EPIGENETICS and PATHOLOGY

Exploring Connections Between Genetic Mechanisms and Disease Expression

Editor
Kasirajan Ayyanathan, PhD

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ABOUT THE EDITOR

KASIRAJAN AYYANATHAN, PhD

Kasirajan Ayyanathan received his PhD degree from the Department of Biochemistry, Indian Institute of Science, one of the premier research institutions in India. Subsequently, at Temple University School of Medicine, USA, he conducted post-doctoral research on the signal transduction by purinergic receptors, a class of G-Protein Coupled Receptors (GPCR), in erythroleukemia cancer cells. Next, he was trained as a staff scientist at the Wistar Institute, USA, for almost ten years and studied transcription regulation, chromatin, and epigenetic regulatory mechanisms in cancer before becoming an Associate Professor at Florida Atlantic University (FAU). Currently he is at the Center for Molecular Biology and Biotechnology as a Research Associate Professor at FAU. He is the recipient of Chern memorial award and Howard Temin career research award.

Dr. Ayyanathan is well trained in molecular biology, cell biology, biochemistry with main focus on studying transcription factors, and gene regulation. He has contributed to several projects such as generation of conditional transcriptional repressors that are directed against the endogenous oncogenes to inhibit malignant growth, establishment of stable cell lines that express chromatin integrated transcriptional repressors and reporter genes in order to study the epigenetic mechanisms of KRAB repression, and identification of novel SNAG repression domain interacting proteins in order to understand their roles in transcriptional repression and oncogenesis. Dr. Ayyanathan has published several research articles in peer-reviewed articles in these subject areas.

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The chapters in this book were previously published in various places and in various formats. By bringing them together here in one place, we offer the reader a comprehensive perspective on recent investigations into the connections between epigenetics and pathology. Each chapter adds to a more complete image of this research topic.

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INTRODUCTION

The term "epigenetics" describes cellular modifications caused by mechanisms other than DNA sequence variations that can be heritable and modified by environmental stimuli. Since the beginning of Mendel's era, the influence of genetic changes (alterations in the underlying DNA sequence) on gene expression remains the main thrust of geneticists. For this reason, it is also widely understood. Mendelian inheritance, named after its discoverer Gregory Mendel, refers to the process of transmission of genetic traits from the parents to the offspring. Since then, several researchers have shown that genes serve as blueprints in almost all living organisms from simple prokaryotes to multicellular eukaryotes, evidenced by the dearth of research articles and textbooks on the subject.

However, two other distinct factors can bring out similar changes in gene expression. One such influencing factor is conferred from the inside of a cellular milieu, namely epigenetic modification. Selective epigenetic changes can specifically alter the chromatin environment and thus result in altered gene expression. This also underlies the basis of Lamarckism. Similarly, from the outside of a cellular milieu, several environmental factors can influence gene expression and result in rapid changes in gene expression. This is mostly prevalent in gene expression observed in prokaryotes, wherein quick adjustment to its immediate environment is mandatory for them to thrive effectively.

A growing body of evidence points towards epigenetic mechanisms being responsible for a wide range of biological phenomena, from the plasticity of plant growth and development to the nutritional control of caste determination in honeybees and the etiology of human disease (e.g., cancer). With the (partial) elucidation of the molecular basis of epigenetic variation and the heritability of certain of these changes, the field of evolutionary epigenetics is flourishing. Despite this, the role of epigenetics in shaping host–pathogen interactions has received comparatively little attention. Yet there is plenty of evidence supporting the implication of

epigenetic mechanisms in the modulation of the biological interaction between hosts and pathogens. The phenotypic plasticity of many key parasite life-history traits appears to be under epigenetic control. Moreover, pathogen-induced effects in host phenotype may have transgenerational consequences, and the bases of these changes and their heritability probably have an epigenetic component. The significance of epigenetic modifications may, however, go beyond providing a mechanistic basis for host and pathogen plasticity. Epigenetic epidemiology has recently emerged as a promising area for future research on infectious diseases. In addition, the incorporation of epigenetic inheritance and epigenetic plasticity mechanisms to evolutionary models and empirical studies of host–pathogen interactions will provide new insights into the evolution and coevolution of these associations. The goal of this volume is to bring together into a coherent whole some of the most current and relevant research being done in this field.

In chapter 1, "The Epigenetics of Host-Pathogen Interactions," authors Goméz-Díaz et al review the evidence available for the role epigenetics on host–pathogen interactions, and the utility and versatility of the epigenetic technologies available that can be cross-applied to host–pathogen studies. They conclude with recommendations and directions for future research on the burgeoning field of epigenetics as applied to host–pathogen interactions.

In chapter 2, "The Molecular Mechanisms of Epigenetic Regulation," Golbabapour and his colleagues explore the epigenetic regulatory events that occur during the gametogenesis, embryogenesis and placental development. The epigenetic modifications that modulate expression of genes and subsequent reprogramming of the somatic nucleus to pluripotent state are also briefly discussed. The authors' purpose in this chapter is to summarize effective epigenetic events that could increase efficiency of SCNT and to emphasize recent epigenetic findings. They briefly look into transition techniques and highlight epigenetic modifications that happen during the nucleus reprogramming.

Kim and his colleagues focus on the amnion in chapter 3, "Mutation Rate and DNA Methylation in the Human Genome." To investigate the importance of epigenetic events in this tissue in the physiology and pathophysiology of pregnancy, they performed genome-wide DNA methylation

profiling of human amnion from term (with and without labor) and preterm deliveries. Using the Illumina Infinium HumanMethylation27 BeadChip, they identified genes exhibiting differential methylation associated with normal labor and preterm birth. Their work provides preliminary evidence that DNA methylation changes in the amnion may be at least partially involved in the physiological process of labor and the etiology of preterm birth, suggesting that DNA methylation profiles, in combination with other biological data, may provide valuable insight into the mechanisms underlying normal and pathological pregnancies.

Chapter 4, "Causal Relationships in Gene-Environment Interactions," offers a conceptual framework to assess causal relationships in clinical genomics and, particularly, for evaluating the etiopathogenic significance of gene-disease associations and gene-environment interactions (i.e., a framework to assess the validity and significance of such environment-host-gene relationships in the etiology of human diseases). The framework includes a two-step approach that combines the causal criteria of Austin Bradford Hill with graphical models such as directed acyclic graphs (DAGs). The approach Geneletti and her colleagues propose thus helps, first, to untangle the web of interactions amongst several exposures and characteristics (environmental, clinical and genetic) and a disease, using criteria to assess causality that have long been used in clinical and epidemiological research. More generally, chapter 4 is an example of integrative research, i.e., research that integrates knowledge, data, methods, techniques, and reasoning from multiple disciplines, approaches and levels of analysis to generate knowledge that no discipline alone may achieve.

Because enzymes that catalyze acetylation are also transcriptional co-activators, which coordinate with transcription factors in regulating gene expression—underscoring the integration of transcription with metabolism—such enzymes present potential therapeutic targets. In chapter 5, "Lysine Acetylation in Transcriptional Programming and Metabolism," the overall goal authors Patel, Pathak and Mujtaba is to highlight the most recent advances in the field of acetylation biology that could spark new perspectives and illuminate novel research avenues.

In chapter 6, Delcuve, Khan and Davie take an in-depth look at the role histone deacetylation plays in epigenetic regulation. The scope of their review concerns emerging concepts regarding the roles of HDACs in

modulating chromatin structure and function as revealed by studies with HDAC inhibitors. The zinc-dependent mammalian histone deacetylase (HDAC) family comprises 11 enzymes, which have specific and critical functions in development and tissue homeostasis. Mounting evidence points to a link between misregulated HDAC activity and many oncologic and nononcologic diseases. Thus the development of HDAC inhibitors for therapeutic treatment garners a lot of interest from academic researchers and biotechnology entrepreneurs. Numerous studies of HDAC inhibitor specificities and molecular mechanisms of action are ongoing, and in one of these studies, mass spectrometry was used to characterize the affinities and selectivities of HDAC inhibitors toward native HDAC multiprotein complexes in cell extracts. Such a novel approach reproduces *in vivo* molecular interactions more accurately than standard studies using purified proteins or protein domains as targets and could be very useful in the isolation of inhibitors with superior clinical efficacy and decreased toxicity compared to the ones presently tested or approved. HDAC inhibitor induced-transcriptional reprogramming, believed to contribute largely to their therapeutic benefits, is achieved through various and complex mechanisms not fully understood, including histone deacetylation, transcription factor or regulator (including HDAC1) deacetylation followed by chromatin remodeling and positive or negative outcome regarding transcription initiation. Although only a very low percentage of protein-coding genes are affected by the action of HDAC inhibitors, about 40% of noncoding microRNAs are upregulated or downregulated. Moreover, a whole new world of long noncoding RNAs is emerging, revealing a new class of potential targets for HDAC inhibition. HDAC inhibitors might also regulate transcription elongation and have been shown to impinge on alternative splicing.

In chapter 7, "Aberrant Epigenetic Silencing and Gene Expression," Oyer and his colleagues have developed a system to directly test the hypothesis that a transient reduction in gene expression can sensitize a promoter to undergo epigenetic silencing. Their results demonstrate that this principle is correct. Additionally, they find that induction of silencing is dependent on histone deacetylase activity, but does not require DNA methylation. Silenced alleles readily reactivated spontaneously or after treatment of cells with inhibitors of histone deacetylation and/or DNA

methylation, but re-silencing of reactivated alleles did not require a new round of Dox exposure. Inhibition of histone deacetylation inhibited both the induction of silencing and re-silencing, whereas inhibition of DNA methylation had no such effect

Blenn, Wyrsch and Althaus discuss in chapter 8, "RNAi Silencing in Poly (ADP-Ribose) Research," the potential of RNAi to manipulate the levels of PARPs and PARG, and consequently those of PAR and ADPR. They compare the results of their studies with those obtained after genetic or chemical disruption.

Chapter 9, "MicroRNA Deregulation in Rhabdomyosarcoma and Neuroblastoma," focuses on current knowledge about miRNAs deregulated in RMS and NB by epigenetic modifications. Romania et al highlight miRNAs' role in developmental pathways, highlighting RMS and NB tumorigenesis. They discuss the translational implications and challenges of miRNAs modulation in these pediatric tumors, explaing that gene expression control mediated by microRNAs and epigenetic remodeling of chromatin are interconnected processes often involved in feedback regulatory loops. These strictly guide proper tissue differentiation during embryonal development. Altered expression of microRNAs is one of the mechanisms leading to pathologic conditions, such as cancer, and several lines of evidence point to epigenetic alterations as responsible for aberrant microRNA expression in human cancers. Rhabdomyosarcoma and neuroblastoma are pediatric cancers derived from cells presenting features of skeletal muscle and neuronal precursors, respectively, blocked at different stages of differentiation. Consistently, tumor cells express tissue markers of origin but are unable to terminally differentiate. Several microRNAs playing a key role during tissue differentiation are often epigenetically downregulated in rhabdomyosarcoma and neuroblastoma and behave as tumor suppressors when re-expressed. Recently, inhibition of epigenetic modulators in adult tumors has provided encouraging results causing re-expression of anti-tumor master gene pathways. Thus, a similar approach could be used to correct the aberrant epigenetic regulation of microRNAs in rhabdomyosarcoma and neuroblastoma. Chapter 9 highlights the current insights on epigenetically deregulated microRNAs in rhabdomyosarcoma and neuroblastoma and their role in tumorigenesis and developmental pathways. The translational clinical implications and challenges regarding

modulation of epigenetic chromatin remodeling/microRNAs interconnections are also discussed.

In chapter 10, "Epigenetic Effects of Environmental Chemicals," Singh and Shoei-Lung Li investigate the epigenetic effects on DNA methylation, histone modification, and expression of non-coding RNAs (including microRNAs) of environmental chemicals such as bisphenol A (BPA) and phthalates, expanding our understanding of the etiology of human complex diseases such as cancers and diabetes. Multiple lines of evidence from *in vitro* and *in vivo* models have established that epigenetic modifications caused by *in utero* exposure to environmental toxicants can induce alterations in gene expression that may persist throughout life. Epigenetics is an important mechanism in the ability of environmental chemicals to influence health and disease, and BPA and phthalates are epigenetically toxic. The epigenetic effect of BPA was clearly demonstrated in viable yellow mice by decreasing CpG methylation upstream of the Agouti gene, and the hypomethylating effect of BPA was prevented by maternal dietary supplementation with a methyl donor like folic acid or the phytoestrogen genistein. Histone H3 was found to be trimethylated at lysine 27 by BPA effect on EZH2 in a human breast cancer cell line and mice. BPA exposure of human placental cell lines has been shown to alter microRNA expression levels, and specifically, miR-146a was strongly induced by BPA treatment. In human breast cancer MCF7 cells, treatment with the phthalate BBP led to demethylation of estrogen receptor (ESR1) promoter-associated CpG islands, indicating that altered ESR1 mRNA expression by BBP is due to aberrant DNA methylation. Maternal exposure to phthalate DEHP was also shown to increase DNA methylation and expression levels of DNA methyltransferases in mouse testis. Further, some epigenetic effects of BPA and phthalates in female rats were found to be transgenerational. Finally, the authors describe the ways in which available new technologies for global analysis of epigenetic alterations will provide insight into the extent and patterns of alterations between human normal and diseased tissues. *In vitro* models such as human embryonic stem cells may be extremely useful in bettering the understanding of epigenetic effects on human development, health and disease, because the formation of embryoid bodies *in vitro* is very similar to the early stage of embryogenesis.