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DNA Methylation and Cancer Therapy

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LANDES BIOSCIENCE / EUREKAH.COM
GEORGETOWN, TEXAS
U.S.A.

KLUWER ACADEMIC / PLENUM PUBLISHERS
NEW YORK, NEW YORK
U.S.A.

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Landes Bioscience / Eurekah.com
Kluwer Academic / Plenum Publishers

Printed in the U.S.A.

Kluwer Academic / Plenum Publishers, 233 Spring Street, New York, New York, U.S.A. 10013
http://www.wkap.nl/

Please address all inquiries to the Publishers:
Landes Bioscience / Eurekah.com, 810 South Church Street
Georgetown, Texas, U.S.A. 78626
Phone: 512/ 863 7762; FAX: 512/ 863 0081
www.Eurekah.com
www.landesbioscience.com

DNA Methylation and Cancer Therapy, edited by Moshe Szyf, Landes / Kluwer dual imprint / Landes series: Medical Intelligence Unit

ISBN: 0-306-47848-X

Library of Congress Cataloging-in-Publication Data

DNA methylation and cancer therapy / [edited by] Moshe Szyf.
p. ; cm. -- (Medical intelligence unit)
Includes bibliographical references and index.
ISBN 0-306-47848-X
1. DNA--Methylation. 2. Cancer--Molecular aspects. I. Szyf, Moshe. II. Series: Medical intelligence unit (Unnumbered : 2003)
[DNLM: 1. Neoplasms--genetics. 2. DNA Methylation. 3. Neoplasms--therapy. QZ 200 D629 2004]
QP624.5.M46D625 2004
616.99'406--dc22

2004022708

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

CONTENTS

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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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