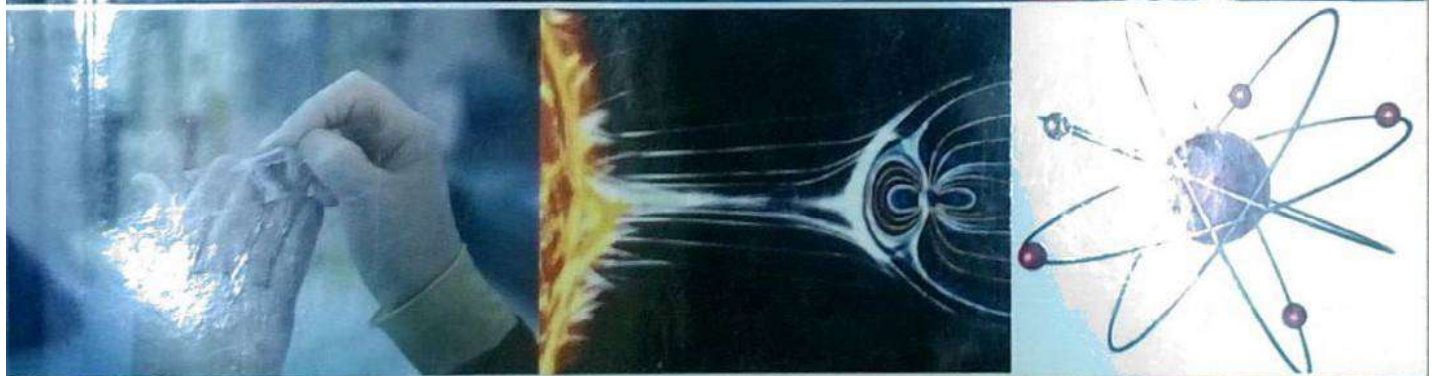




*Trends in*  
**EXPERIMENTAL  
BIOLOGY**



*Editor*  
**Dr. L. Kma**

*Trends in*  
**EXPERIMENTAL  
BIOLOGY**

*Editor*  
**Dr. L. Kma**

  
**INDIA PUBLISHERS**  
EXCEL INDIA PUBLISHERS  
NEW DELHI

## **List of Contributors with Affiliations**

### **Jeny Laskar**

M.Sc., Department of Biotechnology,  
Assam University, Silchar, India-788011

### **Sayantana Nath**

M.Sc., Department of Biotechnology,  
Assam University, Silchar, India-788011

### **Sankar Kr. Ghosh**

Ph.D., Department of Biotechnology,  
Assam University, Silchar, India-788011

### **Mahuya Sengupta**

Ph.D., Department of Biotechnology,  
Assam University, Silchar, India-788011

### **Yashmin Choudhury**

Ph.D., Department of Biotechnology,  
Assam University, Silchar, India-788011

### **Dr. Surya Bhan**

Ph.D., Department of Biochemistry,  
North-Eastern Hill University, Shillong, India-793022

### **Dr. Lakhana Kma**

Ph.D., Department of Biochemistry,  
North-Eastern Hill University, Shillong, India-793022

### **Dr. Neha Chaurasia**

Ph.D., Department of Biotechnology and Bioinformatics,  
North-Eastern Hill University, Shillong, India-793022

### **Dr. Sreedhar Bodiga**

Ph.D., Department of Biochemistry, Kakatiya University,  
Warangal, Telangana, India-506009

### **Dr. Vijaya Lakshmi Bodiga**

Ph.D., Institute of Genetics & Hospital for Genetic Diseases,  
Osmania University, Begumpet, Telangana, India-500016

### **Dr. Madhukar Rao Kudle**

Ph.D., Department of Biochemistry, Kakatiya University,  
Warangal, Telangana, India-506009

# Contents

## CHAPTER 1

Targeting Metabolic Pathways for Disease  
Therapy

*J. Laskar, S. Nath, S.K. Ghosh,  
M. Sengupta and Y. Choudhury*..... 1

## CHAPTER 2

Significance of Apoptosis in Diseases

*S. Bhan*..... 38

## CHAPTER 3

MicroRNA as Molecular Target in Cancer  
Detection and Therapy

*L. Kma*..... 62

## CHAPTER 4

Cyanobacterial Genes as a Potential Targets for  
Removing Heavy Metal Contamination from  
Agroecosystems

*N. Chaurasia*..... 142

## CHAPTER 5

Effect of Vitamin Supplementation on  
Cisplatin-induced Intestinal Epithelial Cell  
Apoptosis in Chronic Vitamin-restricted Rats

*V.L. Bodiga, M.R. Kudle and S. Bodiga*..... 160

*AUTHOR INDEX*..... 180

**INTRODUCTION**

The gradual sequence of organized biochemical reactions catalyzed enzymes that convert preliminary substrate molecule or molecules to a final product or products through a series of metabolic intermediates is referred to as a metabolic pathway. Metabolism is the sum total of biochemical processes in living organisms that either produce or consume energy. These essential metabolic pathways are divided into three classes:

- Anabolic pathways involved in the synthesis and polymerization of simple molecules into complex macromolecules,
- Catabolic pathways involved in degradation of molecules to release energy, and
- Waste disposal pathways which govern elimination of toxic waste.

Core metabolism includes pathways for the synthesis and breakdown of carbohydrates, fatty acids, and amino acids, which are the most vital processes for energy homeostasis and macromolecular synthesis in humans.

Illustrating these pathways and understanding their physiological roles have been among the most fruitful pursuits in biological research. The "Golden Age of Biochemistry" between the 1920s and 1960s defined almost all the metabolic processes responsible for nutrient consumption and energy production in humans as well as in other organisms. These included glycolysis, respiration, the tricarboxylic acid (TCA), urea cycle, glycogen catabolism, oxidative phosphorylation, and the supremacy of ATP in energy transfer reactions and many more. Biochemistry and the analysis of metabolic pathways dominated basic and medically oriented research during these decades, with some 15 Nobel Prizes in either Physiology/Medicine or Chemistry awarded for work related to energy balance or basic metabolic pathways. The driving force behind metabolic research was the realization that metabolic perturbations-often genetically programmed-accompany several

# Computational Epigenetics and Diseases

Translational Epigenetics Series

Volume 9

Edited by  
Loo Keat Wei



Copyrighted material



Translational Epigenetics

Volume 9

# Computational Epigenetics and Diseases

**Edited by**

**Loo Keat Wei**

Universiti Tunku Abdul Rahman, Kampar, Malaysia



**ACADEMIC PRESS**

An imprint of Elsevier

Academic Press is an imprint of Elsevier  
125 London Wall, London EC2Y 5AS, United Kingdom  
525 B Street, Suite 1650, San Diego, CA 92101, United States  
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States  
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Copyright © 2019 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: [www.elsevier.com/permissions](http://www.elsevier.com/permissions).

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

#### **Notices**

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

#### **Library of Congress Cataloging-in-Publication Data**

A catalog record for this book is available from the Library of Congress

#### **British Library Cataloguing-in-Publication Data**

A catalogue record for this book is available from the British Library

ISBN: 978-0-12-814513-5

For information on all Academic Press publications visit our website at  
<https://www.elsevier.com/books-and-journals>



*Publisher:* Andre Wolff

*Acquisition Editor:* Rafael Teixeira

*Editorial Project Manager:* Megan Ashdown

*Production Project Manager:* Punithavathy Govindaradjane

*Cover Designer:* Greg Harris

Typeset by TNQ Technologies



# Contents

Contributors .....	xvii
<b>CHAPTER 1 Computational Epigenetics and Disease.....</b>	<b>1</b>
<i>Loo Keat Wei</i>	
Introduction.....	1
Computational Approaches in DNA Methylation .....	1
Computational Approaches in Histone Modifications.....	3
Computational Approaches in miRNAs.....	4
Computational Epigenetics in Metabolic and Cardiac Disorders .....	4
Computational Epigenetics in Neurological Disorders .....	5
Computational Epigenetics and Cancer .....	6
Conclusions.....	7
Acknowledgment .....	7
References.....	7
<b>CHAPTER 2 Computational Methods for Epigenomic Analysis.....</b>	<b>11</b>
<i>Ho-Ryun Chung</i>	
Introduction.....	11
Unbiased Detection of ChIP-Enrichment.....	12
Segmentation of the Epigenome Into Chromatin States.....	16
The Differential Epigenome.....	19
References.....	21
<b>CHAPTER 3 Statistical Approaches for Epigenetic Data Analysis.....</b>	<b>23</b>
<i>Thorsten Dickhaus</i>	
Introduction.....	23
Statistical Modeling.....	24
Statistical Methodology.....	25
Formulation of Multiple Test Problems .....	25
Test Statistics and Their Limiting Null Distributions.....	26
Multiple Test Procedures: Closure Principle .....	27
Finite Sample Modification: Studentized Permutation Approach.....	28
Real Data Analysis .....	29
Discussion .....	30
Acknowledgments.....	31
References.....	31

<b>CHAPTER 4</b>	<b>Bioinformatics Methodology Development for the Whole Genome Bisulfite Sequencing</b> .....	<b>33</b>
	<i>Deqiang Sun</i>	
	Introduction .....	33
	Results .....	35
	Beta-Binomial Hierarchical Model for Both Sampling and Biological Variations .....	35
	Credible Methylation Difference (CDIF) Is a Single Metric for Both Statistical and Biological Significance of Differential Methylation .....	35
	Functions and Performance of the MOABS Pipeline .....	37
	Simulated BS-seq Data Reveal the Superior Performance of MOABS .....	39
	MOABS Improves the Detection of Allele-Specific DNA Methylation .....	44
	MOABS Reliably Reveals Differential Methylation Underlying TFBSs .....	47
	MOABS Detects Differential 5hmC in ES Cells Using RRBS and oxBS-seq .....	52
	Discussion .....	57
	Methods .....	57
	Distribution for Difference of Two Binomial Proportions .....	57
	Distribution for Difference of Difference .....	58
	Distribution for Measurements With Replicates .....	58
	Acknowledgments .....	59
	References .....	59
	Supplementary Methods .....	61
	Methylation Ratio of One Locus Follows a Beta Distribution .....	61
	CI for Single Binomial Proportion .....	62
	CI for Difference of Two Binomial Proportions in Detail .....	63
	Identification of DMCs for Two or More Samples .....	64
	Identification of DMRs for Two Samples by Simply Grouping DMCs .....	65
	Identification of DMRs for Two Samples by Hidden Markov Model .....	65
	Identification Hypomethylated Regions from One Sample .....	66
	Supplementary References .....	66
<b>CHAPTER 5</b>	<b>Data Analysis of ChIP-Seq Experiments: Common Practice and Recent Developments</b> .....	<b>67</b>
	<i>Qi Zhang</i>	
	The Design of ChIP-Seq .....	68
	The Quality of ChIP-Seq Data .....	69
	Mapping ChIP-Seq Reads .....	69
	Peak Calling .....	70
	Differential Enrichment Detection .....	71

	All-in-One Data Analysis Pipelines for ChIP-Seq .....	72
	Beyond the Standard Pipeline: Allelic-Imbalance Detection From ChIP-Seq .....	73
	Summary .....	75
	References.....	75
<b>CHAPTER 6</b>	<b>Computational Tools for microRNA Target Prediction .....</b>	<b>79</b>
	<i>Nurul-Syakima Ab Mutalib, Siti Aishah Sulaiman and Rahman Jamal</i>	
	Introduction.....	79
	Principles of microRNA Target Prediction .....	80
	Seed Sequence Complementarity.....	81
	Free Energy.....	82
	G–U Wobble .....	82
	Evolutionary Conservation Status .....	82
	3' UTR Compensatory Binding.....	82
	Target-Site Accessibility.....	83
	Target-Site Abundance .....	83
	Local AU Flanking Content .....	83
	Machine Learning.....	84
	Pattern-Based Approach.....	84
	microRNA Target Prediction Tools .....	84
	Conclusion and Future Direction .....	86
	References.....	98
	Further Reading .....	105
<b>CHAPTER 7</b>	<b>Integrative Analysis of Epigenomics Data .....</b>	<b>107</b>
	<i>Cenny Taslim, Stephen L. Lessnick and Simon Lin</i>	
	Introduction.....	107
	Quality Control and Data Preprocessing.....	109
	Relationship Between Histone Modification Pattern, Transcription Factor Binding, and mRNA Expression Level.....	110
	Regression Analysis.....	111
	Mixture Model.....	112
	Identification of Functional Regulatory Regions.....	114
	Association Between Multiple Transcription Factors Using Self-Organizing Map (SOM).....	115
	Prediction of Chromatin and Transcription Binding Sites Directly From DNA Sequences Using Deep Learning .....	116
	Discussion.....	117
	Acknowledgments.....	118
	References.....	118

<b>CHAPTER 8</b>	<b>Differential DNA Methylation and Network Analysis in Schizophrenia</b> .....	<b>121</b>
	<i>Huang Kuo Chuan</i>	
	Introduction.....	121
	Methodology for DNA Methylation.....	121
	Methylation Schizophrenia Network.....	123
	Novel Prediction Applications .....	123
	Candidate Genes in Schizophrenia.....	123
	SDMGs and Disease Mechanism of Schizophrenia .....	123
	Corresponding Pathways and Schizophrenia .....	125
	Schizophrenia and Epigenetic Review.....	126
	Findings Highlight the Significance of Antipsychotic Drugs on DNA Methylation in Schizophrenia Patients.....	127
	References.....	128
<b>CHAPTER 9</b>	<b>Epigenome-Wide DNA Methylation and Histone Modification of Alzheimer's Disease</b> .....	<b>131</b>
	<i>Ankush Bansal and Tiratha Raj Singh</i>	
	Background.....	131
	Epigenetics Association With the Nervous System.....	131
	Epigenetic Mechanisms in AD.....	132
	Epigenetic Changes in AD.....	132
	Epigenetic Modifications.....	133
	DNA Methylation .....	133
	Hypomethylation in AD .....	134
	Hydroxymethylation in AD.....	134
	Gene-Wise DNA Methylation Changes in AD.....	134
	Genome-Wide DNA Methylation Alternations in AD .....	135
	DNA Repair and Methylation in AD .....	135
	Histone Modifications.....	136
	Histone Acetylation Changes in AD.....	136
	Gene-Wise Histone Alterations in AD.....	136
	Epigenomics.....	137
	Molecular Mechanisms Linking Genomic Risk Factors to AD.....	137
	Polymorphisms and AD .....	137
	Systems Level Modules for AD.....	138
	Future Directions .....	141
	References.....	142
	Further Reading .....	148

<b>CHAPTER 10</b>	<b>Epigenomic Reprogramming in Cardiovascular Disease</b> .....	<b>149</b>
	<i>Yang Zhou, Jiandong Liu and Li Qian</i>	
	Introduction.....	149
	Decipher Histone Codes of CM Transcription .....	150
	Identify Chromatin Modification Landscapes and Dynamics During Heart Development .....	151
	Dynamics of Regulatory cis-Elements in Heart Disease.....	152
	DNA Methylation During Heart Development and in Disease .....	153
	DNA Methylation Is Orchestrated in Normal Heart.....	153
	DNA Methylation Is Potential Therapeutic Target in Heart Disease.....	154
	DNA Hydroxymethylation Regulates Gene Expression in Cardiac Development and Hypertrophy.....	155
	Chromatin Conformation in Cardiomyocytes.....	155
	Rapid Chromatin Switch During Somatic Reprogramming.....	156
	Conclusion .....	157
	References.....	157
<b>CHAPTER 11</b>	<b>Bioinformatic and Biostatistic Methods for DNA Methylome Analysis of Obesity</b> .....	<b>165</b>
	<i>Sarah Amandine Caroline Voisin</i>	
	Which DNA Methylation Assessment Technique Should I Use?.....	165
	Which Software and Data Sets Should I Use to Analyze DNA Methylation Data in the Context of Obesity? .....	167
	How Do I Annotate My DMRs to Specific Genes? .....	169
	What Does a Difference of 5% in Methylation Mean?.....	170
	How Do I Know Whether My DMRs Are a Cause or a Consequence of Obesity? .....	171
	How Can I Be Sure That My DMRs Are Not Due to Differences in Cell Type Proportions? .....	172
	References.....	173
<b>CHAPTER 12</b>	<b>Epigenomics of Diabetes Mellitus</b> .....	<b>181</b>
	<i>Ivanka Dimova</i>	
	Basics of Epigenetics.....	182
	Epigenetic Regulation in Type 2 Diabetes Mellitus .....	185
	Epigenetics in Vascular Complications of Type 2 Diabetes Mellitus .....	187
	Epigenetics and Cancer Development in Type 2 Diabetes Mellitus .....	188
	Role of microRNAs (miRNAs) in Type 2 Diabetes Mellitus.....	191
	Future Perspectives and Epigenetic Drugs.....	192
	Conclusion .....	193
	References.....	194

<b>CHAPTER 13</b>	<b>Epigenetic Profiling in Head and Neck Cancer .....</b>	<b>201</b>
	<i>Javed Hussain Choudhury, Sharbadeb Kundu, Fazlur Rahaman Talukdar,</i>	
	<i>Ruhina S. Laskar, Raima Das, Shaheen Laskar, Bishal Dhar, Manish Kumar,</i>	
	<i>Sharad Ghosh, Rosy Mondal, Yashmin Choudhury and Sankar Kumar Ghosh</i>	
	Introduction.....	201
	Epigenetic Alterations in Cancer .....	202
	DNA Methylation Profiling in Head and Neck Cancer.....	204
	Techniques Available for Epigenetic Profiling of HNC .....	206
	Methylation Specific PCR .....	206
	Combined Bisulfite Restriction Analysis Assay .....	206
	Bisulfite Sequencing .....	206
	Pyrosequencing .....	208
	Whole Genome Bisulfite Sequencing .....	208
	Array or Bead Hybridization Techniques for Epigenetic Profiling.....	208
	Enrichment-Based Methods .....	209
	Methylated DNA Immunoprecipitation .....	209
	Computational Epigenetics Analysis .....	209
	Bioinformatics Tools for Computational Epigenomics .....	210
	Methods for Analyzing and Interpreting the DNA Methylation Data .....	210
	Conclusion and Future Perspectives .....	214
	References.....	215
<b>CHAPTER 14</b>	<b>Epigenome-Wide DNA Methylation Profiles in Oral Cancer .....</b>	<b>219</b>
	<i>Raghunath Chatterjee, Shantanab Das, Aditi Chandra and Baidehi Basu</i>	
	Introduction.....	219
	Epigenetic Regulation in Oral Cancer .....	220
	Need for Computational Tools in Epigenetics Study .....	221
	Available Methods and Computational Tools for Oral Cancer Methylomics.....	221
	Tools for Methylomics by Bisulfite-Sequencing Method.....	221
	Tools for Methylomics by Bisulfite-Microarray Method .....	223
	Tools for Methylomics by Enrichment-Based Method.....	223
	DNA Methylation Data Visualization .....	224
	DNA Methylomics in Oral Cancer .....	224
	DNA Methylation Biomarker for OSCC .....	224
	Advancement in DNA Methylation Study in OSCC.....	227
	Conclusion .....	228
	References.....	228

<b>CHAPTER 15</b>	<b>Computational Epigenetics for Breast Cancer .....</b>	<b>233</b>
	<i>Juan Xu, Yongsheng Li, Tingting Shao and Xia Li</i>	
	Introduction.....	233
	DNA Methylation in Breast Cancer.....	233
	Histone Modification in Breast Cancer.....	235
	Noncoding RNA Regulation in Breast Cancer.....	238
	Epigenetic Databases.....	240
	Epigenetic Tools in Cancer.....	240
	Future Directions.....	243
	References.....	244
<b>CHAPTER 16</b>	<b>Integrative Epigenomics of Prostate Cancer.....</b>	<b>247</b>
	<i>Madonna Peter, Shivani Kamdar and Bharati Bapat</i>	
	Prostate Cancer: An Overview.....	247
	Genomic Alterations in PCa.....	247
	Epigenomic Alterations in PCa.....	248
	DNA Methylation.....	249
	DNA Hydroxymethylation.....	249
	Histone Modifications.....	250
	microRNA and Long Noncoding RNA.....	250
	Rationale for Integrative Analysis.....	251
	Emerging Integrative Analysis Tools Utilized in PCa.....	252
	Future Directions and Potential Applications for PCa.....	255
	Concluding Remarks.....	256
	Acknowledgments.....	256
	References.....	257
<b>CHAPTER 17</b>	<b>Network Analysis of Epigenetic Data for Bladder Cancer .....</b>	<b>265</b>
	<i>Bor-Sen Chen</i>	
	Introduction.....	265
	Materials and Methods.....	269
	Data Preprocessing of Omics Data.....	269
	Construction of the Stochastic Regression Models for the IGEN System.....	270
	Identification of the TF Regulatory Ability $a_{ij}$ , the miRNA Repression Ability $c_{ij}$ , and the Protein Interaction Ability $d_{jk}$ and Their Statistical Significance Testing.....	271
	Principal Genome-Wide Network Projection.....	273
	Design of a Multiple Drug Combination With Minimal Side Effects for the Treatment of Bladder Cancer.....	276

Results and Discussion.....276

    Construction of IGEN.....276

    Projection of the Core Network Biomarkers into Biological Processes  
    and Signaling Pathways to Investigate Carcinogenic Mechanisms  
    of Bladder Cancer .....278

    The Impact of Aging, Smoking, and miRNA and Epigenetic Regulation  
    on Bladder Carcinogenesis Through the Core Network Biomarkers .....279

    miR1-2 and miR200b Mediate the Reduction of Cell Proliferation and  
    Metastasis Through KPNA2 and ECT2, Respectively.....280

    The Smoking-Related Protein HSP90AA1 and DNA Methylation  
    of ECT2 Mediate the Metastasis of Bladder Cancer .....280

    Functional Module Network Analysis in Bladder Carcinogenesis .....281

    Two Separate Drug Combinations for Treating Stage 1 and Stage 4  
    Bladder Cancer Cells With Minimal Side Effects .....283

Conclusion .....285

References.....286

Further Reading .....288

**CHAPTER 18 Epigenome-Wide Analysis of DNA Methylation in Colorectal Cancer..... 289**

*Nurul-Syakima Ab Mutalib, Rashidah Baharuddin and Rahman Jamal*

Introduction.....289

Approaches to Analyze DNA Methylation in Colorectal Cancer .....291

Epigenome-Wide Analysis of DNA Methylation in Colorectal Cancer .....292

DNA Methylation Biomarkers in Colorectal Cancer .....292

    Blood-Based DNA Methylation Biomarkers .....292

    Stool-Based DNA Methylation Biomarkers .....295

    Prognostic Biomarkers .....296

Computational Tools for DNA Methylation .....296

Workflow for DNA Methylation Analysis in CRC.....299

Conclusion .....303

Acknowledgment .....304

References.....304

Further Reading .....310

**CHAPTER 19 Integrative Omic Analysis of Neuroblastoma ..... 311**

*Kamalakaran Palanichamy*

Introduction.....311

    Neuroblastoma .....311

    Omics: Genomics, Transcriptomics, Proteomics, Epigenomics, and  
    Metabolomics .....312



	Integrative Omics.....	313
	Tools for NGS Data Analysis and Integrative Omics.....	313
	Workflow.....	313
	Neuroblastoma Omics .....	316
	Transcriptome and Epigenome.....	319
	Integrative Omics.....	320
	Network Modeling, Reverse Engineering Modeling, and Dynamic Modeling .....	321
	Machine Learning-Based modeling .....	321
	Summary and Future Directions .....	322
	References.....	322
<b>CHAPTER 20</b>	<b>Computational Analysis of Epigenetic Modifications in Melanoma .....</b>	<b>327</b>
	<i>Ming Tang and Kunal Rai</i>	
	Introduction.....	327
	DNA Modifications.....	328
	Histone Modifications and Chromatin States .....	330
	Higher-Order Chromatin Structure .....	332
	Nucleosome Positioning .....	333
	Future Perspective .....	334
	References.....	334
<b>CHAPTER 21</b>	<b>DNA Methylome of Endometrial Cancer .....</b>	<b>343</b>
	<i>Golnaz Asaadi Tehrani</i>	
	Introduction.....	343
	Molecular Signaling Pathways of Endometrial Carcinoma.....	345
	PI3/AKT/mTOR .....	345
	MAPK/ERK.....	346
	WNT/ $\beta$ -Catenin .....	346
	VEGF/VEGFR.....	346
	HER-2/neu .....	347
	Epigenetic Alternations in Endometrial Carcinoma.....	347
	Enzyme Digestion-Based Methods .....	347
	Affinity Enrichment-Based Methods.....	348
	Bisulfite Conversion-Based Methods .....	348
	DNA Mismatch Repair Genes.....	350
	Steroid Receptor Genes .....	354
	Tumor Suppressor Genes.....	354
	Other Related Genes.....	356

microRNA Aberrant Methylation in Endometrial Carcinoma .....356  
 TS-miRNAs Involved in Endometrial Cancer With Their Function  
 Including miR-129-2, miR-152, miR-124, miR-126, miR-137,  
 and miR-491 .....357  
 DNA Methylation Machinery in Endometrium .....358  
 Application of DNA Hypermethylation for Treatment .....359  
 Future Directs and Conclusion .....360  
 References .....361  
 Further Reading .....364

**CHAPTER 22 Epigenetics and Epigenomics Analysis for Autoimmune Diseases..... 365**

*Bhawna Gupta, Kumar Sagar Jaiswal, Arup Ghosh and Sunil Kumar Raghav*  
 Study Design and Data Acquisition Methods .....367  
 Microarray-Based Detection .....368  
 Next-Generation Sequencing .....370  
 Epigenetic Changes in Autoimmune Diseases .....373  
 Rheumatoid Arthritis .....373  
 Systemic Lupus Erythematosus .....375  
 Multiple Sclerosis .....375  
 Type 1 Diabetes .....376  
 Analyzing Epigenetic Changes in Autoimmune Diseases .....376  
 DNA Methylation .....376  
 Histone Modification Analysis .....380  
 miRNA and Targets Prediction .....382  
 Epigenetic Databases .....384  
 Histome .....385  
 MethylomeDB .....385  
 MethBase .....386  
 miRWalk2.0 .....386  
 Roadmap Epigenomics .....386  
 Conclusion .....386  
 References .....387

**CHAPTER 23 Computational Epigenetics in Lung Cancer ..... 397**

*S. Babichev, V. Lytvynenko, M. Korobchynskiy and I. Sokur*  
 Introduction .....397  
 Conceptual Basis of the Objective Clustering Inductive Technology .....398  
 Affinity Metric and Clustering Quality Criteria to Estimate the Proximity  
 of Gene Expression Profiles .....399

Simulation of the Objective Clustering Process Using Lung Cancer Patients’ Gene Expression Profiles .....	405
Practical Implementation of SOTA and DBSCAN Clustering Algorithms Within the Framework of the Objective Clustering Inductive Technology.....	408
Results of the Simulation and Discussion.....	411
Hybrid Model of Cluster–Bicluster Analysis of Gene Expression Profiles .....	413
Conclusions.....	415
References.....	416
Index .....	419

# EPIGENETIC PROFILING IN HEAD AND NECK CANCER

# 13

Javed Hussain Choudhury<sup>1</sup>, Sharbaddeb Kundu<sup>1</sup>, Fazlur Rahaman Talukdar<sup>2</sup>, Ruhina S. Laskar<sup>2</sup>, Raima Das<sup>1</sup>, Shaheen Laskar<sup>1</sup>, Bishal Dhar<sup>1</sup>, Manish Kumar<sup>1</sup>, Sharad Ghosh<sup>3</sup>, Rosy Mondal<sup>4</sup>, Yashmin Choudhury<sup>1</sup>, Sankar Kumar Ghosh<sup>1,5</sup>

<sup>1</sup>Department of Biotechnology, Assam University, Silchar, India; <sup>2</sup>International Agency for Research on Cancer (IARC), Lyon, France; <sup>3</sup>Kalinga Institute of Industrial Technology (KIIT), Bhubaneswar, India; <sup>4</sup>Institute of Advanced Study in Science and Technology (IASST), Guwahati, India; <sup>5</sup>University of Kalyani, Nadia, India

## INTRODUCTION

One of the fundamental questions regarding the diversity of phenotypes within a population is why monozygotic twins or cloned animals can have different phenotypes and disease susceptibility despite their identical DNA sequences; classic genetics is unable to explain these phenomena. However, the concept of epigenetics offers a partial explanation of these phenomena. In 1939, C. H. Waddington introduced “the causal interactions between genes and their products, which bring the phenotype into being.” Later on, the term *epigenetics* was described as the study of heritable changes in gene expression without any changes in the DNA sequences. Epigenetic gene patterns play a fundamental role in diverse biological development including embryonic changes, X-chromosome inactivation, and genetic imprinting [1,2]. Unlike genetic changes, epigenetic alterations are reversible, and the key processes involved in epigenetic regulation include DNA methylation, chromatin modification (covalent alteration in core histones), nucleosome positioning, and posttranslational gene expression regulation by noncoding RNAs. Epigenetic changes occur more often than genetic mutation and may persevere for the entire cell life and even for multiple generations. Disruptions of these epigenetic processes can cause aberrant gene expression and function, which may lead to initiation, development, and progression of cancer [3].

Head and neck cancer (HNC) is a broad term that refers to a heterogeneous group of malignancies that arise in the oral cavity, larynx, pharynx, nasal cavity, and paranasal sinuses. Globally, HNC is the sixth most frequent malignancy, accounting for more than 650,000 new cases and 350,000 deaths annually [4]. The development of HNC is a multistep process modulated by genetic, epigenetic, and environmental factors. The environmental risk factors such as tobacco smoking and chewing, in addition to HPV infection, may influence a wide range of genetic and epigenetic alterations that promote genomic and epigenetic instability and endorse tumor development. Epigenetics is a bridge between genotype and phenotype, a phenomenon that changes the ultimate outcome of a genetic locus

**INTRODUCTION**

The gradual sequence of organized biochemical reactions catalyzed enzymes that convert preliminary substrate molecule or molecules to a final product or products through a series of metabolic intermediates is referred to as a metabolic pathway. Metabolism is the sum total of biochemical processes in living organisms that either produce or consume energy. These essential metabolic pathways are divided into three classes:

- Anabolic pathways involved in the synthesis and polymerization of simple molecules into complex macromolecules,
- Catabolic pathways involved in degradation of molecules to release energy, and
- Waste disposal pathways which govern elimination of toxic waste.

Core metabolism includes pathways for the synthesis and breakdown of carbohydrates, fatty acids, and amino acids, which are the most vital processes for energy homeostasis and macromolecular synthesis in humans.

Illustrating these pathways and understanding their physiological roles have been among the most fruitful pursuits in biological research. The "Golden Age of Biochemistry" between the 1920s and 1960s defined almost all the metabolic processes responsible for nutrient consumption and energy production in humans as well as in other organisms. These included glycolysis, respiration, the tricarboxylic acid (TCA), urea cycle, glycogen catabolism, oxidative phosphorylation, and the supremacy of ATP in energy transfer reactions and many more. Biochemistry and the analysis of metabolic pathways dominated basic and medically oriented research during these decades, with some 15 Nobel Prizes in either Physiology/Medicine or Chemistry awarded for work related to energy balance or basic metabolic pathways. The driving force behind metabolic research was the realization that metabolic perturbations-often genetically programmed-accompany several

# Chapter 11

## Detection of p16 Promoter Hypermethylation by Methylation-Specific PCR

Javed Hussain Choudhury, Raima Das, Shaheen Laskar, Sharbadeb Kundu, Manish Kumar, Partha Pratim Das, Yashmin Choudhury, Rosy Mondal, and Sankar Kumar Ghosh

### Abstract

DNA methylation plays a decisive role in the regulation and control of gene expression. DNA methylation is a covalent modification, in which a methyl group is attached to the 5th carbon of the cytosine ring of a CpG dinucleotide that is located upstream from the promoter region of a gene. Promoter hypermethylation (gain of DNA methylation) of the *p16* gene may cause silencing of gene expression and plays an important role in cancer. Therefore, detection of the methylation status of *p16* gene is an important tool in epigenetic studies of various human cancers. The methylation-specific PCR (MSP) is the most commonly used technique for studying DNA methylation. This technique is based on bisulfite modification of DNA, which converts unmethylated cytosine (C) into uracil (U) and leaving methylated cytosine (C<sup>m</sup>) unchanged. Here we describe the bisulfite modification of DNA samples and detection of promoter methylation of *p16* gene from bisulfite-treated DNA using MSP. In MSP, modified DNA samples are subjected to PCR amplification using methylated and unmethylated specific primers for the *p16* gene separately. The PCR amplified products are then analyzed in a 2.5–3% agarose gel containing ethidium bromide. The PCR amplified band generated by specific sets of primers is used to determine the methylation status of the *p16* gene.

**Key words** DNA methylation, *p16* gene hypermethylation, Bisulfite modification, Specific primers, Methylation-specific PCR, Agarose gel electrophoresis

### 1 Introduction

DNA methylation plays a crucial role in the regulation and control of gene expression. DNA methylation is a covalent modification, in which a methyl group is attached to the 5th carbon of the cytosine ring of a CpG dinucleotide (at CpG Islands) by the enzyme DNA methyltransferases (DNMTs). The CpG dinucleotide is located upstream from the promoter region of a gene [1]. Promoter hypermethylation (gain of DNA methylation) can cause silencing of tumour suppressor's pathway genes (such as *p16*, *p53*, *DAPK*, *ECAD*, and *RASSF1A*) in various human cancers. Therefore,