

**MEDICAL
INTELLIGENCE
UNIT**

DNA Methylation and Cancer Therapy

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This book is dedicated to my parents
and Vicky whose support has enabled me to delve
into the secrets of the epigeome.

CONTENTS

Preface	xiii
1. DNA Methylation: Three Decades in Search of Function	1
<i>Aharon Razin</i>	
DNA Methylation in Prokaryotes	1
DNA Methylation in Eukaryotes	2
Methylation and Embryogenesis	2
DNA Methylation and Imprinting	4
DNA Methylation in Gene Silencing	4
DNA Methylation and Disease	5
Reflections	8
2. Epigenetic Mechanisms of Gene Regulation: Relationships between DNA Methylation, Histone Modification, and Chromatin Structure	13
<i>Keith D. Robertson</i>	
The Mammalian DNA Methyltransferases (DNMTs)	14
Interaction between DNMTs and Other Proteins	16
DNA Methylation and DNA Replication	20
Links between DNA Methylation and Histone Modification	20
DNA Methylation and Histone Methylation	21
DNMTs As Transcriptional Corepressors	22
DNA Methylation and Chromatin Remodeling	24
3. DNA Hypo- vs. Hypermethylation in Cancer: Tumor Specificity, Tumor Progression, and Therapeutic Implications	31
<i>Melanie Ehrlich and Guanchao Jiang</i>	
Are There Tumor-Specific DNA Hypomethylation Profiles Like the Tumor-Specific DNA Hypermethylation Profiles?	32
Is There a Relationship between Cancer-Associated DNA, Hypomethylation and DNA Hypermethylation?	34
Is DNA Hypomethylation, Like DNA Hypermethylation, Sometimes Associated with Tumor Progression?	35
Might There Be Deleterious Consequences of Introducing DNA Hypomethylation in the Genome As a Cancer Therapy?	37
4. DNA Methylation in Urological Cancers	42
<i>Wolfgang A. Schulz and Hans-Helge Seifert</i>	
An Overview of Urological Cancers	42
A Description of DNA Methylation Changes in Urological Malignancies	44
A Global View of DNA Methylation Alterations in Urological Cancers	47
Causes of Altered DNA Methylation in Urological Cancers	48
Consequences of Altered DNA Methylation in Urological Cancers	50
DNA Methylation in Diagnosis and Therapy of Urological Cancers	51

5. DNA Methylation in Colorectal Cancer	59
<i>Jeremy R. Jass, Vicki L.J. Whitehall, Joanne Young and Barbara A. Leggett</i>	
Relevance of Familial Models of Colorectal Cancer to Sporadic Neoplasia	60
DNA Microsatellite Instability	60
Mechanisms Underlying Microsatellite Instability in Colorectal Cancer	61
The Methylator Phenotype in Colorectal Neoplasia	62
Are Subjects Genetically Predisposed to Methylation of Colorectal Mucosa?	64
Morphologic Counterparts of the Methylator Phenotype	64
Serrated Pathway of Colorectal Neoplasia	65
Can DNA Methylation Be Reversed Spontaneously?	65
 6. CpG Island Hypermethylation of Tumor Suppressor Genes in Human Cancer: Concepts, Methodologies and Uses	 69
<i>Michel Herranz and Manel Esteller</i>	
Concepts	69
Methodologies	76
Translational Studies of CpG Island Hypermethylation: From the Bench to the Bedside	80
 7. The Loss of Methyl Groups in DNA of Tumor Cells and Tissues: The Immunochemical Approach	 85
<i>Alain Niveleau, Chandrika Piyathilake, Adriana de Capoa, Claudio Grappelli, Jean-Marc Dumollard, Lucien Frappart and Emmanuel Drouet</i>	
Results	86
 8. Identifying Clinicopathological Association of DNA Hypermethylation in Cancers Using CpG Island Microarrays	 107
<i>Susan H. Wei, Timothy T.-C. Yip, Chuan-Mu Chen and Tim H.-M. Huang</i>	
Genomic Targets for DMH and MTA	109
DMH and Its Applications	109
MTA and Its Applications	113
 9. Methylation Analysis in Cancer: (Epi)Genomic Fast Track from Discovery to Clinical Routine	 117
<i>Carolina Haefliger, Sabine Maier and Alexander Olek</i>	
DNA Methylation and Carcinogenesis	117
Methylation Profile in Human Cancer	118
Technological Approaches for DNA Methylation Study: High Throughput Analysis	119
Sensitive Detection	122

10. Regulation of DNA Methyltransferases in Cancer	125
<i>Nancy Detich and Moshe Szyf</i>	
Transcriptional Regulation of DNMTs	126
Regulation by the RAS Signaling Pathway	127
APC-TCF Pathway	128
Feedback Regulation	129
Differential Regulation of the DNMTs during Cell Growth	129
Regulation by Viral Infection	130
Simian Virus 40 (SV40)	131
Epstein-Barr Virus (EBV)	132
Human Immunodeficiency Virus Type 1 (HIV-1)	132
Cell Differentiation and DNMT1	132
Upregulation of DNMT1	132
Downregulation of DNMT1	133
Regulation through Protein Interactions	134
PCNA	134
Rb	135
Interactions between the DNMTs	135
11. Inhibition of Poly(ADP-Ribosyl)ation Allows	
DNA Hypermethylation	142
<i>Anna Reale, Giuseppe Zardo, Maria Malanga, Jordanka Zlatanova and Paola Caiafa</i>	
Poly(ADP-Ribosyl)ation	143
Block of Poly(ADP-Ribosyl)ation Induces in Vivo DNA	
Hypermethylation	146
Atomic Force Microscopy (AFM) Studies of the Effect of DNA	
Methylation on Chromatin Fiber Structure	148
How Is Poly(ADP-Ribosyl)ation Involved in Protecting DNA	
Methylation Pattern	149
12. The Role of Active Demethylation in Cancer and Its	
Therapeutic Potential	156
<i>Moshe Szyf, Paul M. Campbell, Nancy Detich, Jing Ni Ou, Stefan Hamm and Veronica Bovenzi</i>	
Global Hypomethylation in Cancer	157
Global Hypomethylation in Cancer; Single Copy and Multiple Copy	
Sequences Are Hypomethylated in Multiple Tumor Types	157
Mechanisms Responsible for Hypomethylation in Cancer	158
The Possible Role of a Demethylase; DNA Methylation	
Is a Reversible Reaction	158
Resolving the Paradox of the Coexistence of Regional	
Hypermethylation and Global Hypomethylation in Cancer;	
Role of Chromatin Structure	162
Possible Role of Global Hypomethylation in Cancer	165
Mechanisms Whereby Hypomethylation Enhances Tumorigenesis ...	167
The Therapeutic Implications of Global Hypomethylation	169
MBD2/Demethylase As an Anticancer Target	171

13. Purine Analogues and Their Role in Methylation and Cancer Chemotherapy	178
<i>Katherine L. Seley and Sylvester L. Mosley</i>	
DNA Methylation and SAHase Inhibition	178
Nucleoside Inhibitors	180
Carbocyclic Nucleoside	181
Enzyme Inhibition and Cell Differentiation	182
14. DNA Methyltransferase Inhibitors: Paving the Way for Epigenetic Cancer Therapeutics	187
<i>Gregory K. Reid and A. Robert MacLeod</i>	
DNA Methylation: Discovery of the First Epigenetic Modifier	187
DNA Methylation and Cancer: A Correlation	188
The DNA Methyltransferase Family of Enzymes	190
DNA Methylation an Active Player in Oncogenesis: Validation of DNMT1 As a Therapeutic Cancer Target	191
Isotype-Selective Inhibition of DNMTs: Antisense to siRNA	192
Medicinal Chemistry of Oligonucleotides: Towards Antisense Drugs ...	195
DNA Methyltransferase Inhibitors: Cancer Specificity and Potential Therapeutic Window	196
Methylation-Independent Mechanisms of DNMT1 Depletion	196
Implications of Tumor DNA Methylation in Clinical Oncology	197
DNA Methylation: Diagnostics and Rationally Designed Combination Therapy	197
DNMT Inhibitors: The First of Many Epigenetic Therapeutics	198
Clinical Experience with Demethylating Agents for Cancer Therapy ...	198
Antisense Cancer Therapy Targeting DNMT1	198
Clinical Development of MG98	199
15. Preclinical and Clinical Studies on 5-Aza-2'-Deoxycytidine, a Potent Inhibitor of DNA Methylation, in Cancer Therapy	205
<i>Richard L. Momparler</i>	
Historical Perspective	205
Pharmacology of 5-Aza-2'-Deoxycytidine (5-AZA)	206
Evaluation of Antineoplastic Activity in Animal Models	208
Clinical Trials in Leukemia	209
Clinical Trials in Tumors	210
Future Perspectives on 5AZA in Cancer Therapy	211
16. Anticancer Gene Therapy by in Vivo DNA Electrotransfer of MBD2 Antisense	218
<i>Pascal Bigey and Daniel Scherman</i>	
Delivery Principle	219
In Vivo DNA Electrotransfer: Targeted Tissues	220
MBD2 Antisense Electrotransfer	224
Epilogue	230
<i>Moshe Szyf</i>	
Index	235

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PREFACE

DNA methylation has bewildered molecular biologists since Hotchkiss discovered it almost six decades ago (Hotchkiss RDJ. *Biol Cem* 1948; 175:315-332). The fact that the chemical structure of our genome consists of two components that are covalently bound, the genetic information that is replicated by the DNA replication machinery and DNA methylation that is maintained by independent enzymatic machinery, has predictably stimulated the imagination and curiosity of generations of molecular biologists. An obvious question was whether DNA methylation was a bearer of additional information to the genetic information and what was the nature of this information? It was tempting to speculate that DNA methylation applied some form of control over programming of the genome's expression profile. Once techniques to probe the methylation profile of whole genomes as well as specific genes became available, it became clear that DNA methylation patterns are gene and tissue specific and that patterns of gene expression correlate with patterns of methylation. DNA methylation patterns emerged as the only component of the chemical structure of DNA that exhibited tissue and cell specificity. This data seemingly provided an attractively simple explanation for the longstanding dilemma of how could one identical genome manifest itself in so many different forms in multicellular organisms? The DNA methylation pattern has thus become the only known factor to confer upon DNA a unique cellular identity. This important set of data provided strong support for the hypothesis that DNA methylation played an important role in controlling tissue specific gene expression. However, the naïve early models that predicted that DNA methylation would ultimately explain cell specific gene expression programs were later replaced by confusing and complex sets of data, cynicism and sarcasm. The fact that lower organisms such as flies and nematodes developed elaborate gene expression programs in the absence of any detectable DNA methylation has further shaken the belief that DNA methylation played any role in gene regulation. It became clear that proteins such as transacting and trans repressing factors interacting with *cis* acting factors in DNA as well as chromatin, chromatin modifications and the proteins that modify histones were principally responsible for cell specific gene expression profiles. However, recent advances in the field of chromatin modification and the discovery of methylated binding proteins are starting to clarify how DNA methylation is integrated with other epigenomic factors in regulating programmed gene expression. This clearer picture of the factors involved in regulating gene expression has brought DNA methylation back to the forefront of molecular biology. The first two chapters of the book by Razin and by Robertson will provide a review of our current understanding of how DNA methylation is integrated with the complex machinery, which controls gene expression in vertebrates.

An additional issue that is obviously coupled with the question of the functional role of DNA methylation is to understand the mechanisms responsible for generating and maintaining the DNA methylation pattern. The fact that gene expression and DNA methylation patterns correlate does not necessarily imply that there is a causal relation between DNA methylation and gene expression. The reverse possibility that DNA methylation is

directed by gene expression or that a common factor determines both DNA methylation patterns and gene expression is also consistent with such a correlation. To address this issue we need to understand what defines DNA methylation patterns. This obviously has important implications for our understanding of the changes in DNA methylation seen in cancer as will be discussed below.

The first unresolved question is the enzymology of DNA methylation. A number of DNA methyltransferases were discovered and cloned as will be discussed by Robertson. The presence of an enzyme that reverses the DNA methylation reaction has been extremely controversial. It has been long believed that DNA methylation is an irreversible reaction and that an enzyme that truly demethylates DNA and reverses the methylation reaction does not exist. The reason behind this strong dogma is that the bond between the methyl moiety and the cytosine ring is considered to be a strong bond that could not be broken by an enzymatic process. Our entire understanding of DNA methylation is based on this assumption. I will discuss recent data suggesting that DNA methylation is a reversible reaction and that the steady state DNA methylation pattern is an equilibrium of DNA methylation and demethylation reactions. This clearly changes our conception of DNA methylation patterns and how they are formed and maintained.

It is clear that none of the enzymes that catalyze either DNA methylation or demethylation show distinct specificity. This raises the question of what determines the specificity of the DNA methylation reactions. New data suggests an important relation between the chromatin modifying proteins and enzymes that catalyze either DNA methylation or demethylation, which could also explain the correlation between DNA methylation and chromatin structure. This will be discussed in Robertson's and my chapter.

The tight correlation between DNA methylation and programmed gene expression begs the question whether DNA methylation aberrations play a role in cancer. There is now overwhelming data supporting the conclusion that alterations in DNA methylation are a hallmark of cancer. This has both diagnostic and therapeutic applications, which will be discussed in this book. Four chapters in this book will focus on discussing the nature of DNA methylation alterations in cancer. The paradox of DNA methylation patterns in cancer is the coexistence of global hypomethylation and regional hypermethylation. The chapter by Ehrlich will focus on this issue. Three other chapters by Schulz et al, by Jass et al, and by Esteller et al will discuss specific methylation aberrations observed in cancer.

Another aberration of the DNA methylation machinery observed in cancer is the deregulation of expression of DNA methyltransferases, which will be discussed by Detich. Demethylases and their potential clinical application will be discussed. Caiafa et al will discuss the possible role of poly ADP ribosylation in hypermethylation.

The changes in DNA methylation observed in cancer have potentially important implications in therapeutics as well as diagnostics in addition to challenging us scientifically. The diagnostic applications of DNA methylation in cancer will be discussed as well as novel methods to measure global and regional hypermethylation in cancer. Niveleau et al will discuss immunochemistry approaches whereas Wei et al and Haefliger et al will discuss the use of new microarray technology and bioinformatics to unravel profiles of DNA methylation that can potentially serve as diagnostic tools for cancer, cancer stages and predictors of clinical progression of the disease.

The last part of the book will focus on preclinical and clinical attempts to target the DNA methylation machinery in cancer therapy. Seley et al will discuss the synthesis of novel DNA methylase inhibitors. Momparler will discuss the lessons derived from preclinical and clinical trials with the DNA methyltransferase inhibitor 5-aza-CdR and Scherman and Bigey will discuss the use of electrotransfer for knockdown of methylated DNA binding protein 2.

DNA methylation patterns and their relation to cancer have confused and bewildered us on one hand and stimulated our curiosity and enchanted us on the other hand. Recent advances in DNA methylation enzymology, methylated DNA binding proteins and chromatin have begun to clarify the role of DNA methylation in gene expression and cancer. They also raised the attractive possibility that enzymes of the DNA methylation machinery might serve as targets for anticancer drugs. More work needs to be done and future trials will determine whether the pioneering work with DNA methylation modulators will indeed translate to first-rate anticancer therapeutics. We hope that this book unravels some of these advances and therapeutic potential of DNA methylation as well as inspires the reader to further understanding of this emerging field in cancer biology and therapeutics.

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