makes up only a small percentage of the total cell-free DNA (cfDNA), generally inferred by the detection of somatic variants [10]. In 1948, Mandel and Métais discovered non-cellular nucleic acids in the bloodstream of cancer patients [20], and this was the first time cfDNA was reported [3]. cfDNA may present outside of cells in body fluids such as plasma, urine, and cerebrospinal fluid (CSF) [21]. The majority of cfDNA in plasma originates from leukocytes, while only a minor percentage is tumour□derived (ctDNA) [21].

ctDNA fragments are slightly shorter nucleosome-associated fragments (80-200bp) [22]. However, the size of ctDNA fragments is still unknown, some researchers believe it is longer than its corresponding non-tumor cell-free DNA (cfDNA), while others believe it is shorter [1]. Recently Jiang *et al*. noted that the plasma of liver cancer patients had both long and short DNA molecules (<150 bp, 150-180 bp, and >180 bp), with the short fragments containing the tumor-relevant copy number aberrations [23]. The proportion of DNA fragments shorter than 150 bp and the tumour DNA fraction in plasma had a positive correlation, whereas the proportion of DNA fragments with sizes between 150 and 180 bp and tumor DNA fraction in plasma had no correlation. The proportion of DNA longer than 180 bp and the tumour DNA fraction in plasma were found to have a negative correlation. A similar phenomenon was also described by Madhavan *et al*. in patients with breast cancer [24].

In plasma or serum, ctDNA can be found as single-stranded or double-stranded DNA [1]. In principle, ctDNA contains genetic abnormalities that are identical to those found in tumour cells [25]. Plasma samples are preferred over serum

CHAPTER 7

Circulating Tumour Cells in Solid Cancer

Sharmin Aktar[1,2], Tracie T. Cheng[1], Sujani M. K. Gamage[1,3], Vinod Gopalan[1] and **Farhadul Islam[4,5,*]**

[1] *School of Medicine, Menzies Health Institute Queensland, Griffith University, Gold Coast, Australia*

[2] *Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Tangail-1902, Bangladesh*

[3] *Department of Anatomy, Faculty of Medicine, University of Peradeniya, Galaha Rd, 20400, Sri Lanka*

[4] *Griffith University, Institute for Glycomics, Gold Coast, Australia*

[5] *Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

Abstract: Circulating tumour cells (CTCs), as 'liquid biopsy", has a major benefit over traditional tissue biopsy and has the potential to become a less invasive and more cost-effective cancer biomarker. The presence of CTCs in the circulation indicates the presence of a tumour and the possibility of metastatic spread. Hence, the characterisation of CTCs is expected to provide crucial insights into the mechanisms of metastasis. It can also provide useful information about the future use of CTCs as a surrogate endpoint biomarker in diagnosis, prognosis, and treatment response prediction by minimizing the limitations of tissue biopsies. Also, it provides a new horizon for the development of novel targeted therapies. However, the lack of specific and effective methods is the key limitation in CTC detection and isolation in patients with cancer. Therefore, more responsive methods and approaches may be needed to improve the accuracy of CTC measurements. Herein, this book chapter will provide a current picture of CTCs as surrogate biomarkers for disease diagnosis, prognosis and predicting therapy response, along with the risk of relapse in cancers.

Keywords: Circulating tumour cells (CTCs), Diagnosis, Liquid biopsy, Overall Survival, Prognosis, Progression Free Survival, Risk of Relapse, Therapeutic Targets.

INTRODUCTION

Cancer was the second leading cause of death globally in 2020 [1]. The high mor-

* **Corresponding author Farhadul Islam:** Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh; E-mail: farhad_bio83@ru.ac.bd

tality rate of this disease is mostly attributed to its late diagnosis and the emergence of anti-therapy resistance [2]. Circulating tumor cells (CTCs) are cells that escape from a primary tumour into the vasculature or lymphatics and seed at distant sites to metastasize [3]. CTCs had recently been gaining widespread attention as an alternative and a promising non-invasive biomarker with minimal cost and risk over the current traditional disease assessment methods [4]. The discovery of CTCs in peripheral blood was first reported by Thomas Ashworth in 1869 [5]. However, their clinical implications for better management of cancer patients have come into focus in recent years. For example, a number of studies have demonstrated that CTCs had the potential to be used as a "liquid biopsy" and could be used in clinical settings for better diagnosis and predictor of clinical prognosis and treatment efficacy of the disease [6, 7].

CTCs are very rare cells (1 CTC per 10^5–10^7 peripheral blood mononuclear cells) [8], however, they can be isolated from blood by non-invasive approaches, such as using their distinctive physical (size, density, *etc*.) characteristics or biological (immunoaffinity-based, *e.g*., cell surface antigens-EpCAM, HER2, and MUC1]) properties or combinations of these features [9, 10]. As CTCs are believed to be a key player in the process of metastasis, they can be used to monitor the progression of cancer over time in patients with advanced stages. This can provide insightful information to better understand tumour cells' dissemination during metastasis [7, 11]. In addition, studies showed that CTCs have uniquely attributed to therapy resistance, thereby, studying CTCs could provide a better understanding of therapy resistance, leading to establishing better therapeutic strategies. Furthermore, analysis of CTCs has a promising potential for patients with early stages [7, 12]. As CTCs can be detected in patients with early-stage of cancer and have been reported to become a potential diagnostic biomarker and an independent prognostic factor for reduced progression-free survival (PFS) and overall survival (OS) [13 - 16]. Tumour-derived CTCs in the peripheral fluids of patients with cancers have the potential to be used as independent prognostic biomarkers for predicting metastatic relapse, monitoring treatment efficacy, as well as understanding metastasis development in various cancers, including breast, prostate, lung, colorectal, ovarian, pancreatic, head and neck, bladder cancer and melanoma as well as haematological malignancies [7, 10, 17]. For instance, patients with metastatic breast cancer had shown basal CTC count equal to five or more than five in 7.5 ml of blood [18]. These patients had shown shorter progression-free and overall survival when compared with patients having less than five CTC counts in 7.5 ml of blood [18]. For routine clinical implementation, however, adequate clinical and technological validation for specificity and sensitivity have not yet been achieved. Thus, a promising and reliable outcome is still scarce for the potential use of CTCs as surrogate endpoint biomarkers in cancer patients. Extensive research is going on to overcome technological

limitations. This chapter will focus on the potential role of CTCs as a novel biomarker and their effectiveness as an important diagnostic and prognostic tool in different types of cancer.

Circulating Tumour Cells (CTCs): Cytomorphology, Biology and Isolation Techniques

Circulating tumour cells (CTCs) leave the primary or metastatic tumour site, invade and circulate into the circulation, and if they survive, they migrate to secondary organs, where they can seed metastasis [3]. CTCs are a highly dynamic and heterogeneous subpopulation. The heterogeneity of CTCs is due to the accumulation of various genetic and epigenetic changes in its different subpopulations [19]. Also, the phenotypic and genotypic characteristics of CTCs can change during the course of the disease by microenvironmental factors and /or therapeutic selective pressures [20]. These cells are very rare cells, approximately 1 CTC per 10^5–10^7 peripheral blood mononuclear cells or even lower in solid tumours during local growth (8). They can be found as a single cells or clusters of cells containing an aggregation of a heterogeneous population of 3 or more cancer cells [21]. These clusters of CTCs are known as circulating tumor microemboli (CTM). It is thought that CTMs appear from the aggregation of adjacent cells within the primary tumour mass, rather than from single CTCs aggregating together after shedding [22].

Currently, there is no uniform information regarding the morphological characteristics of CTCs, and recent studies have showed the morphological heterogeneity of CTCs [23 - 25]. For example, a recent study reported that CTCs have a relatively larger dense basophilic and irregular nucleus (generally 13 to 15 μm in diameter) compared to leukocytes, a high nucleus-to-cell ratio, and a pale-bluish ring of cytoplasm encircling the nucleus [23]. Another study noted that CTCs have a relatively big nucleus (more than 8 μm) and prominent nucleoli [25]. It is very difficult to propagate *in vitro* culture of CTCs due to high transcriptional activities, and they usually survive up to 14 days [26].

CTCs are involved in the metastatic cascade, starting from the dissemination of tumour cells into the bloodstream and then e-seed for metastasis [11]. During this process, disseminating tumour cells (CTCs) must fight against several mechanical or environmental factors, such as shear forces, oxidative stress in the bloodstream, and the immune surveillance to extravasate into the distant site and colonize locally to form metastasis or remain dormant [20, 27]. The complete process of metastasis is unclear, however, it is believed that CTCs undergo the epithelial-mesenchymal transition (EMT) [28]. EMT is a normal physiological phenomenon that occurs during embryogenesis. During EMT, the epithelial cells lose the cell-

Part 3: Protein/Enzyme Biomarkers

CHAPTER 8

Protein Cancer Biomarkers

Sarath S. Joseph[1], Dan H. V. Tran[1], Farhadul Islam[2] and **Vinod Gopalan[1,*]**

[1] *Molecular and Cellular Pathology, School of Medicine and Dentistry, Griffith University, Gold Coast, Australia*

[2] *Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

Abstract: Cancer is one of the leading causes of death worldwide and it is becoming increasingly important to be able to efficiently identify and map the progression of cancers. The study of the diagnostic, predictive and prognostic value of protein biomarkers has become one of the main aspects at the forefront of cancer research. The diversity of various biomarkers for different cancers and their varying roles in each disease presents a continual challenge for researchers to understand, with new biomarkers still being discovered today. Understanding the role of protein biomarkers ensures patients are diagnosed with greater confidence and helps clinicians with treatment regimes. This chapter aims to discuss the clinical significance of various protein biomarkers in terms of their diagnostic, prognostic, and predictive value in the treatment of their respective cancers.

Keywords: Biomarker, BRCA, Cancer, Calretinin, CD117, Desmin, Diagnosis, ER/PR receptor, GFAP, HER2, Inhibin, Keratin, Protein, Prognosis, Predictive marker, S100 protein, TTF-1.

INTRODUCTION

Even with advancements in therapeutic interventions, the prognostic outcome of most cancers is poor. The low survivability of cancer can be mainly attributed to the difficulty in detecting it at an early stage and the insufficient tools available to map the progression of the disease. At the time of clinical presentation, more often than not, the tumour has metastasized, making it difficult to excise. Imaging, such as mammography with high sensitivity for breast cancer [1], is routinely utilised as screening tools to detect a select range of common tumours before they metastasize. However, the diagnostic and prognostic utility of imaging is questionable for other cancers, not to mention other limitations, including difficulty in tracing macro and micro metastasis. Current limitations result in poor

[*] **Corresponding author Vinod Gopalan:** Molecular and Cellular Pathology, School of Medicine and Dentistry, Griffith University, Gold Coast, Australia; Email: v.gopalan@griffith.edu.au

Farhadul Islam (Ed.)

surveillance of identified tumours, often resulting in a lack of understanding of the progression of cancer and the regrettable late-stage detection of cancer spread.

The involvement of protein-encoding genes and proteins in tumour growth has been established as a promising front for understanding the development of cancer. Utilising proteins generated from mutated genes during cancer pathogenesis can reveal a great amount of information regarding the identity of the tumour, its origin and its progression. This potentially opens a role for these protein biomarkers to act as predictive markers in clinical use and could offer a real-time assessment of therapeutic efficacy, revolutionising the prognostic role of a simple serum sample. Thirty-seven well-established proteins in today's literature that act as biomarkers (Table **1**), with significant diagnostic, prognostic and clinical value, will be discussed in this chapter.

Table 1. Summary of the diagnostic and prognostic biomarkers with their associated cancers.

Protein Biomarkers	Associated Cancers	References
α-SMA	Oral tongue squamous cell carcinoma, Lung adenocarcinoma, Ovarian cancer	[1, 2, 3, 4, 6]
BRAF	Colon adenocarcinoma, Melanoma, Lung adenocarcinoma, Thyroid papilloma	[7, 9, 10, 11, 12, 13, 14]
BRCA1,BRCA2	Breast cancer, Ovarian cancer, Prostate cancer, Fallopian tube cancer, Endometrial cancer, Pancreatic cancer, Prostate cancer, Colorectal cancer, Melanoma	[17, 18, 19, 20, 21, 22, 23, 24, 25]
Calretinin	Epithelial pleural mesothelioma	[27, 28, 29, 30]
CD117	Gastrointestinal stromal tumour	[32, 33, 34, 35, 36]
CD20	B cell lymphoma, Leukemia	[40,41,42,44]
CD30	B Cell lymphoma	[46, 47, 48]
Chromogranin A	Neuroendocrine tumour	[52, 53, 54]
Cytokeratin (TPA, TPS & CYFRA 21.1)	Lung cancer, Squamous cell carcinoma of Oesophagus, Metastatic breast cancer, Colorectal cancer	[57, 58, 60]
Desmin	Colorectal cancer, Gastrointestinal stromal tumour, Embryonal sarcoma, Gall bladder cancer	[62,63, 64, 65, 66, 67]
EGFR	Lung adenocarcinoma, Glioblastoma, Breast cancer, Colon adenocarcinoma	[68, 69, 70, 71]
EML4/ALK	Lung adenocarcinoma	[72, 73 74]
ER/PR	Breast cancer, Endometrial cancer, Ovarian cancer	[75, 76, 77, 79, 80, 85]
FIP1L1-PDGFRα	Chronic eosinophilic leukemia	[86, 87]
FLI-1 protein	Ewing sarcoma, Vascular tumour, Lymphoblastic lymphoma	[88, 89]

(Table 1) cont.....

Protein Biomarkers	Associated Cancers	References
GFAP	Glioma, Glioblastoma	[91, 92, 93]
GCDFP-15	Breast cancer	[94, 95, 96]
HER2/neu	Breast cancer, Gastric cancer	[97, 98, 99, 100, 101, 102]
hPG80	Colorectal cancer, Renal cell carcinoma, Hepatocellular carcinoma	[103, 104, 107]
HMB-45	Melanoma	[108, 109]
Inhibin	Ovarian cancer	[110]
Keratin-17	Gastric cancer, Breast cancer, Ovarian cancer, Endometrial cancer, High-grade cervical cancer, Bladder cancer	[112, 113, 114, 115, 116, 117]
Keratin-19	Breast cancer, Hepatocellular carcinoma	[118, 119]
KRAS	Lung adenocarcinoma, Pancreatic adenocarcinoma, Colon adenocarcinoma	[122, 123, 124, 125, 126]
Mart-1	Melanoma	[127, 128, 129]
MyoD1	Rhabdomyosarcoma, Breast cancer, Colorectal cancer, Gastric cancer, Head and neck cancer, Lung cancer, Retinoblastoma, Medulloblastoma, Colorectal cancer	[130, 131, 133]
MSA	Paediatric rhabdomyosarcoma, Laryngeal leiomyosarcoma	[134, 135]
Neurofilament	Breast cancer, Gastric cancer, Prostate cancer, Brain metastases from lung cancer	[136, 137, 138, 139]
PDGFR	Ovarian cancer, Breast cancer, Gastrointestinal stromal tumour	[140, 141, 143, 144]
PML/RARα	Promyelocytic leukemia	[148, 149]
S100 protein	Melanoma, Ovarian cancer, Pancreatic cancer, Colorectal cancer, Glioblastoma	[150, 151, 152, 154]
Synaptophysin	Paraganglioma, Pheochromocytoma, Neuroendocrine epithelial tumours	[155, 156]
TTF-1	Thyroid cancer, Lung adenocarcinoma	[157, 158, 159, 161]
Vimentin	Thyroid cancer, Breast cancer, Melanoma, Renal Cell carcinoma, Central neuron system cancer, Gastrointestinal cancer, Cervical cancer, Endometrial cancer, Hepatocellular cancer, Non-small cell lung cancer	[162, 163, 164, 165, 166]

PROTEIN BIOMARKERS

α-Smooth Muscle Actin (α-SMA)

α-SMA is overexpressed in several cancers. Firstly, α-SMA is overexpressed in

CHAPTER 9

Enzymes: Tumour Associated Biomarker

Farhadul Islam[1,*]

[1] *Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

Abstract: Enzymes catalyse biochemical reactions and tightly regulate biophysical and metabolic pathways to maintain cellular homeostasis. However, the unregulated activity of these enzymes results in metabolic disorders and genetic diseases, including cancer. In cancer, significant alteration of enzyme levels and/or activity can be detected during malignant transformation, thus, it can be used as a potential biomarker in clinical applications. For example, serum levels of lactate dehydrogenase (LDH), neuron-specific enolase (NSE) and thymidine kinase 1(TK1), alkaline phosphatases (ALPs), tumour M2-PK, hexokinase (HK), *etc*., significantly increased in patients with various cancers, such as metastatic breast cancer, intracranial germ cell tumours, ovarian serous carcinomas, oesophagus, cervical, gastrointestinal, prostate, renal cell carcinoma, head and neck and lung cancers. Also, they are associated with various clinicopathological factors, such as stage, grade, lymph node metastasis, distant metastasis, *etc*. In addition, overexpression of carbonic anhydrase XII (CAXII), matrix metalloproteinases (MMPs) and aldehyde dehydrogenase 1 (ALDH1), in cancer tissues, is associated with the presence of several cancers and correlated with the progression of the diseases. Therefore, screening of these enzymes at the point-of-care settings could facilitate better management of patients with cancer. This chapter summarizes the roles of cancer associated-enzymes, especially emphasizing their clinical significance in patients with various cancers.

Keywords: Alkaline phosphatase, Aldehyde dehydrogenase 1, Biosynthesis, Carbonic anhydrase XII, Cancer bioenergetics, Cancer metabolism, Ghrelin O-Acyl Transferase, Glucose-6-phosphate dehydrogenase, Hexokinase, Lactate dehydrogenase, Matrix metalloproteinases, Metabolic enzymes, Neuron-specific enolase, Receptor-interacting protein kinase, Thymidine kinase, Tumour M2-PK, Urokinase-type plasminogen activator, Warburg effects.

[*] **Corresponding author Farhadul Islam:** Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh; Email: farhad_bio83@ru.ac.bd

Farhadul Islam (Ed.)

INTRODUCTION

Enzymes are biocatalysts, accelerating the biochemical reactions in almost all metabolic processes in the cells at a rate fast enough to sustain life [1]. Individual steps of metabolic pathways are catalysed and regulated by enzymes in cells, thus, tight control of enzymatic activity is essential for cellular homeostasis. Any malfunction, such as mutations, overproduction, underproduction or deletion *etc*., of a single critical enzyme, can lead to metabolic, such as Tay-Sachs disease and genetic diseases, including cancers [2, 3]. For example, malfunction of enzymes involved in DNA repair causes cancers due to less capability to repair mutations in their genome, thereby slowing the accumulation of genetic alterations, resulting in cancer development [4].

Altered metabolism, such as altered bioenergetics (aerobic glycolysis, reduced oxidative phosphorylation), increased biosynthesis (production of biosynthetic pathways intermediates), redox balance, *etc*., are evident in cancer cells in comparison to most normal tissue cells. These changes support the acquisition and maintenance of malignant properties known as cancer metabolism. In cancer, these reprogrammed activities improve cellular fitness to provide selective advantages during tumour formation either by supporting cell survival under stressful microenvironment or by allowing uncontrolled growth and proliferation [5].

Metabolic reprogramming (enhanced or suppressed metabolic activity) and oncometabolite (metabolites increased significantly in cancer) are two key phenomena in cancer as a consequence of malfunction of metabolic enzymes due to tumorigenic mutations and /or other factors [6]. Aerobic glycolysis, or Warburg effect- constitutive glucose uptake and production of lactate regardless of oxygen availability, is a prominent example of metabolic reprogramming in cancer [7]. Enhanced glycolytic flux in cancer cells produces increased glycolytic intermediates, which provide the metabolic demands of other pathways in proliferating cells. Also, the increased flux of tricarboxylic acid (TCA) cycle intermediates is used as the precursor for macromolecule synthesis in cancer cells [8]. Thus, metabolic reprogramming of cancer cells allows increased biosynthesis of biomolecules for uncontrolled proliferating cells. In addition, alteration of metabolic enzymes generates oncometabolite, such as D-2-hydroxyglutarate, citrate, *etc*., which in turn stabilize hypoxia-inducible factor 1, nuclear factor-like 2, thus, can inhibit p53, prolyl hydroxylase 3 along with regulation of DNA/histone methylation [9]. These activities induce cellular growth and proliferation by activating cell growth signalling. Also, the oncometabolite promote glutaminolysis, glycolysis and generation of reactive oxygen species (ROS), resulting in increased cellular growth and proliferation. Furthermore,

epigenetic and genetic alterations of metabolic enzymes are associated with increased fatty acid β-oxidation, which can induce epithelial-mesenchymal transition (EMT). Thus, alterations of metabolic enzymes stimulate carcinogenesis by activating cell proliferation and survival signalling.

Additionally, the detachment of cancer cells from the extracellular matrix (ECM), a step in cancer metastasis (the main reason for cancer-related mortality), is critically regulated by enzymatic activity [10]. Altered cellular metabolism can induce the death of detached cells by elevated production of ROS due to low glucose influx during ECM detachment. Importantly, the metabolic enzyme receptor-interacting protein kinase (RIPK) family induces necrosis of ECM-detached cells by mitophagy (selective degradation of mitochondria by autophagy)-mediated increased ROS production [10]. Also, suppression of RIPK1-mediated mitophagy stimulates *in vivo* tumour growth. In addition, RIPK1 higher expression is associated with improved survival of patients with lung cancer [10].

Enzymes play crucial roles in cancer pathogenesis and distant metastasis in various cancers. Thus, the implications of enzymes as cancer biomarkers hold promise for the better management of patients with cancers. Hence, this chapter discusses the clinical significance and biomarker roles of cancer-associated enzymes in disease diagnostic, prognostics and predictive perspective.

Enzyme Biomarkers in Cancer

Enzymes have the potential to be used as biochemical markers for cancer screening, detection, prognosis, and therapeutic response predictions, as the alterations of gene expression encoding enzymes during malignant transformation can be detected in resulting cancers. Although, a single cancer-specific enzyme with clinical applications for all tumours is yet to be identified, however, alterations of enzymes activity and/or levels in cancer compared to the normal tissues may have the potential for cancer screening, prognosis, monitoring treatment response and clinical stratifications of patients with cancers. The enzymes that have biomarker implications in various cancers are summarized in Table **1**. In the following sections, we discuss the biomarker roles of important enzymes in cancer.

Table 1. Enzymes with cancer biomarker significance.

Enzymes	Clinical Significance	References
GOAT	Diagnostic and predictive marker for biological aggressiveness of cancer in patients prostate cancer	12-14

CHAPTER 10

Glycoproteins and Cancer Biomarkers

Md Abedul Haque[1,*]

[1] *The University of Texas MD Anderson Cancer Center, Houston, Texas-77033, USA*

Abstract: Glycoproteins or glycosylated proteins are carbohydrates (oligosaccharide chains or glycan's) linked proteins and execute important functions in the biological systems, such as embryonic development, cell-to-cell recognition, adhesion, pathogen identification and immune functions. It is evident that the alteration of glycoproteins in cells are associated with a number of human diseases, including cancer, rheumatoid arthritis, inflammatory diseases as well as immunodeficiency diseases. Recent advances in modern technologies in cancer treatment are promising. However, researchers and clinicians are still searching for appropriate biomarkers for the early detection and management of patients with cancer. Altered glycoprotein levels are associated with critical events in cancer pathogenesis and progression. Also, abnormal glycosylation of protein is a common regulatory event in carcinogenesis, therefore, aberrant glycosylation could act as a promising resource in identifying a cancer biomarker for diagnosis and monitoring of the progression of patients with cancers. This chapter summarizes the major clinically approved glycoproteins utilized for screening, diagnosis, and monitoring of the treatment response of patients with cancers.

Keywords: α-fetoprotein, Biomarkers, Breast Cancer, Cancer antigen, Carbohydrates, Cancer Diagnosis Glycoprotein, Cell, Colon cancer, Glycosylation, Glycan, Lung cancer, Microenvironment, Mucins, Neoplasia, N-Glycosylation, Ovarian Cancer, O-glycosylation, Proteins, Prostate cancer, Prostate-specific antibody, Targeting therapy.

INTRODUCTION

Cancer is one of the major public health concerns and leading causes of death worldwide [1]. Besides health concerns, the economic burden of medical costs for cancer treatments is also expected to increase significantly in the future due to less improvements in overall survival rate, changes in treatment patterns and other related medical costs following a cancer diagnosis. Still, the management of cancers is posing the greatest threat and challenge to the modern medical sciences, thus, the development of correct screening and diagnosis strategies and treatment

[*] **Corresponding author Md. Abedul haque:** The University of Texas MD Anderson Cancer Center, Houston, Texas-77033, USA; Email: MHaque@mdanderson.org

Farhadul Islam (Ed.)

and monitoring approaches for cancers remain a major field of biomedical research throughout the world. The advances in ongoing cancer treatment, including surgical resection, chemo-radiotherapy, hormonal therapy, immune therapy and targeted therapy, are appreciable, however, the overall disease-free survival rate and complete recovery are still disappointed due to heterogeneity of tumor population and acquired drug resistance [2]. Therefore, proper management of patients with cancer-specific biomarkers, molecularly targeting therapy and effective medication plan to prolong disease-free survival is a promising approach to save millions of lives as well as billions of dollars.

Previous studies indicated that neoplastic transformation results from a series of cellular events, which in turn could alter the cellular growth, development, proliferation, survival and general physiology of the affected tissues or organs. Traditionally, genetic mutations are believed to be strongly related to the pathogenesis of neoplastic disease development, however, changes in non-genetic materials, such as epigenetic alterations, could also significantly contribute to the neoplastic transformations. Epigenetic changes such as acetylation, methylation, and histone glycosylated proteins act as key regulatory components of neoplastic-transformation and progression in many cancers [3]. Glycoproteins are a group of post-translationally modified proteins in which oligosaccharide chains or glycans are covalently liked to amino acid side chains. Glycoproteins are ubiquitously distributed and play an important role in various biological processes, including cell signalling, cell-cell interaction, immune recognition, cell proliferation, and differentiation [4, 5]. Therefore, altered levels of glycoproteins possess a close relationship with the progression of many diseases, such as autoimmune disease, rheumatoid arthritis, inflammatory diseases, Alzheimer's disease, and cancers [6, 7]. Proteins are glycosylated in cancer cells either in benign or malignant conditions, and induce cancer development, progression and metastasis [5, 8]. So, glycoproteins represent promising biomarkers to detect, diagnose, and manage various cancer types [9].

Aberrant glycoproteins levels are observed in several human cancers, such as prostate, colorectal, breast, and hepatocellular carcinoma (HCC) [10 - 14]. Identification of the changes of glycoproteins at early stages of cancers is helpful for clinicians in treatments plan that can enhance survival rates. This chapter summarizes the major clinically approved glycoproteins, which are not only aberrantly expressed in different stages of cancers but also create potential opportunities for researchers and clinicians to use these aberrant glycoproteins as a biomarker for screening, diagnosis and monitoring tools for cancer treatments.

Protein Glycosylation in Cancer

During neoplastic transformation, abnormal glycosylation of proteins is one of the epigenetic (or post-translational) modifications of proteins that manipulate the biological activity and appear to regulate cellular growth and proliferation, survival, as well as mediate metastasis in several cancers [3, 15]. The protein glycosylation required a coordinated presence of a complex array of enzymes, organelles, and other factors, *i. e.,* factors for post-translational modifications. The complex process can enzymatically add sugar molecules to the protein either as a linkage of monosaccharides or as whole oligosaccharides (glycans)] to the specific residue (amino acids) of the glycoprotein. Approximately 50% of amino acids in a protein (*e.g.,* 9 out of 20) can be modified either by a single monosaccharide or a glycan chain with oligosaccharides. In the case of mammals, the most frequent glycan's consist of 10 monosaccharides building blocks—Glc (Glucose), Gal (Galactose), GlcNAc (N-acetylglucosamine), GalNAc (N-acetylgalactosamine), Fuc (Fucose) Fuc, Man (Mannose), Xyl (Xylose), GlcA (Glucuronic acid), IdoA (Iduronic acid), and Neu5Ac (5-N-acetylneuraminic acid or sialic acid). They are derivatives of glucose found in all cells (Fig. **1**) [16].

In mammalian cells, two types of protein glycosylation are mainly observed, such as N -Glycosylation and O -Glycosylation. Both types of glycosylation often coexist in the same protein. N-glycosylation involves the attachment of glycan's ranging in complexity to select asparagine (or less commonly, arginine) residues. The addition of N-glycan`s initiated in the endoplasmic reticulum (ER) and further diversified in the Golgi apparatus [17]. While in O-glycosylation, single sugars (N- acetylglucosamine) or glycan's are added to serine, threonine, and, less often, to tyrosine or other hydroxyl-containing residues. O-glycosylation of proteins is primed in ER and Golgi-apparatus or cytosol by stepwise enzymatic transfer of monosaccharides [18]. Increased N-glycosylation rate is associated with cancer growth and metastasis. For example, β1, 6 N-acetyl glucosaminyl transferase V (GnT-V; MGAT5) is one of the most relevant glycosyl transferases associated with the migration, invasion, and metastasis of cancer. On the other hand, the expression of β1–6 branched oligosaccharides regulated by GnT-V serves as a marker for tumor progression, metastasis, and poor prognosis in breast and colorectal cancers [19, 20]. Similarly, aberrant O- glycosylation's correlated with the pathogenesis of lung and pancreatic cancers. For instance, the expression of N-acetyl galactosaminyl transferase (GALNT) 3 is a potential diagnostic and prognostic marker for lung and pancreatic cancers [21, 22]. In addition, GALNT6 can glycosylate and stabilize onco-protein MUC1 (mucin 1), thereby contributing to mammary carcinogenesis by disruption of β-catenin and E-cadherin. Also, GALNT6-fibronectin signalling is an important component for the development and procreation of breast cancers [23, 24].

Part 4: Hormone & Small Molecular Biomarkers

CHAPTER 11

Hormones as Cancer Biomarkers

Plabon Kumar Das[1, 2] and **Farhadul Islam**[1, 2,*]

[1] *Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

[2] *Institute for Glycomics, Griffith University, Gold Coast, QLD-4222, Australia*

Abstract: Among all the cancer biomarkers, hormones are less discussed despite having the ability to be used as potential biomarkers in the diagnosis and prognosis of various cancers. When a tissue, normally produces hormones in lesser quantity, produces a hormone in excess levels, then hormones can be used as tumour biomarkers. Sometimes it is also seen that a hormone is produced by the tissue, which is not normally associated with the secretion of that hormone. For example, calcitonin, a protein hormone produced by the thyroid gland, is reported to be increased in production in thyroid carcinoma. Another protein hormone, namely human chorionic gonadotropin (hCG), is used as a biomarker in choriocarcinoma, testicular tumors, *etc*. On the other hand, a lower level of testosterone hormone is found in prostate cancer, indicating its role in prostate cancer prognosis. There are other peptidase and steroid hormones, such as insulin, glucagon, estrogen and progesterone which significantly contribute to various tumours and are used as valuable biomarkers in the diagnosis and prognosis. Taken into consideration, in this chapter, we discuss the roles of multiple peptides and steroid hormones in the diagnosis and prognosis of various cancer types.

Keywords: Hormones, Biomarkers, Breast cancer, Cancers, Calcitonin, Choriocarcinoma, Diagnosis, Estrogen, Gastrin, Glucagon, Gonadotropin, Human chorionic gonadotrophin, Insulin, Prognosis, Peptide hormones, Progesterone, Prolactin, Steroid hormone, Thyroid cancer, Thyroid stimulating hormone, *etc*.

INTRODUCTION

Tumour biomarkers are biomolecules produced by tumours or noncancerous tissues in response to the presence of tumours [1]. Those biomolecules are altered genetic, epigenetic material, proteins and hormones. Hormones are the chemical messengers that regulate crucial functions. For example, they maintain energy metabolism, growth, stress and reproduction. They bind to the receptors on plasma membrane and initiate the signalling cascade for the cellular process [2].

[*] **Corresponding author Farhadul Islam:** Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh; Email: farhad_bio83@ru.ac.bd

Farhadul Islam (Ed.)

Their actions are very wide and can maintain growth either positively or negatively.

In cancer, they can regulate apoptosis and immune response in patients, thus can contribute to the development and progression of various cancers either positively or negatively. Levels of hormones, such as estrogen, progesterone may act as the risk factor of some common cancers, including breast cancer and prostate cancer [3]. A hormone can act as tumour biomarker when it is produced in excess by diseased tissue compared to normal tissue, or even when ectopically produced by other tissues which are not normally involved in the production of that hormone [1]. For example, calcitonin, a hormone produced by the parafollicular cells (also kwon as C-cells) of thyroid gland, has been observed to produce in excess in medullary thyroid carcinomas [4]. Human chorionic gonadotropin (hCG), a placental hormone primarily produced by syncytiotropoblastic cells during pregnancy, however, can be generated in testicular cancers, thereby can be o used as a maker of testicular cancers as a panel of alpha-phetoprotein, lactate dehydrogenase and hCG [5]. In addition, changes of hormonal level was noted in major types of cancers, such as breast, prostate, endometrial and thyroid cancers [6]. For example, Luteinizing hormone (LH) was reported to be upregulated in breast, prostate, endometrial and thyroid cancers, whereas thyroid stimulating hormone (TSH) and prolactin is upregulated in ovarian, pancreatic and endometrial cancers [7 - 9]. Moreover, another hormone known as adrenocorticotropic hormone (ACTH) was upregulated in ovarian and endometrial cancers [2]. Thus, a number of hormones could have the potential to be used as the biomarkers for various cancers (Table **1**). Therefore, measuring the level of these hormones in cancer tissue or nearby normal tissue will help us to diagnose cancer early and determine the prognosis effectively. In this chapter, we describe the roles of various hormones as potential biomarkers and their clinical application in the diagnosis as well as in monitoring the disease process.

Table 1. Hormones as tumour biomarkers.

Hormones	Types	Function as Biomarkers	References
Calcitonin	Peptide	Aids in the diagnosis of medullary thyroid cancer	[13, 14]
hCG	Peptide	Diagnosis of choriocarcinoma, testicular tumors, and ovarian germ cell tumors (GCTs)	[18]
Insulin	Peptide	Helps in the diagnosis of insulinoma	[26]
Gastrin	Peptide	Aids in the diagnosis of Gastrinoma	[30, 31]
Glucagon	Peptide	Diagnosis of glucagonomas	[36]
Prolactin	Peptide	Aids in the diagnosis of ovarian cancer	[38]
TSH	Peptide	Aids in the diagnosis of thyroid cancer and breast cancer	[44]

(Table 1) cont.....

Hormones	Types	Function as Biomarkers	References
Estrogen	Steroid	Higher level is seen in breast, endometrial, prostate and ovarian and thyroid cancers and laryngeal squamous cell carcinoma	[46 - 50]
Progesterone	Steroid	Increases cancer stem cell characteristics in breast cancer	[54, 55]
Testosterone	Steroid	Lower level is seen in prostate cancer	[62 - 64]

HORMONES AS BIOMARKERS IN VARIOUS CANCERS

Peptide Hormones as Cancer Biomarkers

Peptide hormones, made of amino acids, are water soluble and act on the surface of target cells *via* second messenger [10]. After releasing into the circulation, these hormones interact with receptors on plasma membrane of target cells (Fig. **1**). This interaction initiates intracellular signalling pathways involved in the regulation of various physiological processes, including energy metabolism, growth, stress, and reproduction [10]. These hormones are a vital component in many cellular processes, however, excessive amount of circulating peptide hormones is often associated with various tumours, such as pancreatic cancer, medullary thyroid cancer, *etc*. Thus, these hormones could have potentially be used as a tumour biomarker for the specified types of cancers. The roles of various peptide hormones as biomarkers in cancers will be discussed in the following sections.

Calcitonin

Calcitonin, a linear peptide consisting of 32 amino acids, is released from the parafollicular cell, also known as C-cells of the thyroid gland. Circulating calcium drives the secretion of calcitonin and regulates calcium homeostasis [11, 12]. Excessive calcitonin is seen in medullary thyroid cancer, a predominant thyroid malignancy in United States, accounting for 1%–2% of thyroid malignancies [13]. It was noted that serum calcitonin concentration was associated with tumour size inpatients with medullary thyroid cancer [14]. However, the level of serum calcitonin is not recommended as a diagnostic tool for medullary thyroid cancer due to the fact that calcitonin concentrations could also be associated with other conditions, such as acute and chronic renal failure, hypercalcemia, pulmonary disease, and other malignancies [11, 15]. Thus, the level of serum calcitonin could have the potential to be used as a prognostic marker to monitor therapy response in patients with medullary thyroid cancer.

CHAPTER 12

miRNAs as Epigenetic Cancer Biomarker

Afraa Mamoori[1,*]

[1] *Department of Pathology and Forensic Medicine, School of Medicine, University of Babylon, Hillah, Iraq*

Abstract: Despite the fact that the mortality rate of many types of cancer has decreased in the last decades, cancer remains one of the most challenging diseases in the world. The number of newly diagnosed cases with advanced stages in different types of cancer is still high because available tests are not efficient enough to be used for screening. In addition, the available diagnostic tests failed to diagnose certain types of cancer until late presentation. Furthermore, therapeutic agents currently in clinical use to treat a certain type of malignant tumours still show a high rate of resistance in some patients. Many types of available cancer biomarkers failed to manage and resolve this problem because of the lack of both sensitivity and specificity of these markers. Advanced researches in epigenetics highlight the importance of certain non-coding genes in diagnosing and follow-up of patients with different types of cancer. One of these substances is microRNAs (miRNAs) which showed high sensitivity and specificity as cancer biomarkers. miRNAs are highly stable and expressed in different types of human body samples; some of them are tissue specific. These features make them available as cancer biomarkers, and they are started to be in clinical use recently.

Keywords: Biomarkers, Cancer, Diagnosis, Prognosis, Epigenetic, Clinical, Facts, Specificity, Sensitivity, Non-coding RNAs, Fluid, Sample, Tissue, Commercialized miRNAs, miRview test, miRNAs, Therapeutic resistance, Epithelial-mesenchymal transition.

INTRODUCTION

Cancer biomarkers can simply describe as substances like DNA, RNA, protein and peptides present in the human body and its products, which have the ability to identify cancer development, cancer progression and response to the therapeutic agents. These substances become extremely important in clinical practice after the increase in genomic profiling and molecular target therapy technology. Cancer biomarkers can be classified into three types depending on their clinical use [1]. The first type is called diagnostic biomarkers, which are used to identify certain

* **Corresponding author Afraa Mamoori:** Department of Pathology and Forensic Medicine, School of Medicine, University of Babylon, Hillah, Iraq; E-mail: afraajawad@yahoo.com

Farhadul Islam (Ed.)

types of cancer. The second type is named prognostic biomarkers, which are clinically used to determine the risk and recurrence of cancer. The last type is the predictive biomarkers, which are used to predict the usefulness of drugs in the treatment of various types of cancer [2, 3].

The usual cancer biomarkers which are in clinical practice for several years show limitations for the use as diagnostic, prognostic and predictive biomarkers because they are low in both specificity and sensitivity towards certain types of cancer. For example, Prostate Specific Antigen (PSA), which has been in clinical use as a prognostic and diagnostic biomarker for prostate cancer for many decades, is being increasingly criticised for its false positive diagnostic rate [4]. Moreover, CA19-9, which is the only biomarker approved to use in clinical practice for pancreatic cancer diagnosis and prognosis, showed only 79% sensitivity and 82% specificity with poor predictive value in the symptomatic patients reaching only 0.5-0.9% [5].

High throughput sequencing in epigenetic researches raises the opportunity of using the microRNAs as cancer biomarkers with more sensitivity and specificity than the old cancer biomarkers. Thus, this chapter will discuss the importance of using these molecules in different types of cancer as diagnostic and prognostic markers. Herein, we will identify the important research findings in the field and show the miRNAs introduced to clinical practice recently.

MiRNAs and Epigenetics

The term “Epigenetics” can be defined as heritable alterations in gene expression without changes to the DNA bases sequence; they play an integral part in genomic stability and gene transcription [6]. Epigenetics is composed of 3 components, *i.e.* DNA methylation, Histone modifications, and Non-coding RNAs [7]. The term “non-coding RNAs” (ncRNAs) can be defined as the species of RNAs which are not coding for proteins; various forms of them are essential for the regulation of cellular processes, including target genes transcriptions and translations and thereby controlled cellular proliferation, differentiation, aging, longevity and so on [8]. Non-coding RNAs can be divided into two main categories (long non-coding RNAs and short non-coding RNAs) depending on the nucleotide's sequence length of their mature form. Short non-coding RNAs include (PIWI-interacting RNAs (piRNAs), circular RNAs (circRNAs), transfer RNAs (tRNAs)-derived small RNAs (ts RNAs), small nucleolar RNAs (snoRNAs) and micro RNAs (miRNAs).

Micro RNAs (miRNAs) are short non-coding RNAs. In their mature form, they are 20-24 nucleotides in length. Since their initial discovery, it has been demonstrated that this class of non-coding RNA is involved in regulating many

biological pathways in a variety of multicellular organisms, including humans [9]. In relation to cancer, miRNAs can act as oncogenes or tumour suppressor genes, depending on their regulatory targets [10]. Also, miRNAs have been implicated as robust regulators in both normal and cancer stem cells (CSCs). Cancer stem cells are suggested to be responsible for the initiation and promotion of cancers as well as for enabling resistance to chemotherapeutic treatments [11 - 13].

Mode of Action of MicroRNAs

miRNAs act by post-transcriptional repression and target mRNA degradation, through a complex process. Following their transcription in pri-miRNA form, they are converted into pre-miRNAs consisting of a stem-loop structure and finally processed into the mature form of a short, double-stranded RNA of about 20 nucleotides. After this process of biogenesis, the mature miRNAs are rendered single-stranded and assembled into ribonucleoprotein complexes called (miRNAPs) or miRNA-induced silencing complex (miRISC) in association with a protein family called argonaute (AGO) [14, 15]. There are four types of AGO protein in mammals (AGO1 to AGO4), and they are mainly involved in the gene expression-repression process mediated by miRNA [16, 17]. During the process of repression, miRNAs bind to the 3' UTR of target mRNA to form base pairing in a 2-8 nucleotide long sequence (the seed region), which facilitates the complete miRNA-mRNA association [18]. miRNA binding sites are available in multiple copies which are necessary for effective repression processes [19, 20]. In addition, the degree of miRNA-mRNA complementarity is crucial for the strength of the regulatory mechanism. A perfect miRNA-mRNA complementarity will result in the destruction of the mRNA strand by AGO, while central mismatches in the miRNA sequence will promote repression of mRNA translation by strand cleavage processes [21]. This process of post-transcriptional repression of mRNAs can be achieved at either translation initiation or at a post-translational stage.

Targets Selection by MicroRNAs

Single miRNAs can regulate the function of hundreds of target genes, and one target gene can also be regulated by several miRNAs. The process of target selection for a miRNA is mainly driven by seed sequence complementarities between the miRNA and its target mRNAs [22, 23]. It is understood that there are also other factors that may participate effectively in the process of target selection, factors which we are beginning to elucidate. Titration of miRNA from its target gene *via* a phenomenon called competing endogenous RNA (ceRNA) is one of the controlling factors in determining target selection [24 - 25]. An example of such titration in cancer would be through the expression of pseudogenes to decoy

Part 5: Novel Methods for Cancer Biomarker Detection

CHAPTER 13

Electrochemical and Optical Detection of MicroRNAs as Biomarkers for Cancer Diagnosis

Riham Zayani[1], Amira Ben Hassine[1], Amal Rabti[1], Amal Raouafi[1] and **Noureddine Raouafi[1,*]**

[1] *Sensors and Biosensors Group, Analytical Chemistry & Electrochemistry Lab. (LR99ES15), Faculty of Science, University of Tunis El Manar, Tunis El Manar, Tunisia*

Abstract: According to the miRBase (v 22.1), released on October 2018, there are more than 1900 identified human microRNA mature sequences. MicroRNAs (aka miRNAs or miRs) are a class of short non-coding RNA sequences, which have been detected within the cells or in body fluids. They act as gene expression regulators and intervene in numerous physiologic and development processes. They post-transcriptionally/translationally regulate expression of some proteins by forming miRNA-induced silencing complex (mRISC) by binding to 3'-UTR regions of the target messenger RNA to inhibit the protein synthesis. It has been noted that up- and down-regulation of miRs are associated with the pathogenesis of several types of human cancers since their target proteins are tumor-suppressive or oncogenic ones. This chapter will present a general summary of miRNA biogenesis, their link to cancer, and biological methods for their detection. Thanks to their ease of use and high sensitivity, electrochemical and optical techniques were used to detect miRNAs with or without the assistance of amplification methods. We will review the state-of-the-art electrochemical and optical methods for their detection, emphasizing the progress achieved in the last five years (2015-2020). Finally, we will present the main advantages, challenges, and future prospects for future research on detecting miRNAs for clinical diagnosis or prognosis in cancers.

Keywords: Amperometry, Amplification, Biomarkers, Biosensors, Cancer, Detection, Diagnosis, EIS, Electrochemistry, Fluorescence, Hybridization, miRNAs, Nanodevices, Optical, Prognosis, Potentiometry, Raman, SPR, SERS, UV-visible.

INTRODUCTION

According to the International Agency for Research on Cancer (IARC) data,

[*] **Corresponding author Noureddine Raouafi:** Sensors and Biosensors Group, Analytical Chemistry & Electrochemistry Lab. (LR99ES15), Faculty of Science, University of Tunis El Manar, Tunis El Manar, Tunisia; E-mail: noureddine.raouafi@fst.utm.tn

Farhadul Islam (Ed.)

cancer is the second leading cause of death. In 2018, approximately 18.08 million new cases were diagnosed, and 9.55 million died from various forms of cancer.

Lung, colorectum, stomach, liver and breast cancers account for more than 50% of the death toll [1]. Breast cancer is the leading cause among female gender, while for the male population, it is lung cancer. Projections show that in 2040, the cancer incidence will rise by 63.4% (from 18.08 million in 2018 to 29.53 million in 2040), while the death toll will rise by 71.5% (from 9.55 million in 2018 to 16.83 million in 2040) (Chart **1**). This could be explained by the worldwide population growing and aging. Furthermore, seven among ten deaths are recorded in low- and middle-income countries, linking the proliferation and mortality from cancer to development issues. Fig. (**1**) shows the cancer burden and the necessary steps to reduce it.

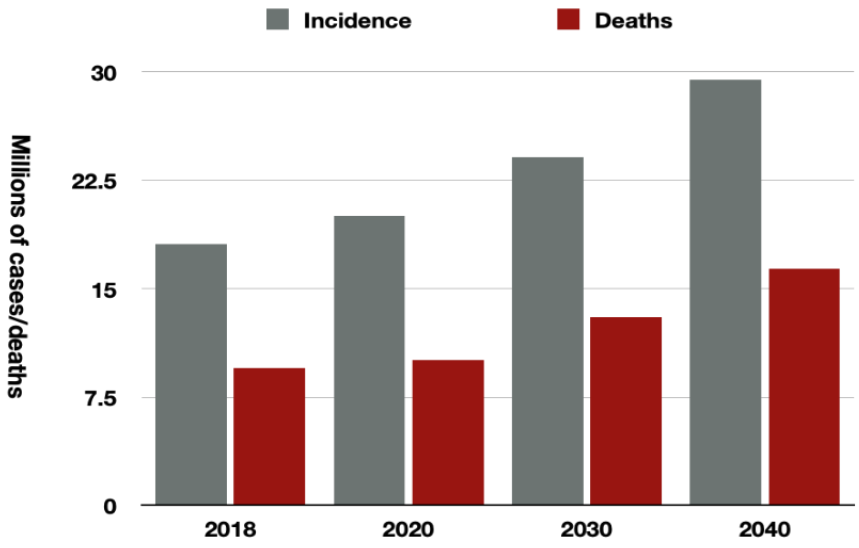


Fig. (1). Estimations of the incidence of cancer and death toll from cancer for the period 2020-2040.

The UICC identified a set of immediate actions for all stakeholders announced in the World Cancer Declaration, aiming to achieve nine concrete targets to reduce premature death from cancer and to improve the quality of life and the survival rates of patients [2]. The sixth key point was the "*Universal access to screening and early detection for cancer*", which can be easily achieved by providing non-invasive and cost-effective methods not requiring sophisticated apparatuses and/or skilled personnel. Furthermore, educating people about the cancer risk factors,

such as tobacco, virus infection by human papilloma and hepatitis B viruses, environmental pollution, carcinogens and radiations, implanting early cancer screening methods in primary healthcare facilities, and the adequate management of cancer patients should also help in reducing substantially the cancer burden in low- and middle-income countries. Early diagnosis can be readily achieved through highly sensitive yet affordable tools, such as biosensors that can be used in low-resource settings and low-income countries [3].

In the last two decades, biosensors have emerged as an appealing alternative to classic analytical methods, such as HPLC, mass spectrometry, ELISA, Western Blot, *etc*. Due to their small size, biosensors can be used for rapid and on-site monitoring to provide real-time information avoiding sampling and analysis in central laboratories. Furthermore, electrochemical biosensors rapidly gained a place of choice since they fit with ASSURED criteria (affordable, sensitive, specific, user-friendly, robust/rapid, equipment-free, deliverable to end-users) for the selection of diagnosis methods [4]. These devices find use in numerous fields, such as healthcare, food safety, environmental monitoring, biosecurity, *etc* [5 - 9].

Since the discovery of CEA in 1965 to be used as the first blood serum biomarker for colon cancer [10], several others such CA 19-9 for pancreatic and colorectal cancer [11], CA 15-3 [12] and CA-125 [13] for breast and ovarian cancers [14] and the PSA as the most reliable biomarker for prostate cancer diagnosis [15]. Nowadays, several other biomarkers are used for screening and diagnostic to predict the recurrence of cancer or the response to therapy, such as HER2 occurring in 20% of breast cancer cases, which tend to be more aggressive than the other forms [16]. Several RNAs are being explored as cancer biomarkers [17], in particular short non-coding RNAs, such as circRNAs [18], miRNAs [19], and piRNAs [20]. So far, miRNAs are the most studied ones [17, 19, 21, 22].

This chapter will present an overview of miRNAs, their biogenesis, biological role and implications in cancer. Later, we will present the electrochemical and optical-based techniques used to detect these biomarkers, and we will conclude and provide some perspectives. The advantages, challenges as well as prospects in that effervescent field of analytical field will be critically discussed.

What are miRNAs?

miRNAs are short (*ca*. 18 to 22 nucleotides), single-stranded, non-coding RNA sequences lacking a polyadenylated tail and a protective group at 3'end [23]. They belong to the small interfering RNAs (RNAi) family, which post-transcriptionally regulates gene expression. They are involved in many biological functions, such as embryonic evolution, cell differentiation and metabolism [24 - 33]. They are also involved in several pathologies. For instance, they act on cell proliferation,

CHAPTER 14

Electrochemical Biosensor for Cancer Biomarkers Detection

Md Arifuzzaman[1], Mostafa Kamal Masud[2,3], Asif Ahmed[4], Md Morsaline Billah[4] and **Md Nazmul Islam[5,6,*]**

[1] *Institute of Tissue Banking and Biomaterial Research, Atomic Energy Research Establishment, Savar, Dhaka-1349 Bangladesh*

[2] *Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Brisbane, QLD, 4072 Australia*

[3] *International Center for Materials Nanoarchitectonics (WPI-MANA), National Institute for Materials Science, Tsukuba, Ibaraki, 305-0044 Japan*

[4] *Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna 9208, Bangladesh*

[5] *School of Health & Life Sciences, Teesside University, Middlesbrough TS1 3BA, United Kingdom*

[6] *National Horizons Centre, Teesside University, Darlington DH1 1HG, United Kingdom*

Abstract: Biosensors are common analytical devices, capable of sensing a myriad of biological analytes, including cancer biomarkers. Although biosensors have different transducer types, electrochemical biosensors provide fast analysis time, high sensitivity, and the ability to perform complex measurements such as multiplexed analysis or screening tests for early diagnosis and prognosis of cancer. This chapter describes the background and theory of electrochemical sensors and introduces the main readout techniques. Innovative electrochemical biosensing strategies for analysis and quantification of important early cancer biomarkers, which include circulating nucleic acids (*e.g.*, circulating tumour DNA, gene mutations, and microRNA) proteins, circulating tumour cells, and extracellular vesicles are discussed with the recent developments to provide an overview of the possible academic and clinical approaches.

Keywords: Biosensor, Early Detection, Electrochemistry, Voltammetry, Impedance, Biomarker, Exosomes, microRNA.

[*] **Corresponding author Md Nazmul Islam:** School of Health & Life Sciences, Teesside University, Middlesbrough TS1 3BA, United Kingdom and National Horizons Centre, Teesside University, Darlington DH1 1HG, United Kingdom; E-mail: mdnazmul.islam@tees.ac.uk

Farhadul Islam (Ed.)

INTRODUCTION

Cancer is one of the leading causes of death and an important barrier to increasing life expectancy across the globe [1]. A failure to improve the survival from cancer is largely a consequence of the failure of the diagnostic system due to late diagnosis. Early detection of cancer holds the greatest promise for reducing the economic and health burden associated with cancer [2]. Despite the recent rapid development of early diagnostic methods and precision medicine, current practices are expensive and laboratory-based methods such as imaging (CT, MRI, X-Ray), and serological tests. However, these diagnostic approaches are considered unreliable due to non-specific and often intermittent symptoms. These also lead to over-investigation and put a significant burden on hospital resources, with the possibility of exposing patients to ionising radiation as well as the risks and discomfort associated with invasive approaches. Therefore, the development of new low-cost diagnostic tools which can facilitate early detection of cancer with high precision, has become a priority [3].

The choice of an accurate biomarker panel is important for early cancer diagnosis and cancer surveillance. An ideal tumour marker should be specific, sensitive, and readily available in biological fluids such as blood and urine. In addition to their correlation with therapeutic monitoring, biomarkers should have a suitable baseline level to lower risk stratification and prognostic evaluation of patients with cancer [3]. Cancer biomarkers are comprised of a variety of molecular entities of cellular, sub-cellular or supramolecular origin, such as nucleic acids (*e.g.*, mutation, altered RNA profiling, epigenetic alteration, *etc*.), proteins and their functional subgroups (*e.g.*, enzymes, glycoproteins, tumour antigens and receptors), cells (*e.g.*, circulating tumour cells (CTCs) and extracellular vesicles (*e.g.*, tumour derived exosomes).

In recent years, circulating biomarkers, such as CTCs, extracellular vesicles, cell-free DNA (cfDNA), circulating tumour DNA (ctDNA) and microRNA have become increasingly popular as suitable candidates for liquid biopsies [4, 5]. The detection approaches for these biomarkers are generally limited to several molecular biology-based methods such as polymerase chain reaction (PCR) and next-generation sequencing (NGS) [5]. Despite their analytical reliability, the scope of these approaches at point of care, as well as resource-limited settings, is much limited.

To address these limitations, there is an increasing interest in the advancement of biosensor-based technologies for the detection of early cancer. Biosensors are analytical instruments capable of calculating the concentration of an analyte of interest in a biological or chemical reaction and can produce signals. Output

single may be quantitative or qualitative depending on functionality and/or biomedical need [6, 7]. Portable electrochemical biosensors represent an inexpensive solution to provide a rapid measurement of biomarkers with clinically relevant sensitivity and specificity [8]. Electrochemical biosensors based on amplification-free direct detection assays are suitable for decentralized laboratory and point-of-care settings. Several electrochemical sensors do not require enzymatic amplification steps, which can provide a faster response and require a simpler workflow [9].

In this chapter, we presented a brief overview of the biosensor with a specific focus on the principle and mechanism of electrochemical biosensors and how they can be utilized for sensing cancer biomarkers.

SOME KEY TERMS IN BIOSENSOR-BASED ASSAYS

Biomarkers and Analytes. Cancer biomarkers could either be a biological molecule or biological processes induced by the tumour itself or by other noncancerous tissues. Tumour biomarkers can be diagnostic (determine the presence and types of cancer), prognostic (give information on the patient's overall cancer outcomes with or without standard treatment), or predictive (to understand which treatment the patient is most likely to respond to or benefit from) [3]. Certain biomarkers can be diagnostic, prognostic, and predictive at the same time. Depending on their localization in the body (*e.g.*, in biological fluids such as blood, urine, or saliva; in tissues; or in cells), the biomarkers can be noninvasive, minimally invasive or invasive. In biosensor-based assays, the target biomarker is known as an 'analyte' [10].

Matrix. Every molecule of a sample except the target analyte is known as a matrix. Detailed knowledge of the matrix is important as the analytical performance of an assay can be significantly influenced by interference of non-specific molecules of the matrix. Matrix interference could arise from the signals caused by non-target molecules or fouling of the sensor due to the adsorption of non-specific molecules on the sensor surface [10].

Transducer. The component of a biosensor that can convert the bio-recognition event into a quantifiable signal is known as a transducer [10]. The extent of target analyte–bioreceptor interactions or biorecognition events is related (usually proportionate) to the signals produced by transducers.

Sensitivity and Specificity. Sensitivity refers to the quantitative ability of a biosensor to detect a true portion of a target analyte within a given sample comprising the total target analyte. In other words, sensitivity reflects a true positive rate of an analyte in a given sample. Specificity can be defined as the

SUBJECT INDEX

A

Acetylglucosamine 197
Acid(s) 181, 187, 197, 284, 285, 292, 323, 325, 350, 353, 356, 362, 363
  nucleic 284, 285, 292, 323, 325, 350, 353, 356, 362, 363
  retinoic 187
  sialic 197
  tricarboxylic 181
Activity 57, 158
  tumorigenic kinase 158
  tyrosine kinase 57
Adenocarcinoma 19, 50, 54, 55, 58, 64, 65, 66, 67, 68, 93, 121, 125, 149, 150, 151, 156, 162, 166, 167, 208, 209, 210, 254, 255, 280, 289
  colon 149, 150, 151, 156, 162
  endocervical 167
  ovarian 50, 68
  pancreatic 19, 150, 210
  pancreatic ductal 121, 125, 280, 289
  renal 50
  tongue 93
Adipogenesis 57
Adjuvant-chemotherapy 207
Albumin 18, 364
  bovine serum 364
Alcohol dehydrogenase 13
Alkaline phosphatases (ALPs) 180, 185
Alveolar rhabdomyosarcoma 55
Alzheimer's disease 196
Amplification 7, 83, 90, 105, 272, 285, 297, 309, 311, 359
  bioassay 285
  nucleic acid 285
  refractory mutation system (ARMS) 105
Anaplastic 87, 157
  lymphoma kinase (ALK) 157
  thyroid cancer (ATC) 87
Androgen deprivation therapy (ADT) 130
Angiogenesis 51, 121, 156, 188, 205, 231, 277
  carcinogenic 156
Angioleiomyoma 91
Anthracyclines 160
Antibodies, monoclonal 156, 166
Antigen 11, 12, 14, 167, 184, 206, 234, 248, 363
  carbohydrate-containing protein 14
  carcinoembryonic 11, 12, 184, 206, 234, 248, 363
  tumour protein 167
Apoptosis 51, 59, 61, 103, 104, 151, 162, 163, 185, 276, 278
Assay 161, 295, 360
  electrochemical methylation 360
  immunohistochemical 161
  sandwich hybridization 295
Assembly 283, 291, 315, 323
  catalytic hairpin 283, 291
  enzyme-mediated mismatched catalyzed hairpin 323
  hairpin DNA 315

B

Basal cell carcinoma 92
Biogenesis 243, 274, 275
Biomarkers 1, 2, 39, 58, 87, 101, 106, 126, 148, 164, 167, 183, 185, 186, 189, 195, 196, 203, 209, 211, 228, 229, 230, 233, 248, 287, 295, 299, 350, 351, 356
  adjunctive detection 186
  cancer-related 295, 299
  genetic 58
  haemoglobin 87
  metastatic 183
  nucleic acid 356
  plasma-based 106
  single-enzyme-based 189
  therapeutic 39
  tumor 287
Biopsies 101, 109
  skin 109

traditional 101
Biosensing 285, 324
method 324
systems 285
Biosensors 274, 286, 287, 288, 294, 299, 300, 301, 303, 308, 309, 313, 315, 316, 320, 322, 328, 349, 351, 352, 353, 354, 367
amperometric 287
amplification-based 299
colorimetric 315
electrochemical microRNA 301, 303
enzyme-free colorimetric 316
fabrication 352, 354, 367
gravimetric 353
miRNA-based 287
sensitive miRNA 313
voltamperometric 300
Blood urea nitrogen (BUN) 18
Bovine serum albumin (BSA) 364
BRAF 88, 151
protein 151
targeted therapies 88
Breast cancer 4, 14, 40, 82, 85, 116, 121, 127, 128, 129, 149, 150, 157, 158, 159, 160, 162, 164, 167, 180, 184, 186, 187, 205, 206, 229, 234, 258, 301
aggressive 162
biosensor 301
metastatic 116, 127, 128, 129, 149, 159, 180, 184
prognosis 167
stem cells 234
Breast carcinomas 183, 189, 206

C

Calcium homeostasis 15, 230
Cancer(s) 15, 57, 62, 85, 93, 123, 124, 125, 149, 150, 152, 153, 163, 182, 183, 186, 187, 188, 197, 202, 230, 234, 243, 246, 256, 258
anaplastic 15
brain 183
esophageal 202, 246
fallopian tube 149, 153
gastrointestinal 150
gynecologic 124
hematologic 125
hypopharyngeal 183
metastasis 93, 182, 188, 197
metastatic 187, 256
neck 123, 150, 163
neuroendocrine 124
pancreatobiliary 186
papilloma 152
stem cell (CSCs) 125, 230, 234, 243, 258
stomach 85
thyroid papilloma 152
uterine 57, 62
Cancer prognosis 5, 122
gastric 122
Cancer syndrome 81, 89, 92
ovarian 89
pancreatic 92
Carbonic anhydrase 180, 187
Carcino-embryonic antigen 201
Carcinogenesis 36, 38, 41, 50, 57, 81, 84, 90, 94, 182, 195, 197
mammary 197
Carcinomas 54, 56, 150, 159, 166, 167, 183, 232, 254
Castrate resistant prostate cancer (CRPC) 25
Cell carcinoma 120, 123, 126, 128, 129, 131, 209, 230, 234, 246, 248
esophageal squamous 120, 123, 126, 128, 129, 131, 248
laryngeal squamous 230, 234
oral squamous 246
Central nervous system (CNS) 150, 167
cancer 150
Chemotherapy 14, 21, 123, 128, 129, 130, 131, 151, 153, 160, 184, 205, 206, 207
platinum-based 184
Cholangiocarcinomas 18, 183, 187, 188, 202
Cholangitis 19
Choriocarcinoma 228, 229, 232
Chronic eosinophilic leukemia (CEL) 149, 158
Chronoamperometry 287, 356
Chymotrypsin 203
Cirrhosis 17
Colon carcinomas 207
Colorectal 92, 160, 207
adenocarcinoma 160
cancer genitourinary 92
carcinomas 207
Colorectal cancer 5, 11, 12, 86, 107, 108, 120, 121, 122, 126, 128, 129, 149, 150, 151, 186, 207, 246
metastatic 5, 107, 108

Complementary DNA probes 326
Computed tomography (CT) 6, 16, 17, 21, 129, 350
Corticosteroids 155
Cowden syndrome 91
Cutaneous tumours syndromes 91
Cyclic enzymatic amplification method (CEAM) 298, 310
Cytokinesis 72
Cytology 4

D

Deregulation 210, 259
of glycoproteins 210
of miRNAs expressions 259
Diabetes mellitus 276
Diagnose hepatitis 126
Diagnosing gonadotroph adenomas 232
Diagnosis 3, 4, 6, 12, 19, 23, 26, 101, 125, 126, 127, 166, 195, 199, 200, 205, 211, 228, 229, 232, 255, 279
biochemical 232
imaging-based tumour 101
Diagnostic 20, 87, 211
algorithm 87
colonoscopy 87
laparoscopy 20
power 211
Disease 1, 2, 4, 6, 12, 14, 24, 40, 92, 93, 106, 120, 122, 152, 164, 188, 195, 196, 199, 201, 203, 204, 205, 210, 245, 276
autoimmune 196
cardiovascular 276
immunodeficiency 195
infectious 204
life-threatening 276
neurodegenerative 164
neurological 276
DNA 33, 34, 35, 36, 37, 43, 57, 58, 92, 102, 103, 152, 284, 292, 293, 298, 302, 304, 312, 313, 320, 359, 360
amplification-free 298
biotinylated detection 302
demethylation processes 34
hairpin 359
hemi-methylated 34
hypomethylation 35, 37
ligase 304
mapping technologies 43
targeted chemotherapy 152
DNA damage 59
detecting 59
proteins 59
DNA methylation 33, 34, 35, 36, 37, 38, 39, 40, 242, 277, 359, 360
mapping technologies 33, 35, 39

E

Electrocatalysis 309
Electrochemical 272, 286, 287, 296, 302, 305, 307, 314, 349, 351, 353, 356, 357, 358, 361, 362, 363, 364, 365, 366
bioassays 314
DNA Sensors 357
impedance spectroscopy (EIS) 272, 287, 305, 307, 356, 363
oligonucleotide-templated reaction (EOTR) 362
RNA sensors 361
sensors 349, 351, 356, 358, 363, 364, 365, 366
signal 296, 302, 357, 358
transducers 286, 353
Electrode, gas-sensing 290
Electron cyclotron resonance (ECR) 360
Electrophoresis 103
Embryogenesis 117, 234
Endometrial 56, 158, 167
carcinoma 158, 167
stromal sarcoma 56
Endometriosis 204
Endoscopic retrograde cholangiopancreatography 20
Enzyme(s) 24, 34, 61, 180, 185, 186, 187, 308
aldehyde dehydrogenase 187
catalyse 180
decorated liposomes 308
glycoprotein serine protease 24
glycosylase 34
linked immunosorbent assay 186
phosphotransferase 185
polymerase 61
Epidermal growth factor (EGF) 14, 90, 151, 156, 157, 160, 162, 201, 205, 206, 209, 363, 366
receptor (EGFR) 90, 151, 156, 157, 160, 162, 201, 205, 206, 209, 363, 366

Epithelial-mesenchymal transition (EMT) 117, 118, 119, 121, 182, 234, 241, 258, 259
Esophageal squamous cell carcinoma (ESCC) 120, 123, 126, 128, 129, 131, 248
Estrogen 231, 234
  hormones 231
  secretion 234

F

Factors 1, 4, 18, 58, 72, 117, 121, 128, 157, 181, 197, 243, 276, 352, 363
  environmental 117, 352
  microenvironmental 117
  transforming growth 121
  vascular endothelial growth 363
Familial adenomatous polyposis (FAP) 86
Fibrofolliculoma 91
Field-effect transistor (FET) 292
Fine-needle aspiration (FNA) 87, 253, 255
Flow cytometry 105
Fluorescence techniques 320

G

Galactose 197, 198
Gall bladder 18, 19, 149, 156
  cancer (GBC) 149, 156
  carcinomas 18, 19
Gardner's syndrome 91, 92
Gastric cancer (GC) 121, 122, 125, 150, 160, 162, 163, 164, 189, 246, 248, 277, 278
Gastrinoma 229, 232
Gastrointestinal 149, 150, 153, 201
  carcinomas 201
  stromal tumour 149, 150, 153
Gene(s) 35, 36, 37, 38, 40, 41, 42, 53, 56, 57, 61, 81, 82, 83, 84, 85, 86, 87, 88, 90, 149, 242, 277
  encoding melanocortin 90
  homologous recombination pathways 85
  housekeeping 35
  miRNA coding 277
  pleomorphic adenoma 56
  protein-encoding 149
  transcription 61, 242
  tumour-suppressor 36, 37, 38
Genetic 86, 87, 196
  mutations 87, 196
  profiling 86
Gestational trophoblastic disease 4
Glutaminolysis 181
Glycogenolysis 233
Glycoproteins 195, 196, 197, 198, 199, 200, 201, 203, 204, 205, 206, 207, 209, 210, 211
  trans-membrane 209
  and cancer biomarkers and sensitivity 211
  in breast cancer 205
  in colon cancer 207
  in liver cancer 199
Growth 12, 165, 208
  factors, hematopoietic 12
  metastatic 165
  promoted lung cancer cells 208
Gynaecological 20, 161
  cancer 20
  malignancy 161

H

Hemithyroidectomy 15, 16
Hepatitis 17, 126, 199, 274
  autoimmune 17
Hepatoblastoma 56, 65
Hepatocarcinoma 288
Hepatocellular 17, 23, 122, 129, 131, 150, 161, 162, 167, 196, 199, 248
  cancer 150
  carcinoma 17, 23, 122, 129, 131, 150, 161, 162, 167, 196, 199, 248
Hodgkin's lymphoma 154
Homeostasis 276, 277
  immune 277
Hormones 229, 231
  adrenocorticotropic 229
  glucocorticoids 231
Hormone therapy (HT) 129
Human 4, 21, 200, 204, 259
  epididymis protein 21, 200, 204
  oral squamous cell carcinoma 259
  papilloma virus (HPV) 4
Hybridization chain reaction (HCR) 285, 286, 317, 319, 322, 325
Hyperparathyroidism 88
Hyperthyroidism 22
Hypoglycemia 232, 233
Hypogonadism 22

Hypomethylation 33, 36, 37

I

Imaging 6, 16
magnetic resonance 16
methods 6
Inflammation 233
Inflammatory bowel disease 13, 86, 87
Inflammatory diseases 40, 195, 196
pelvic 247
Integration of electrochemical biosensors 367

K

Kidney disease 13

L

Lactate dehydrogenase (LDH) 20, 22, 23, 180, 184, 229, 248
Laryngeal squamous cell carcinoma (LSCC) 230, 234
Leukemias 149, 158, 248
chronic eosinophilic 149, 158
chronic lymphocytic 248
Liver 13, 18, 19, 126, 199, 204
cancer 199
cirrhosis 19, 126, 199
diseases 204
metastasis 13
transaminases 18
Lunch syndrome 86
Lung 41, 84, 89, 90, 121, 125, 126, 127, 149, 150, 151, 156, 157, 162, 163, 165, 185, 187, 208, 209, 210, 255
adenocarcinoma 84, 149, 150, 151, 156, 157, 162, 163, 165
cancers 41, 89, 90, 121, 125, 126, 127, 149, 150, 185, 187, 208, 209, 210, 255
Luteinizing hormone (LH) 229
Lymphoblastic lymphoma 149, 159

M

Magnetic-activated cell sorting (MACS) 118
Magnetic resonance 16, 19, 20, 350
cholangiopancreatography (MRCP) 19
imaging (MRI) 16, 19, 20, 350
Malignancies 13, 14, 21, 22, 87, 88, 116, 126, 132, 186, 187, 230, 231, 232
haematological 116
placental trophoblastic 231
Malignant 54, 64, 66, 163, 183, 185, 254
melanoma 54, 64, 66, 163
phaeochromocytomas 183, 185
pleural mesothelioma (MPM) 254
MAPK signalling pathway 88
Masses, suspicious pelvic 21
Medicated mitogen-activated protein kinase 151
Medullary thyroid carcinoma (MTC) 16, 17, 26, 87, 88, 229
Medulloblastoma 92, 150, 163
Meningioma tumorigenesis 68
Mesenchymal chondrosarcoma 159
Metastasis 13, 14, 17, 116, 117, 120, 122, 131, 161, 162, 163, 165, 166, 167, 197, 256, 280
aggressive 256
inhibiting ovarian 280
Metastatic 13, 14, 15, 16, 17, 24, 25, 121, 129, 131, 159, 208
brain tumour 159
castration-resistant prostate cancer 129
disease 13, 14, 15, 16, 17, 24, 25, 121, 208
relapse 131
Methods 51, 354
cytogenetic 51
electrochemical sensing 354
Micrometastasis 102
Mitogen-activated protein kinase (MAPK) 151
Multiple myelomas 125, 187

N

Neoplasia 195
Neoplastic transformations 196, 197
Neuroblastoma 39, 63, 183, 184
Neurodegenerative 40
Neuroendocrine 11, 23, 24, 27, 149, 150, 155, 166, 183, 184, 232
tumours 11, 23, 24, 27, 149, 155, 166, 183, 184, 232
Non-small cell lung cancer (NSCLC) 41, 127, 129, 130, 131, 150, 208, 209, 234, 246, 254, 255, 256
Nucleosomes 103

O

Oral tongue squamous cell carcinoma (OTSCC) 149, 151
Osteomas 91, 92
Osteosarcoma 63, 279
Ovarian 20, 21, 38, 42, 85, 86, 124, 149, 150, 151, 152, 153, 158, 161, 180, 183, 185, 188, 203, 204, 248, 282
  cancer (OC) 20, 21, 38, 42, 85, 86, 124, 149, 150, 151, 152, 153, 158, 161, 183, 188, 203, 204, 248, 282
  carcinoma 20
  neoplasms 20
  serous carcinomas 180, 183, 185

P

Pancreatic 19, 20, 89, 92, 105, 107, 122, 149, 150, 162, 165, 166, 197, 210, 211, 289
  cancer 19, 20, 89, 92, 105, 107, 122, 149, 150, 162, 165, 166, 197, 210, 211
  carcinoma 19, 289
Pancreaticoduodenectomy 20
Pancreatitis 13, 19, 26, 40, 89, 155
  chronic 19, 26, 40
Papillary thyroid cancer (PTC) 87, 88
Paragangliomas 166
Pathogenesis of colorectal cancer 86
Peptide nucleic acid (PNA) 293, 357, 362
Peroxisome proliferator-activated receptor gamma (PPARG) 57
Persistent disease 12
Pheochromocytoma 88, 150, 166
Phosphatase, myosin 70
Prognosis 6, 7, 12, 13, 14, 26, 27, 100, 101, 115, 122, 125, 127, 128, 129, 152, 167, 183, 209, 228, 280
  biomarkers 209
Progression 33, 35, 43, 60, 61, 106, 148, 149, 180, 183, 195, 196, 197, 199, 204, 205, 206, 210, 277
  neoplastic 277
  tumor 106, 197, 210
Promyelocytic leukemia 150
Prostate 24, 25
  biopsies 25
  stem cell antigen (PSCA) 24
Prostatectomy 25
Protein(s) 7, 57, 59, 61, 106, 148, 149, 150, 152, 156, 157, 159, 161, 165, 166, 167, 195, 197, 200, 201, 206, 210, 228, 232, 363
  biomarkers 106, 148, 149, 150, 156, 167, 363
  epsilon 57
  glycosylated 195, 210
  growth-promoting 206
  hormone 228
  metabolism 232
Psychiatric disorders 40
Pulmonary 230, 255
  disease 230
  hemorrhage 255

Q

Quantitative methylation-specific PCR (QMSP) 40, 42

R

Radiation therapy (RT) 25, 130, 131
Radioactive 16
  iodine therapy 16
  iodine treatment 16
Reaction, electrocatalytic 362
Reactive oxygen species (ROS) 181, 182
Receptor-interacting protein kinase (RIPK) 180, 182
Redox reaction 354, 356
Relapse 22, 100, 115, 119, 125, 131, 165, 206
  biochemical 22
  hematologic 165
Renal cell carcinoma (RCC) 38, 68, 161, 166, 167, 180, 183, 185, 256, 257, 278
Replication 58, 59, 61
  protein 61
  stress 58, 59
Repression 243
  post-transcriptional 243
Reverse transcription polymerase chain reaction 281
Rhabdomyosarcoma 163
Rheumatoid arthritis 195, 196
Rituximab therapy 154
RNA polymerases 284

RNAs 2, 7, 84, 92, 241, 242, 274, 284, 285, 310, 320
heterohybrid 310

S

Salivary gland tumour 92
Screen-printed carbon electrodes (SPCEs) 359
Single nucleotide polymorphisms (SNPs) 276, 315, 358, 359
Synaptophysin 166
Syndrome 37, 92
basal cell carcinoma 92
Synovial sarcoma 55, 56, 57, 58, 66
translocation 56
Synthesis 52, 61, 158, 233
oestrogen 158
translesional 61

T

Targeted 87, 88, 89, 90, 91, 100, 102, 160, 196, 255, 256
immunotherapy 87
therapies 88, 89, 90, 91, 100, 102, 160, 196, 255, 256
Technique 105, 106, 281, 283, 284, 285, 287, 291, 294, 305, 320, 356, 365
amperometric 356
biopsy-based 106
electrochemical 287
enzyme-free isothermal amplification 283
Therapy 131, 167
monoclonal antibody 167
neoadjuvant chemo-radiation 131
Thymidine kinase (TK) 180, 185
Thymine DNA glycosylase (TDG) 34
Thyroid 15, 16, 57, 87, 166, 228, 229, 230, 233
adenocarcinoma 57
adenoma 57
carcinomas 166, 228
gland 15, 16, 228, 229, 230, 233
malignancies 87, 230
stimulating hormone (TSH) 228, 229, 233
Thyroid cancers 11, 15, 87, 88, 128, 129, 150, 152, 228, 229, 230, 233, 234
anaplastic 87
follicular 87
Thyroidectomy 15
Transforming growth factor (TGF) 121
Tuberous sclerosis 92
Tumorigenesis 52, 53, 61, 72, 159, 366
Tumors, testicular 228, 229, 232
Tumour apoptosis 101
Tumourogenesis 156
Turcot syndrome 62
Turner's syndrome 68
Tyrosine kinase inhibitor (TKIs) 85, 90, 153, 156, 165, 209, 210

U

Ultrasonography 204
Urokinase 14, 24, 180, 186
plasminogen activator 14, 24
type plasminogen activator 180, 186

V

Vascular endothelial growth factor (VEGF) 363
Voltage-dependent anion channel (VDAC) 188

Farhadul Islam

Dr. Md. Farhadul Islam is currently working as an Associate Professor in the Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh. He has obtained PhD in 2018, in Cancer Molecular Pathology from Griffith University, Australia with Academic Excellence Award. He has been awarded the Chancellor's medal 2019 from Griffith University. He was also awarded Dean's Award, 2019 from University of Rajshahi, Bangladesh and Early Career Researcher award in 2019 from the Institute for Glycomics, Griffith University, Australia. He is working on cancer biology, and cancer marker development for various cancers. His research focus is on cancer genetics, epigenetics, and cancer signalling. In addition, he is working on cancer drug development from natural sources, such as plants and marine products, and peptide based drug development using cell penetrating and cell disrupting peptides. He has published more than 100 peer-reviewed research articles and wrote & published many book chapters on cancer pathogenesis and protocols.