新生物学丛书

肿瘤表观遗传学

EPIGENETICS IN CANCER

（英文版）

Junjiang Fu
Saber Imani

SCIENCE PRESS
Beijing

Narosa

Epigenetics in Cancer

Junjiang Fu & Saber Imani

SCIENCE PRESS
Beijing, China

Narosa
New Delhi, India

Epigenetics in Cancer

Junjiang Fu
Saber Imani

ISBN 978-7-03-054376-9
Science Press Beijing

Responsible Editors: Jing Luo Jing Liu

Preface

About twenty years ago, prof. Junjiang Fu wrote a manual titled *Protocols in Gene Diagnosis* for using in the lab when he was working in the Institute of Reproductive and Stem Cell Engineering, Reproductive & Genetic Hospital of CITIC-Xiangya, State Key Laboratory of Medical Genetics, Central South University Xiangya School of Medicine, Changsha, China. In 2012, he served as the editor for a book titled *Short Protocol in Medical Molecular Biology* published by Chinese Medicine Science & Technology Press. Now, he is interesting to serve as the editor for another book focusing on epigenetics and cancer.

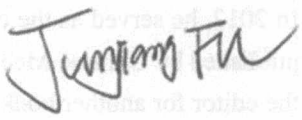
It is well known that as the second leading cause of death cancer has casted a big shadow on the lives worldwide. Many of our loved ones are fighting with different cancers. However and unfortunately, cancers often become the winners in this unjustly war. Both environmental and dietary factors play pivotal roles in cancer initiation and progression partially by altering the epigenetic landscape. Although increasing numbers of publications focusing on the interface of cancer genetics and epigenetics, the understanding of the effects of epigenetics on cancer is far from complete. At the end of 2013, Dr. Saber Imani expressed his interests in cancer epigenetics research and education to Prof. Junjiang Fu. However, it was hard to find a suitable textbook for this purpose and they realized that maybe it was the time to write a book on this topic.

Cancer epigenetics has gradually become the focus of many exciting researches in cancer with many significant advances. Since epigenetics is a rapidly growing field and a fascinating area in cancer research, many of today's undergraduates who are studying cancer biology will become tomorrow's graduates and postgraduates involving in genetic and epigenetic research. This book will cover different aspects of cancer epigenetics ranging from the role of epigenetics in molecular biology and cancer biology to epigenetic drugs either under development or already been used in cancer therapy. The purpose of this book is to provide the most up-to-date information pertaining to the roles and the potentials of epigenetics in cancer. All individuals who are interested in either understanding the underlying mechanisms or the development of anticancer drugs including undergraduate and graduate students, postdoctoral fellows, clinicians, educators, researchers, as well as those working in pharmaceutical companies will find the usefulness of this book in illuminating the most recent advances in the fields of epigenetics and cancer. We hope all the ones who read this book will find it is a rather enjoyable experience in gaining the knowledge pertaining to cancer epigenetics.

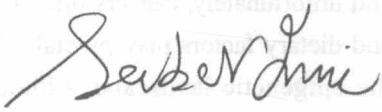
In the acknowledgement, we specified that "if you find this is a valuable book, you should

thank all the authors whose collective contributions to different chapters made this book successful". We also want to thank our family members because without their supporting there would be no *Epigenetics in Cancer*. We definitely want to thank the Science Press and its diligent staff members to make the publication a success. Finally, we want to emphasize that financially this work was partially supported by the National Natural Science Foundation of China (No. 30371493, 81672887, and 81172049).

Best wishes



In Houston, USA



In Luzhou, China

Contributors

Chunli Wei
State Key Laboratory of Quality Research in Chinese Medicines, Macau University of Science and Technology, Macau 999087, China;
Key Laboratory of Epigenetics and Oncology, The Research Center for Preclinical Medicine, Southwest Medical University, Luzhou, Sichuan 646000, China.
E-mail: weichunli2013@163.com

Dhananjaya Saranath
Department of Biological Sciences, Sunandan Divatia School of Science, NMIMS (Deemed-to-be) University, Mumbai 400056, India.
E-mail: dhananjaya.saranath@nmims.edu

Dianzheng Zhang
Department of Bio-Medical Sciences, Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131, USA.
E-mail: dianzheng@pcom.edu

Gordana Supic
Medical Faculty of Military Medical Academy, University of Defense, Belgrade, Serbia, Institute for Medical Research, Military Medical Academy, Crnotravska 17, 11002, Belgrade, Serbia.
E-mail: gogasupic@sezampro.rs

Jingliang Cheng
Key Laboratory of Epigenetics and Oncology, The Research Center for Preclinical Medicine, Southwest Medical University, Luzhou, Sichuan 646000, China.
E-mail: jingliangc@swmu.edu.cn

Junjiang Fu
Key Laboratory of Epigenetics and Oncology, The Research Center for Preclinical Medicine, Southwest Medical University, Luzhou, Sichuan 646000, China;
Hunan Normal University Medical College, Changsha, Hunan 410006, China;
State Key Laboratory of Quality Research in Chinese Medicines, Macau University of Science & Technology, Macau 999087, China.
E-mail: fujunjiang@hotmail.com

Katarina Zeljic
Faculty of Biology, University of Belgrade, StudentskiTrg 3, 11000 Belgrade, Serbia.
E-mail: katjaze@yahoo.com

Marzieh Dehghan Shasaltaneh
Laboratory of Systems Biology and Bioinformatics (LBB), Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran.
E-mail: m_deghan1361@ut.ac.ir

Ruth Borghaei
Department of Bio-Medical Sciences, Philadelphia College of Osteopathic Medicine,

Philadelphia, PA 19131, USA.
E-mail: RUTHB@pcom.edu

Saber Imani
Key Laboratory of Epigenetics and Oncology, The Research Center for Preclinical Medicine, Southwest Medical University, Luzhou, Sichuan 646000, China;
Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, lran.
E-mail: saberimani@swmu.edu.cn

Shangyi Fu
The Honors College, University of Houston, Houston, TX 77204, USA.
E-mail: sfu3@uh.edu

Wendy D'Souza
Department of Biological Sciences, Sunandan Divatia School of Science, SVKM's NMIMS (Deemed-to-be) University, Vile Parle (West), Mumbai 400056, India.
E-mail: wendy.dsouza@nmims.edu.in

Xianqin Zhang
Key Laboratory of Epigenetics and Oncology, The Research Center for Preclinical Medicine, Southwest Medical University, Luzhou, Sichuan 646000, China.
E-mail: sclzzxq@hotmail.com

Zvonko Magic
Medical Faculty of Military Medical Academy, University of Defense, Belgrade, Serbia, Institute for Medical Research, Military Medical Academy, Crnotravska 17, 11002, Belgrade, Serbia.
E-mail: zvonkomag@yahoo.co.uk

Contents

Color Figures

Chapter 1: Overview of Epigenetics

Saber Imani and Junjiang Fu*

Abstract

Over the last couple of decades, studies have introduced Epigenetic as an integral part of cancer research. Linked to phenotype, this has since materialized into the study of complex combinations of DNA methylations, post transcription modifications, noncoding RNAs, histone modifications, chromatin remodelling complexes in genome-wide changes of tumour suppressor gene and oncogene expression. This chapter will provide the most up-to-date and relevant information pertaining to the role and potential of epigenetics modifications in malignancies and solid tumours as well as epigenetic targets for drug discovery and development of novel biomarker in diagnostics or targeted epigenetic therapy.

Key words Cancer epigenetics, DNA methylation, Histone modification, Biomarkers, Epigenetic therapy.

*Corresponding author

1.1 Introduction

Although cancer has been traditionally recognized as a genetic disease, epigenetics sciences has challenged the new concepts of cancer and can provide novel evidence of complex puzzle of cancer [1-3]. In fact, apart from cancer, nearly all human diseases are defined by "Epigenetics" capabilities, that lead up to the development of disease. The epigenetic profile of a cell often dictates cellular differentiation and proliferations, including development, aging, diet, disease and cancer [4-6]. In definition, "Epigenetics" generally describes all meiotically and/or mitotically heritable change in gene expression states that do not have any underlying change in DNA sequence [7-9]. Epigenetic abnormalities in gene expression play an essential role in several parts of cancer process including resisting cell death, neoangiogenesis, evading growth suppressors, uncontrolled proliferation, metastasis and invasion, as well as replicative immortality [10, 11]. Historically, the conception of epigenetics arose in 1942 by *Conrad Hal Waddington* (Waddington C. H., 1905-1975) [12, 13]; study upon development of *Drosophila melanogaster* revealed how genotypes give rise to phenotypes [14, 15]. Moreover, *Robin Holliday* classically developed epigenesist study in 1990 (Holliday R., 1932-2014) with investigations on temporal and spatial control of gene activity during development of complex organisms [16, 17]. But, research over the past two decades cancer epigenetic has been classified particularly into five interacting major fields: DNA methylation, post-translational histone modifications (covalent histone modifications), chromatin remodeling, histone variants and non-coding RNAs (ncRNAs) [15, 18]. All of these modifications lead to gene activation or inactivation [19]. Large number of epigenetic alterations found in cancer cells as well as other rare diseases have been proceeded by a defect in one of the components of three classes epigenetic machinery: a writer that makes modifications to DNA and histone, a reader that deciphers codes and facilitate epigenetic effects; besides an Eraser that eliminates alterations [11, 15]. In addition, "movers" are protein complexes that position the nucleosomes across the genome.

Cancer has a series of hallmarks, the most important of which are listed in the Fig. 1-1. Sustaining proliferative signaling, resisting cell death, evading growth suppressors, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis are the most characteristic cancer hallmarks [20]. These hallmarks generate genome instability, deregulating cellular energetic as well as facilitating immune systems destruction and genetic diversity that expedites their acquisition, inflammation, and mutation [3]. Fig. 1-1 shows the traditional hallmarks of cancer, but supplementing these, additional hallmarks have been described [3]. Interestingly, aberrant epigenetic control can influence cancer hallmarks, some of which we try to cover in this book.

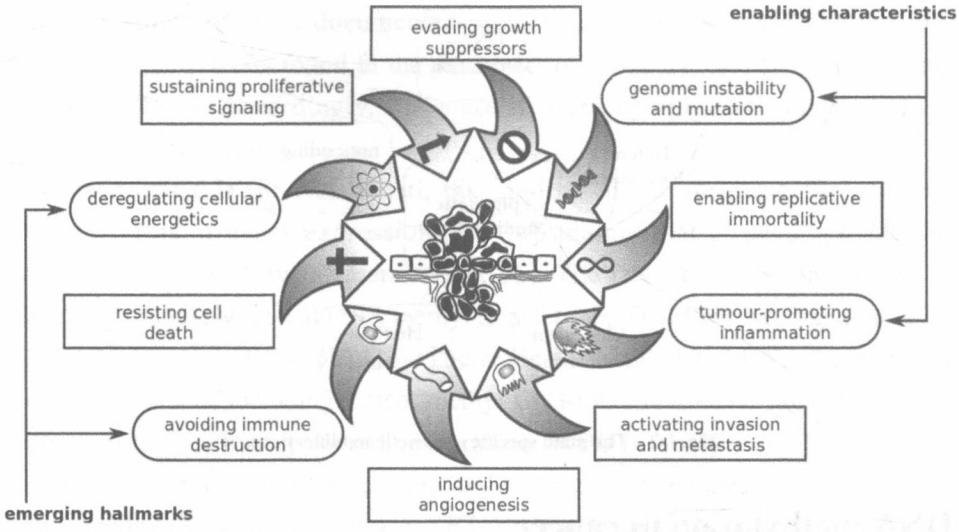


Fig. 1-1 The four hallmark capabilities of cancer.

In the last two decades, epigenetics has witnessed remarkable progress towards understanding the mechanistic underpinnings of each hallmark [21].

In this chapter, the first chapter of *Epigenetics in cancer* book, we will discuss and summarize the general epigenetic mechanisms in more detail. Then, we will give a brief overview of some well-studied epigenetic modifications that have been associated with particular cancers as well as clinical use of epigenetics as cancer biomarkers in diagnosis, treatment, and prevention.

1.2 Specific epigenetic modifications

Specific epigenetic modifications are called "epigenetic marks". These biomarkers have functional consequences for how genes are expressed and how chromatin is packaged [22]. This heritability of gene expression patterns is mediated by epigenetic marks, which generally include methylation of cytosine bases in DNA; posttranslational modifications of histone proteins; ATP-dependent chromatin remodeling complexes (CRCs) shift nucleosomes; histones with varying stabilities or specialist domains that alter the function of the nucleosome as well as ncRNAs; piwi-interacting RNAs (piRNAs); and other short interfering RNAs (siRNAs) [23] (Fig. 1-2). Most heritable changes are established during differentiation that are maintained stable through multiple cycles of cell division, giving cells distinct identities while covering the similar genetic information. In the following sections, we will describe the different epigenetic mechanisms involved in some pathogenic processes to learn how each process work, to truly understand these processes on a molecular level, and to apply this knowledge to other instances [24, 25].

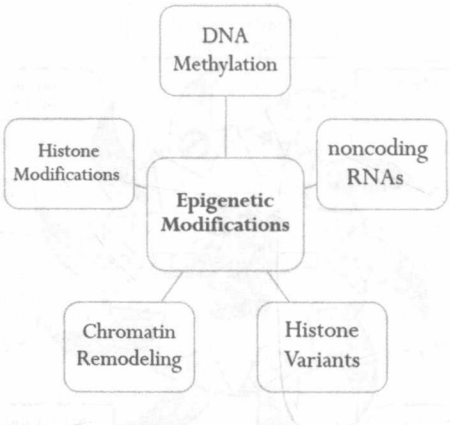


Fig. 1-2 The main specific epigenetic modifications.

1.3 DNA methylation in cancer

DNA methylation is the most well-known and thoroughly studied epigenetic modification in DNA levels. It occurs in approximately 3% of the whole genome of a eukaryotic cell is essential for the proper development of the organisms. Mechanistically, DNA methylation, just as it sounds, is the transfer a methyl group from *S*-adenosyl methionine to position 5 of the cytosine base in the DNA to create 5-methyl cytosine (5 mC). The methyl group is a CHC group that is added directly onto that base. It is well established that, in mammals, DNA methylation occurs almost exclusively at Cs followed by Gs; cytosine followed by guanine is called a CpG dinucleotide, in which the "p" is a phosphate bond between the cytosine and guanine. The CpGs dinucleotides account for 2-6% of the genome, but are not distributed equally throughout the genome. Rather, DNA methylation occurs largely on areas called CpG islands (CGIs) that are CG rich. CGIs are usually found in the promoters of genes that are located mainly within repetitive sequence, such as promoters of tumor suppressor genes (TSGs) as well as short and long interspersed nuclear elements. The CGIs are transcription start site of approximately 60% of human genes [26, 27]. The promoter region of CGIs are defined as a 0.5 to 5 kb stretch of DNA that cover this sequence at a higher frequency than the rest of the genome.

1.3.1 DNA hypermethylation in cancer

Abnormal DNA hypermethylation patterns are present more frequently in cancer cells than to normal cells. The recently finding show that hypermethylation on in CGIs-containing regions of chromosomes 3p, 11p, and 17p that are normally unmethylated in numerous human cancers [28-31]. The methylation of CGIs in the promoter region resulted in transcriptional silencing either by promoting or preventing the recruitment of regulatory proteins to DNA.

For example, methylated DNA documented by histone deacetylases (HDACs) facilitated gene silencing [32, 33]. CGIs found in the promoters of TSGs indicate a cancer cell are able to inactivate the TSGs. Accordingly, TSGs are silenced by epigenetically locking it in a hypermethylate inactive state.

Methylation of CGIs interferes with the binding of transcription factors and then suppresses all forms of genes expression [27, 34], mainly that of developmental genes, imprinted genes, and repetitive sequences [35, 36]. The DNA methylation process occurs through a family of closely related DNA methyl transferases (DNMTs) that functions as writers (DNMT1, DNMT3a, and DNMT3b) [37]. The readers of methylated DNA are methyl-CpG-binding domain proteins, including Kaiso, methyl CpG-binding domain protein 2b (MeCP2), and members of the methyl CpG-binding domains (MBDs) family [36, 38]. Certainly, the DNA demethyltransferase serves as erasing and editing bio-machines, including ten-eleven translocation methyl cytosine dioxygenase (TET) proteins main players.

DNA demethylation could be passive or active: Active DNA demethylation occurs via direct removal of a methyl group, such as MBD2b [39-42]. The passive process takes place during the replication of recently synthesized DNA strands by DNMT1. Base excision repair machinery (BER) and nucleotide excision repair (NER) are important passive DNA demethylation processes, which allow them to be maintained through cell division [43-45]. However, it is still being studied. DNA methylation at CGIs, for the inactive X chromosomes (Xist), will be expanded further in chapter 2 with additional details.

Importantly, DNA methylation is mitotically heritable. Originally supposed to be irremovable, since DNMT (such as TET proteins) recognizes semi-methylated DNA and restores methylation on both strands of DNA during the DNA replications process. These proteins are involved in active demethylation as well as are only expressed during development. This issue is completely covered in chapter entitled "DNA Methylation and Cancer".

In normal cells, the pattern of DNA methylation is conserved after DNA replication and cell division through the methylation of cytosine using a maintenance DNA methylase (DNMT1). DNA methylation of genes occurs mainly in the CGIs of promoter region. As seen in Fig. 1-3, the CGIs are located upstream of the gene. In the normal cells, DNA methylation occurs intergenic regions and repetitive elements. Approximately, all CGIs gene in the normal state are hypomethylated; in contrast, the intergenic regions and repetitive elements of gene promoters are usually hypermethylated which leads to transcriptional silencing. De novo DNA methylation is brought about by DNMT3a and/or 3b that convert cytosine residues of CpG dinucleotides into 5-methylcytosine, whereas DNA methylation is maintained by DNMT1 [46]. Although methylation of DNA in 5′ promoters has been shown to suppress gene expression, recently, DNA methylation

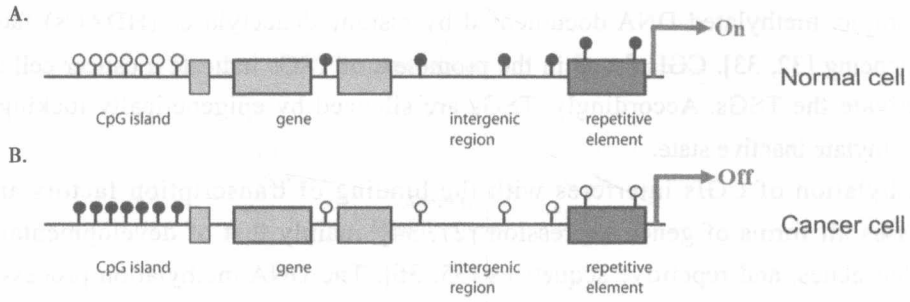


Fig. 1-3 DNA methylation patterns in epigenetic control seen in cancer and normal cells.

A. Methylation of CGIs. In normal cells, actively transcribed TSGs are associated with unmethylated CGIs. Also, most repetitive elements and intergenic region are hypermethylated in normal cells. B. In contrast, during tumorigenesis, many CGIs in TSGs promoters convert hypermethylated, leading to aberrant transcriptional silencing TSGs. Furthermore, in cancer cells, repeat-rich sequences and intergenic region become hypomethylated, which contributes to genomic instability, are hallmark of tumor cells, and tumorigenesis.

was described downstream of the promoters in intergenic regions that maintain genomic integrity or genomic stability [47]. But, in the tumor cells, methylations pattern completely change, where it supports transformation of cells to neoplastic in the TSGs. The aberrant methylation of genes, mainly CGIs' hypermethylation and hypomethylation of intergenic and repetitive elements that suppress tumorigenesis, appears to occur early in tumor development and increases progressively, finally leading to the malignant phenotype (Fig. 1-3).

According to the *Knudson* hypothesis, DNA methylation can actually be the second hit, or one of the hits. *Knudson* hypothesis proposed that multiple hits should occurred before the cancer develops; while methylation of TSGs or one copy of the two alleles of TSGs might be one of these hits, these are not sufficient to cause tumorigenesis. Instead, tumorigenesis needs the removal of both copies of TSGs. In another instance, it also needs to activate an oncogene. Tumorigenesis needs to have many insults for the cell to end up forming a tumor rather than dying. DNA methylation and consequent epigenetic silencing of TSGs can be one of these hits in terms of the *Knudson* hypothesis [48].

The frequent occurrence of hypermethylation has been described for several different types of cancers, where the CGIs methylator phenotype is called CIMP. It is well established that TSGs hypermethylation is a mutation that often occurs more frequently than other ones. In the wide array of cancer disorders, we try to identify the hypermethylated CGIs that varies with the genome-wide DNA methylation studies and is distinct with each tumor type. The CGIs methylation progresses are variable depending on different time periods (Fig. 1-4); they are often confounded by DNA methylation alterations with increases of age. The CGIs hypermethylation is also known as single cancer biomarkers, which can identify a specific

feature of cancer. These biomarkers are more frequently used to distinguish cancer from normal cells in the same sample, e.g. tumor *vs.* benign skin cells, tumor *vs.* normal prostate cells, tumor *vs.* normal cell DNA that are free-floating in the blood existent in normal blood. DNA hypermethylation biomarkers are favored, since detection is more sensitive [49, 50]. The RB in retinoblastoma, MLH1 in colorectal cancer, BRCA1 in breast cancer, and MGMT in gliomas are notable single gene examples of hypermethylation that highlighted in cancer; these biomarkers could be used for prognostics, diagnostics and/or treatments in the outcomes of cancer [51].

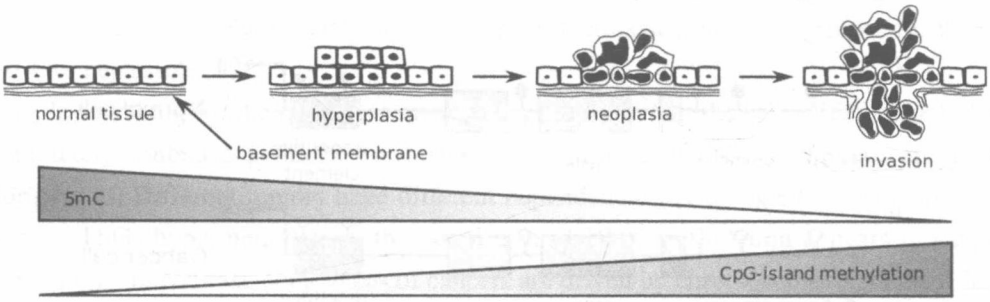


Fig. 1-4 The features of CGIs hypermethylation in during of cancer invasion [56].

The aberrant hypermethylation in RB gene is responsible for retinoblastoma cancer. Mapping of the RB gene hypermethylation in the genealogy of suspicious families can greatly reduce the incidence of this retinoblastoma cancer [52]. For instance, there is he hypermethylation of the MLH1 CGIs promoter in hereditary nonpolyposis colon cancer (HNPCC) and colorectal cancer in Lynch syndrome [53]. We know BRCA1 and BRCA2 are responsible for a fairly large proportion of hereditary breast cancer. They seem to be mutated in sporadic cases as well. Similarly, BRCA1 can be hypermethylated rather than mutated [54]. Finally, MGMT can be hypermethylated, DNA is found to be hypermethylated in glioma as well as in colorectal tumors. The colorectal CIMP is tend to be have defective MLH1 function in older patient age, mostly females [55].

1.3.2 DNA hypomethylation in cancer

Hypomethylation of genome is another kind of aberrant DNA methylation that is established in many cancers. Although hypomethylation was first confirmed in cancer research in 1983, there is little information known about the epigenetic significance of aberrant DNA hypomethylation compared to hypermethylation in gene silencing. Hypomethylation of tumor cells is principally due to the loss of methylation from tandem repetitive regions and interspersed repeats of the genome, including dispersed retrotransposons, heterochromatic DNA repeats, and endogenous retroviral element [57, 58].