Sevgi Durna Daştan
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Editors

EPIGENETICS

Beyond the Genetics and Medicine

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Genetics - Research and Issues

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Contents

Preface

Epigenetics refers to the study of changes in organisms that are caused by modifications of gene expression instead of alteration of the genetic code itself. This volume includes 30 chapters that explore epigenetics from a variety of perspectives, including the role of epigenetics in aging, cancers, fetal brain development, epilepsy, and more.

Chapter 1 - Epigenetics is termed as non-sequence deoxyribonucleic acid (DNA) alterations in the gene expression profile that is heritable. With these, cellular identity is preserved, and gene function is transferred from one cell to another. Epigenetic changes occur with the effect of DNA methylation, chromatin structure changes, and non-coding ribonucleic acids, histone modifications. Even though cells in different tissues have the same DNA, differences may occur between the steps that take place during protein synthesis from DNA, and this can be explained by epigenetic regulation. In addition to the genetic basis, it is known that epigenetic differences are also effective in the development of many diseases, especially cancer. Changes in gene expression should be investigated with epigenomics rather than gene analysis in a particular region. In the investigation of diseases of unknown etiology, such as obesity, insulin resistance, neurodegenerative, cardiovascular, and immune system diseases, it has been determined that many internal and external environmental factors directly affect gene expression. Understanding the differences that cause diseases with epigenetic mechanisms makes it possible to develop molecular diagnostic tests and targeted treatment strategies. In this chapter, basic information about epigenetics will be given and the situations where disruptions in epigenetic mechanisms may lead to related gene expression, and the importance of epigenetics in these situations will be emphasized.

Chapter 2 - Epigenetic changes occur in the phenotype due to differences in the expression of genes while remaining the same in the existing DNA sequence. Disruptions in the promoter methylation pattern, histone modifications, and non-coding RNA irregularities can be given as examples of epigenetic changes. The positive or negative effects of lifestyle changes lead to epigenetic mechanisms and these mechanisms often work together to cause changes that affect all organ systems. These changes can result in autoimmune diseases or syndromes. Therefore, further research on epigenetic mechanisms can significantly contribute to the diagnosis, follow-up, and the treatment of many organ systems diseases.

Chapter 3 - Epigenetic modifications include DNA methylation and covalent modifications of histones. In spite of the fact that they are reversible, these alterations have an unavoidable effect on gene expression and are very stable. Cytosines are more frequently methylated than guanine nucleotides or CpG sites in DNA. In addition to regulating tissue-specific gene expression, DNA methylation also affects genomic imprinting and the inactivation of X chromosomes. It is possible that DNA methylation in different genomic regions may influence gene activity differently depending on the genetic sequence.

Methylation changes in the promoter or first exon can mimic the effects of mutations in various tumor suppressor genes (TSGs) or protooncogenes. Cancer may also develop from abnormal DNA methylation, such as hyper- or hypomethylation of cancer-related genes' promoters or first exons. The transcriptional silencing of a variety of TSGs is caused by hypermethylation of their promoters. Activating the transcription of protooncogenes, retrotransposons, and genes that encode proteins that have a role in genome instability and malignant cell metastasis is accomplished by hypomethylating regulatory DNA sequences.

In carcinogenesis, DNA methylation has a critical role in gene expression. In this chapter, hypo- and hyper-DNA methylation on cancer as well as recurrence risk of human cancers are discussed along with the mechanisms and cell-regulating effects of both hypo- and hyper-methylation.

Chapter 4 - Epigenetics refers to changes that do not affect the DNA sequence but can affect gene expression. Healthy cells transform into malignant cells through some complex mechanisms. The simultaneous evaluation of tumor suppressor genes and oncogenesis with epigenetics makes it easier for us to understand this complex process. Among epigenetic mechanisms, there are two mechanisms that directly control gene expression, which are DNA methylation and histone modification. DNA methylation is involved in cellular control, while histone modifications have functions in transcriptional activity and modifications at the chromatin structure level. It has been determined that epigenetic mechanisms such as DNA methylation and histone modifications also regulate the expression of miRNAs. miRNAs are non-coding RNA molecules composed of twenty-two nucleotide sequences that are short and involved in the development of both physiological and important diseases such as malignancies. Tumor-associated abnormalities in miRNA or epigenetic mechanisms are commonly found in human cancers. Abnormal proliferation, apoptosis, and genetics are involved in the development of malignant cells. miRNAs are considered to be actively involved in the regulation of these processes. Available data reveal that miRNAs, which can be used as biomarkers, are biomarkers that can be used in early diagnosis, tumor subtyping, early treatment, prognosis prediction, and treatment resistance. Thus, it is considered that they can increase the disease-free lifetime and quality of life of cancer patients. In this paper, it was attempted to describe the relationship between dysregulated miRNA and epigenetic mechanisms in cancer.

Chapter 5 - Genetics cannot solely explain genetic variations in humans and disease developments. We see varying differences in phenotypes and disease susceptibility in organisms that have the same genetic make-up, e.g., monozygotic twins and cloned animals. The information carried by the genomic sequence is the blueprint, but the final product requires environmental determinants. Here comes the concept of epigenetics, as it is the framework where biochemical interactions between the genome and the environment blend. We can describe epigenetics as mechanisms that are beyond genetics, as such mechanisms alter the result of the genomic blueprint without altering the information itself, i.e., the sequence. Both epigenetics and epigenomics are trending research fields to better evaluate the genotype and the phenotype. The methylation of genetic material is a well-studied and well-known epigenetic marker. The epigenome, as a term, describes the inheritable changes in both the DNA and histone molecular structures, where the methylation and acetylation mechanisms are studied extensively. As the building blocks of the chromatin structure, nucleosomes depend on epigenetic changes, which lead to becoming either tight or loose, based on the particular mechanisms. Chromatin structure changes directly affect the gene expression, e.g., particular

gene expression becomes silenced if the gene position has DNA hypermethylation and histone hypoacetylation that leads to the condensed form of the chromatin. Inversely, if the said changes were removed, genes in that position would express themselves again. Therefore, epigenetics provides a vigorous and remarkably malleable means for gene expression regulations. Epigenomics includes both the epigenetic mechanisms on the DNA and histones and complicated interactions between the genotype and the phenotype. There are well-known epigenomic changes in DNA, RNA, and protein levels. DNA base changes in somatic cells and chromosome positioning are among the aspects determining the epigenomic scene. Alternative splicing mechanism, RNA processing (editing, capping, Poly-A tailing, etc.), RNA methylation, and regulations conferred by the non-coding RNAs (ncRNAs) are RNA level epigenomic changes. Epigenomic changes at the protein level include various mechanisms of the post-translational modification. Epigenomics research includes total hypomethylation profile of the genome, identification of hypermethylated genes, microRNA-driven gene silencing with DNA methylation, epigenome projects, and DNA-based clinical therapies for different biomedical conditions. This chapter will detail fundamental concepts and basic approaches to both epigenomics and epigenomes.

Chapter 6 - Epigenetic changes are important mechanisms that have a role in the etiology and/or progression of cancer by causing the activation of oncogenes or the silencing of tumor suppressor genes. Epigenetic mechanisms that alter chromatin structure can be separated into four categories: histone modifications, DNA methylation, nucleosome remodeling, and non-coding RNAs (e.g., miRNAs). These mechanisms work together to organise the functioning of the genome by regulating the accessibility of chromatin and altering the structural dynamics of chromatin. These epigenetic mechanisms are necessary for normal mammalian development and regulation of gene expression. Disorders in epigenetic regulators have been determined in both blood and solid cancers, and the significance of epigenetic changes in cancer cells has been emphasized by several groups in different types of cancer.

Chapter 7 - The human is a superorganism called the holobiont, which consists of human cells and a larger proportion of microbial cells. Microbiota is the ecological communities of microorganisms that live in a particular environment. It is known that the human microbiota, especially the gut microbiota, is known to affect the physiology and pathology of the host through various mechanisms. For example, the intestinal microflora regulates many epigenetic pathways, such as modifications to DNA or histones, their metabolites, and noncoding RNAs. Epigenetic factors are also known to regulate the gut microbiota within the host by various mechanisms.

Chapter 8 - The study of epigenetics examines how genetic changes in gene expression are transmitted from one generation to another and are not caused by changes in the DNA sequence. A key role played by epigenetic mechanisms is thought to be in cellular growth, differentiation, and autoimmune diseases. There are several epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNA regulation.

Transcripts that do not have protein-coding capacity in our genome are defined as non-coding RNA (ncRNA). The post-transcriptional regulation of genes is greatly influenced by non-coding RNAs. These ncRNAs are composed of small non-coding RNAs (sncRNA) and long non-coding RNAs (lncRNA) according to their length. Epigenetic mechanisms (genetic imprinting and dosage compensation) have been found to regulate the expression of lncRNAs. The function of centromeres is regulated by sncRNAs, which are involved in gene expression, chromatin organization, and modification.

In this chapter, the potential epigenetic mechanisms of ncRNAs and their associated cellular processes are presented. The importance of non-coding RNAs in the etiology of diseases has been revealed in recent genetic studies. Non-coding RNAs have been shown to have functions ranging from control of gene expression, epigenetic mechanisms, and signal transduction. In particular, it has been determined that some piRNAs, miRNAs, and siRNAs function in common pathways and are involved in regulatory mechanisms.

Chapter 9 - Epigenetics generally describes inherited DNA methylation, histone modifications, and chromatin-based events including chromatin structure that regulate gene expression. In the field of epigenetics, microRNAs have also been shown to have a role in epigenetic regulation. While DNA methylation and histone modifications add a new dimension to gene regulation at the transcriptional level, miRNAs are involved in the regulation at the post-transcriptional level. Analysis of histone modifications is done by chromatin immunoprecipitation (ChIP) and 3C Chromosome conformation capture. There are many methods used for DNA methylation analysis. These methods are hybridization (Southern blot and microarray), restriction enzyme PCR, methylation-sensitive single-nucleotide primer extension, cutting with methylation-sensitive restriction enzymes, bisulfite genomic DNA sequence analysis techniques and epigenetic MR. This chapter offers a reference of various techniques for epigenetic analysis.

Chapter 10 - Epigenetics is a new critical field that has arisen to investigate the effect of elements apart from genes on the character and on the role of an organism. Using computational tools constitutes the core of this new research area and has critical roles because controlling the choice of basic tests, developing recent provable theories from precise examination of complicated genomic knowledge, which is not attainable utilizing classical methods, only possible with computational tools. Epigenomics connects conventional genomics with computer science, biochemistry, mathematics, chemistry, proteomics for the extensive investigation of hereditary diversities in phenotype, and gene activity or gene interpretation, which are not dependent on gene array. Moreover, it helps us to better understand transcriptional adjustment, nuclear arrangement, improvement, and illness. Here, current computational approaches to investigate epigenetic factors are explored. Essential data sources and bioinformatic apparatus in this fast-developing area have been evaluated.

Chapter 11 - Aging is a multifactorial process happening with many biological, biochemical, and physiological changes. There are a lot of theories to explain the nature of aging. There are also many data supporting the relationships between epigenetic modifications and the aging process. Nucleosomal remodeling is frequently stressed by numerous research due to its critical nature in cellular senescence and aging. Different mechanisms are postulated for nucleosomal remodeling, which include (1) modification of nucleosome residency by histone loss, (2) alteration of nucleosome affinity to either DNA or histones induced by histone protein variants, and (3) ATP-dependent nucleosome relocation to modify recruitment of transcriptional factors and other modifiers. Recent advances have revealed aging-related genes that have modifying properties for histone repositioning, which supports the fact that both aging and senescence are directly related to the disruption of heterochromatin and euchromatin ratios in the chromatin landscape. Thus, leading to the dissolution of the clear distinctions between chromatin regions in cellular senescence, which is more pronounced in the aging process. This dissolution correlates with the downregulation of genes in the senescence that are normally in an active state before the senescence period. However, conflicting results are present in terms of the genome-wide heterochromatin association with the aging and senescence processes.

Therefore, specific analyses are necessary of which genomic regions, related loci, and gene expressions are affected. This review will try to describe the intricate associations between cellular senescence and known epigenetic mechanisms, e.g., chromatin remodeling and restructuring. Also, the currently established properties of the aging process with epigenetic mechanisms are described using different models for the aging phenomenon.

Chapter 12 - FMF represents an autosomal recessive hereditary disease, which may have recurrent episodes of fever, serositis, and arthritis/arthralgia and accompanying skin findings. Its prevalence varies from society to society and changes in the range of 1/200-1/1000 on average. The gene responsible for FMF is the MEFV (Mediterranean Fever) gene on chromosome 16, which encodes the pyrin protein. The problem in FMF is the change in pyrin protein due to the MEFV gene mutation. Mutation in pyrin reduces the activation threshold of the inflammasome, which excessively increases IL-1 secretion and initiates the inflammatory process. Mutations of M694V, V726A, M680I, and M694I are observed most frequently worldwide, and these are known as pathological mutations. The most feared complication is secondary AA-type amyloidosis. With the use of colchicine in the treatment, attacks are prevented, subclinical inflammation between attacks is taken under control, and the risk of amyloidosis is eliminated. Alternative treatments should be considered in the presence of partial remission with colchicine therapy, in cases of unresponsiveness to colchicine, or in cases of colchicine intolerance. TNF-a inhibitors, IL-1 inhibitors (anakinra, rilonacept, and canakinumab), and Janus kinase inhibitors are among alternative treatments.

It has been recently revealed that changes in DNA cannot be explained only by genetics, and the role of epigenetic mechanisms is also quite significant. In epigenetics, the DNA sequence does not change; however, since the promoter region of the gene changes, quantitative and qualitative changes emerge in gene transcription. As a result of transcriptional changes, the transcription of the relevant gene can be silenced or activated. These quantitative and qualitative changes are not permanent and can be regulated when needed. Furthermore, these alterations in gene transcription can be transferred to the following generations. When epigenetic mechanisms are mentioned, DNA methylation, histone acetylation, histone methylation, histone ubiquitination, histone phosphorylation, histone citrullination, histone ribosylation, non-coding RNA, and chromatin re-modeling come to mind.

Epigenetics tries to explain different phenotypes of FMF patients with the same genotype (patients with the same mutation and individuals from the same family), the absence of the clinical presentation of a patient with the mutation or the FMF clinical presentation of patients without the mutation, drug response or drug resistance. Histone modification, DNA methylation, and microRNA (miRNA) represent the epigenetic mechanisms that are accused the most of the correlation between FMF and epigenetics.

Chapter 13 - Genetic and environmental interactions have a significant role in the evolution, development, and functioning of the central nervous system. Epigenetic mechanisms including, DNA methylation, non-coding RNAs, and histone modifications are important to clarify how genetic and environmental interactions alter neurobiology and behavior. Alterations in epigenetic mechanisms induce remarkable changes in cognitive and behavioral phenotypes. It is becoming apparent that the epigenetic machinery is involved in the pathogenesis of neurobehavioral and neurobiological disorders. A better understanding of the altered epigenetics mechanism underlying these disorders is important to inventing new therapies for neurobiological disorders.

Chapter 14 - The physiological and behavioral development of an individual is a dynamic event that includes the interaction between genes and the environment. The brain and nerve development period of the baby in the womb is between the 4th and 10th weeks of pregnancy. Although the early stages of the brain development are affected by genetic factors, the decision of where and how genes are utilized is determined by environmental factors. This situation solely depends on the pregnancy period of the mother, meaning it is tightly related to the level of food intake and stress exposure which affects the early phases of brain architecture. Environmental factors and experience leave traces on certain parts of genes, and these epigenetic changes can change the activity or expression of the genes.

Chapter 15 - Epilepsy is a common neurological disorder manifested by recurrent seizures, and its underlying causes could be significantly associated with genetic. Epigenetic hereditary changes in gene expression that are not caused by changes in the DNA sequence.

DNA methylation, post-transcriptional changes of histones, and the action of non-coding RNA molecules are currently the most well-researched epigenetic mechanisms. These processes control gene expression, and interruption of these molecular pathways can lead to disease development. Investigating the epigenetic processes involved in epilepsy is a potential avenue of search that will lead to a better understanding of the etiology and treatments of epilepsy.

Chapter 16 - Epigenetic mechanisms occurring in DNA methylation, histone modification, and RNA-based mechanisms may generate heritable phenotypic changes without a change in DNA sequence. The disruption of gene expression patterns governed by epigenetics may lead to various diseases such as cardiovascular diseases, autoimmune diseases, and cancers. Genetic studies guide for preventive measures, appropriate treatment selection, drug interactions, drug efficacy, and patient compliance. Cardiovascular diseases (CVDs) are the most common cause of mortality worldwide. The increase in risk factors is predictive of the fact that the frequency of CVDs will further increase. Thus, detailed genetic data screening will become important in the diagnosis and prevention of CVD in the near future.

Chapter 17 - Obesity is an important health problem that has reached the level of pandemic today, has important effects on high prevalence diseases such as cardiovascular, diabetes, and cancer, and even leads to deaths. Obesity occurs from multifactorial effects, primarily genetic and environmental factors. Studies have identified a large number of genes that cause obesity, but the rapid increase in obesity in a short time cannot be explained only by genetic factors alone. Epigenetic changes that occur through environmental factors such as nutrition and physical activity also have an important role in the current increase in the incidence of obesity. Gene-environment interaction causes different phenotypic variations to occur in organisms. These different phenotypes, which occur without any changes in the DNA sequence, are explained by epigenetics. Therefore, the view that interindividual differences in susceptibility to obesity are due to epigenetic factors has recently gained considerable importance. Many studies have shown that epigenetic mechanisms such as DNA methylation, histone modifications, and non-coding RNAs are closely related to obesity.

The aim of this review is to present the relationship between epigenetics and obesity and the effects of epigenetic mechanisms on obesity.

Chapter 18 - Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia resulting from a partial or absolute decrease in insulin secretion, with varying degrees of peripheral resistance to the impact of insulin. The prevalence of type 2 diabetes mellitus increases exponentially every day, along with an increase in obesity and bad eating habits.

Prolonged hyperglycemia causes microvascular (retinopathy, nephropathy, neuropathy, and cardiovascular diseases) and macrovascular (cardiovascular diseases) complications and major morbidity and mortality in patients. The causes of disease in DM, which is such an important public health problem, are largely based on the interaction of genetic and environmental factors. Nevertheless, the etiopathogenesis of the disease has not been completely elucidated. Since epigenetics can provide a molecular link between genetic, environmental factors and diabetes and can be transmitted over generations, it is promising in elucidating the etiopathogenesis of diabetes and identifying new treatment options for its etiology. Even if hyperglycemia is corrected, epigenetic mechanisms are included in metabolic memory, and in the long term, complications secondary to DM persist. Furthermore, studies on epigenetics and metabolic memory demonstrate that diabetes and its complications can be prevented by changing environmental factors such as calorie control.

Chapter 19 - Osteoporosis is a disease that is more common in old age nowadays, significantly reduces bone quality and causes severe morbidity with fractures and in the development of which many factors have a role. It has extensive etiopathogenesis, and it has been investigated for many years. Along with the increasing knowledge of genes, the evolving technological developments open new windows, and the adventure that starts with this small window turns into a large world. New approaches, new possible drugs, and new etiopathogenesis for osteoporosis are present in this new world of epigenetic science. Determining this cycle of bone tissue, which starts with gene sequences and turns into the most robust tissue of people, through genetic, epigenetic, and molecular mechanisms has still been discussed in studies. The fact that epigenetic factors are modifiable, editable, and recyclable causes this world to be much more interesting.

Chapter 20 - Osteoarthritis (OA) is a common degenerative joint disease worldwide. Considering the large number of people it affects, it creates serious limitations on patients and creates a serious burden on the healthcare system. Obesity, gender, genetic predisposition, trauma, and some systemic diseases can be listed as the most common causes of OA. However, in recent years, with the research on the concept of epigenetics and its relationship with various diseases, there have been serious changes in the view of the etiology of OA. The imbalance of anabolic and catabolic events that have a role in cartilage physiology is the most important element in the pathogenesis of OA. Epigenetic factors are also blamed for the disruption of this balance. DNA methylation, Histone modification, and non-coding RNAs are the most widely accepted epigenetic mechanisms in OA. Many published studies have revealed the relationship of each of these mechanisms with the pathogenesis of OA. New doors will likely be opened in the prevention and treatment of OA with the studies that have been conducted and will be conducted in this regard.

Chapter 21 - Degenerative disc disease (DDD) is one of the most common spine-related diseases. Its etiology is not exactly known, but recent studies have elucidated that this disease is a complex and multifactorial disease resulting from the interaction of genetic and environmental factors. In general terms, these studies focus on changes in genes encoding the structural components of the intervertebral disc.

Chapter 22 - Spinal deformity in neuromuscular scoliosis is the result of various neurological or muscular pathologies. Loss of muscle tone and weakness, which are often the main symptoms. Sometimes in addition to the clinical picture of muscle retraction, sensitivity disorder, accompanied by mental retardation, and digestive, heart, or respiratory problems. DNA modifications are thought to have a role in epigenetic deterioration and disease formation

in congenital scoliosis (CS) that occurs with congenital deformity of the vertebrae. When DNA is extracted from the hemivertebra and spinal process during surgical correction operations, aberrant DNA methylation has been found to be associated with the development of hemivertebrae and congenital scoliosis. In studies conducted in recent years, TGFR1, EGFR, IGF1R, and GHR are in spinal growth stages; Chondrogenic alteration of the SOX9, PAX1, PAX9, and IHH genes and during differentiation; In the cartilage matrix structure of the ACAN, LUM, VCAN, COL1A1, COL2A1, and HAPLN1 genes; It has been shown that the SLC26A2, CHST1, and CHST3 genes are involved in the formation of the extracellular matrix in vivo. In a different study, it has been observed that there is a predisposition to spinal pathologies in the CALM1 gene polymorphism, which is involved in muscle contraction and bone synthesis. Statistical differences were found in the polymorphic distribution of the rs2234693 region of the ER1 gene in patients with double curve, Cobb angle $\geq 40°$, and thoracic curve. In addition, it was determined that there was a difference in the polymorphic distribution of the rs12885713 region in the CALM1 gene in patients with double curves and in the polymorphic distribution of the same gene in patients with thoracic curves.

Chapter 23 - The endometrium is a functional tissue under the control of ovarian steroid hormones. The endometrium shows cyclic changes including biochemical and morphological. These cyclic changes occur with the growth, proliferation, and death of endometrial stromal and glandular cells. Cyclic changes of endometrial tissue are regulated by levels and nuclear receptors of ovarian steroid hormones. During the normal menstrual cycle, epigenetic mechanisms, especially DNA methylation, have important roles in gene expression regulation and influence functional changes.

Epigenetic mechanisms affect gene expression with transcriptional regulation and control endometrial cell proliferation, angiogenesis, desidualization, and embryo implantation. DNA methylation is an essential modification for the normal cell cycle. Abnormal DNA methylation can be associated with abortus and other endometrial pathologies. Recent studies focus also on the combination of epigenetic changes with genetic changes in the development of endometrial pathologies such as endometriosis and endometrial cancer.

The most known epigenetic mechanisms are DNA methylation, post-translational modifications, and non-coding RNAs. Understanding the epigenetic mechanisms and identifying the methylation profile can help create personalized treatment for each patient.

Chapter 24 - Polycystic ovary syndrome (PCOS) is a common disease in women of reproductive age. Recently, research has shown that the interaction of genomic and environmental factors can modify the clinical condition through epigenetic modifications in the pathophysiology of this disease.

Chapter 25 - Preeclampsia (PE) is a disease that affects pregnant women at rates ranging from 2% to 10%, causing perinatal and maternal morbidity as well as maternal and perinatal mortality. PE is typically characterized by the onset of new hypertension with proteinuria at and after the 20th week of pregnancy; It may be associated with various causes such as inflammatory cytokines and poor trophoblast invasion. Several epigenetic changes can be associated with PE, such as histone modifications, environmental factors, microRNAs (miRNAs), and DNA methylation. In this perspective, the most important epigenetic factor associated with PE; is abnormal DNA methylation during placentation. In addition, acetylation-like histone modifications and low regulation of miRNAs or the effect of ovarian regulation and long non-coding RNAs in various signaling pathways may be involved in the etiology of PE and its epigenetics. PE is associated with low birth weight (LBW) and intrauterine growth

restriction (IUGR). When children born as a result of pregnancies with a diagnosis of PE were followed, it was determined that these children were at risk of cardiovascular disease. The cause of PE, which carries serious risks for the mother and the child, is unfortunately not known precisely, and there is no effective treatment to cope with the acute and chronic consequences of the disease. Although it is an obvious fact that the placenta is essential for fetal development, it is known that epigenetic factors have a role in placental processes such as spiral artery remodeling and trophoblast invasion.

Epigenetic mechanisms leading to changes in placental gene expression in PE mediate processes that contribute to the development of placental malfunction, impaired fetal growth, and IUGR, a critical mission in the onset of PE. Lightening the epigenetic processes that conduce to routine placental growth and the triggering incidents in the etiopathogenesis of PE may contribute to the clear etiology of the disease and may lead to the discovery of new treatments.

This review aims to shed light on the epigenetic relationship with PE in light of these perspectives.

Chapter 26 - Pain and its chronicity in the following process are the main subjects of numerous studies nowadays with the adverse impacts of pain on quality of life, loss of workforce it causes, and still not completely explained pathophysiological mechanisms. The difficulties in treating symptoms that accompany pain and individual differences in response to applied treatment methods have accelerated genetic and epigenetic research. Epigenetics, which we have heard frequently in recent years, is the formation of different genetic variations under the influence of various factors and is now accepted as a significant mechanism in the unknown etiology of numerous diseases. Different epigenetic modifications have been described in the formation, typing, and treatment of pain, and new information is obtained with ongoing studies. The difficulties experienced in treatment due to changes at the molecular level caused by the chronic pain process and the activation of sensitization mechanisms have brought about epigenetic mechanisms both in etiopathogenesis studies and in the development of novel treatment approaches.

Chapter 27 - Connective tissue diseases (CTDs) are defined as chronic inflammatory diseases that can affect all organs and systems, primarily the joints. The underlying genetic background is very important for the development of these diseases, and environmental factors also contribute to this process. The clinical spectrum can vary considerably from patient to patient in individuals with CTDs, and even responses varying from person to person are observed in treatment responses. The emergence of such different phenotypic characteristics in this group of patients with relatively similar genotypic characteristics has caused the need to investigate them in different models that may contribute to the underlying pathogenetic process.

It has been recently revealed that changes in DNA cannot be explained only by genetics, and the role of epigenetic mechanisms is also quite large. In epigenetics, the DNA sequence does not change. However, since the promoter region of the gene changes, differences occur in gene transcription, in other words, in the end product of the gene. There is no clear factor that induces this change, and epigenetic modifications such as histone modification, DNA methylation, and microRNA (miRNA) that probably occur under the influence of environmental factors have led to the emergence of a new field to explain different phenotypic characteristics in patients.

In this chapter, the characteristics of epigenetic mechanisms in general and the reflections of these modifications on common CTDs, both at the cellular level and clinically, will be discussed.

Chapter 28 - Rheumatoid arthritis (RA) is chronic, autoimmune, erosive inflammatory rheumatism that impacts especially the female population between the ages of 20-50. Its prevalence varies between 0.5-1% worldwide. The etiology of rheumatoid arthritis is multifactorial. The risk factors include genetic, epigenetic, allergic, hormonal, neuroendocrine, reproduction-related factors, comorbid conditions, smoking, air pollution, socioeconomic status, lifestyle, diet, inhalation of dusts such as silica-asbestos-glass powder-textile dyes, microbiota, and infectious agents. After the diagnosis is established with physical examination, laboratory, and imaging methods, an appropriate treatment regimen must be quickly arranged to prevent disability. Pharmacological and non-pharmacological treatments are used to treat RA. There are patient education, psychosocial support, orthoses, exercise program, physical therapy agents, nutritional support and diet, prevention of osteoporosis and other comorbidities among non-pharmacological treatments. Pharmacologically, conventional DMARDs (disease-modifying anti-rheumatic drugs) are initially used (methotrexate, leflunomide, sulfasalazine, and hydroxychloroquine). In cases unresponsive to conventional treatments, TNF-a inhibitors (adalimumab, golimumab, infliximab, certolizumab pegol, and etanercept) or non-TNF biologics (rituximab, tocilizumab, anakinra, abatacept, baricitinib, tofacitinib) are utilized. Epigenetics refers to changes in gene expression without any change in DNA sequence. Changes occur in the chromosome, various phenotypes emerge, and the resulting changes can be passed on to the next generations by mitosis or meiosis. Environmental stimuli such as drugs, smoke, and cigarettes can induce epigenetic modifications. Hence, the link between the genome and the environment is ensured by epigenetic mechanisms. Epigenetic mechanisms include DNA methylation, post-translational histone modifications (histone methylation, histone acetylation, histone phosphorylation, histone ubiquitination, histone citrullination, and histone ribosylation), and the expression of non-coding RNAs (ncRNAs) such as microRNA (miRNA). DNA hypomethylation, histone acetylation, histone methylation, micro-RNAs, and long non-coding RNAs are the most accused in the relationship between RA and epigenetics. Nowadays, it is thought that these epigenetic mechanisms detected in RA will be employed as a prognostic factor and will have a role in diagnosis and treatment follow-up and in determining the susceptibility to RA.

Chapter 29 - Inflammatory bowel diseases (IBDs) represent a group of diseases, which have a chronic course, cause a decrease in a person’s quality of life, and whose etiopathogenesis is still not fully explained. The risk factors include the environment, smoking, diet, genetic factors, a person’s immunological status, and the intestinal microbiota. Epigenetic mechanisms have recently begun to take their place in the occurrence mechanism of diseases. The mechanisms described under three main headings, DNA methylation, histone modification, and miRNA, have taken a significant place in the diagnosis and follow-up of many diseases and the treatment protocol. In light of all this information, the association between epigenetics and inflammatory bowel diseases and its place in the future will gain even more importance with new studies.

Chapter 30 - In spite of the decreasing incidence rates of gastric cancer in many industrialized countries, it is still an important cause of death from cancer around the world. Gastric cancer takes the fourth place among causes of death due to cancer worldwide. Gastric cancer incidence in Turkey was detected at 14.2/100,000 among males and 3.4/100,000 among

females. Risk factors for gastric cancer involve numerous unmodifiable variables, e.g., sex, age, and race/ethnicity. The remaining risk factors, including smoking, infection with Helicobacter pylori (Hp) bacteria, and diets with high nitrate and nitrite contents, are among the controllable causes. Hp represents a Gram-negative microaerophilic bacterium infecting almost half of the population in the world and is considered the main etiologic agent of gastric cancer. Dietary habits and different environmental factors, including Hp infection, and irregularities in genetic and epigenetic mechanisms are involved in forming gastric cancer. While genetic changes lead to loss of function or protein expression in metabolic pathways in different ways, epigenetic alterations cause differentiation in the expressions of tumor suppressor genes and oncogenes.

In gastric cancer surgery, the only curative method in localized gastric cancers is the complete resection of the regional lymph nodes and tumors. Functional surgical methods can be preferred in suitable patients. Pancreas and spleen-preserving D2 dissection is preferred as a standard approach in serosa-positive and/or lymph node-positive cases.

Chapter 5

The Concepts of Epigenomics and Cell Epigenomes

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Abstract

Genetics cannot solely explain genetic variations in humans and disease developments. We see varying differences in phenotypes and disease susceptibility in organisms that have the same genetic make-up, e.g., monozygotic twins and cloned animals. The information carried by the genomic sequence is the blueprint, but the final product requires environmental determinants. Here comes the concept of epigenetics, as it is the framework where biochemical interactions between the genome and the environment blend. We can describe epigenetics as mechanisms that are beyond genetics, as such mechanisms alter the result of the genomic blueprint without altering the information itself, i.e., the sequence. Both epigenetics and epigenomics are trending research fields to better evaluate the genotype and the phenotype. The methylation of genetic material is a well-studied and well-known epigenetic marker. The epigenome, as a term, describes the inheritable changes in both the DNA and histone molecular structures, where the methylation and acetylation mechanisms are studied extensively. As the building blocks of the chromatin structure, nucleosomes depend on epigenetic changes, which lead to becoming either tight or loose, based on the particular mechanisms. Chromatin structure changes directly affect the gene expression, e.g., particular gene expression becomes silenced if the gene position has DNA hypermethylation and histone hypoacetylation that leads to the condensed form of the chromatin. Inversely, if the said changes were removed, genes in that position would express themselves again. Therefore, epigenetics provides a vigorous and remarkably malleable means for gene expression regulations. Epigenomics includes both the epigenetic mechanisms on the DNA and histones and complicated interactions between the genotype and the phenotype. There are well-known epigenomic changes in DNA, RNA, and protein levels. DNA base changes in somatic cells and chromosome positioning are among the aspects determining the epigenomic scene. Alternative splicing mechanism, RNA processing (editing, capping, Poly-A tailing, etc.), RNA methylation, and regulations conferred by the non-coding RNAs (ncRNAs) are RNA level epigenomic changes. Epigenomic changes at the protein level include various mechanisms of the post-translational modification. Epigenomics research includes total hypomethylation profile of the genome, identification of hypermethylated genes, microRNA-driven gene silencing

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with DNA methylation, epigenome projects, and DNA-based clinical therapies for different biomedical conditions. This chapter will detail fundamental concepts and basic approaches to both epigenomics and epigenomes.

Keywords: DNA modifications, epigenetics, epigenome, epigenomics, epigenotype, RNA regulations

Abbreviations

HPLC:	High-performance Liquid Chromatography
HPCE:	High-performance Capillary Electrophoresis
LUMA:	Luminometric Methylation Assay
MAPKs:	Mitogen-activated protein kinases
PI3K:	Phosphatidylinositide- 3-kinase
AKT:	Protein kinase B isozymes
PLK1:	Polo-like kinase 1
PARP:	Poly (ADP-ribose) polymerase
HATs:	P300/CBP CREB binding protein coactivators.

Introduction

A terminology overview would be beneficial for a better understanding of the concept of epigenomics by providing the conceptual similarities and differences. Epigenetics as a whole encompasses all the mechanisms that result in inheritable changes without sequence alterations. These mechanisms are responsible for the creation of different cell lines at the stage of developmental differentiation, of which X-chromosome inactivation (Barr body) and genomic imprinting in mammalian organisms are particularly pronounced in disease developments. The epigenetic code denotes the scheme of genomic methylation and histone modifications related with the expression regulation. The epigenetic code combines with the genetic code to provide an effective modulation of gene expression patterns. Epigenetic inheritance describes the heritable patterns of the somatic cell lines and epigenetic information transmitted through the germ-line cells. Epigenetic marks indicate region-specific DNA and histone modifications that are inheritable and able to regulate the expression of localized genes (Meissner and Walter, 2015). Epigenetic reprogramming describes the returning of epigenetic marks into a neutral state, which would then lead to transitioning into another cell line or developmental stage, e.g., reverting into the ground state of primordial germ-line cells. Such reprogramming course is also seen in the nuclear transfer of somatic cells. Global epigenetic status represents the epigenome, which is different in each developing cell line during the embryonic development phase. Epigenome maps are virtual representations of the chromosomes that reveal DNA methylation and other modifications seen in the chromatin structure. The Epigenotype describes the entire epigenetic marks found in a specific cell line or during a specific developmental phase. Epimutations indicate deviations from normally expected epigenetic marks in a given region that would alter gene expression (Meissner and Walter, 2015).

Epigenetics research currently focuses on the following methodologies for revealing changes in gene expression profiles or chromatin restructuring; DNA methylation patterns, histone modifications, nuclear positioning, ncRNA-driven regulations, and microRNA regulations (Chen and Rajewsky, 2007; Fraser and Bickmore, 2007; Martin-Subero and Siebert, 2009; Esteller, 2009; Qui, 2006). On the other hand, if the research efforts are for revealing epigenetic marks in a specific sequence, then epigenomics would indicate the separation of possible modifications from the rest of the genome (Callinan and Feinberg, 2006; Fazzari and Greally, 2004; Pennisi, 2005; Martin-Subero and Siebert, 2009). Various research point to the susceptibility of the epigenome to environmental determinants that would make it more prone to change than the robust genome, such as nutritional factors (e.g., royal jelly, folic acid, etc.), chemical pollutants, external stimulation of neurons, and psychiatric conditions such as stress (Martin-Subero and Siebert, 2009; Esteller, 2009). Initial studies on cancer revealed that DNA methylation changes gradually throughout the lifespan; however, recent studies suggest different results by showing the alterability of the DNA methylation during the first week following the birthing in mice and sustained DNA methylation patterns in brain tissue associated with aberrant behaviors (Sato et al., 2003; Callinan and Feinberg, 2006). Research revealed that epigenetic changes formed by fetal malnutrition in rats lead to disease developments in the adult stage (Fazzari and Greally, 2004; Qui, 2006; Weaver et al., 2004). Epigenomic changes are present in humans, as revealed by comparative epigenomics conducted in monozygotic twins (Pennisi, 2005; Esteller, 2007; Martin-Subero and Siebert, 2009; Suzuki et al., 2013). The reversible nature of epigenetic mechanisms makes them a verifiable alternative to genomic imprinting, where the maternal epigenetic marks in a given imprinted gene are shown to return to paternal epigenetic marks in the progeny (Weaver et al., 2009; Fraga and Esteller, 2002; Laird, 2003; VanSteensel and Henikoff, 2003; Lillycrop et al., 2005; 2008; Fraga et al., 2005). This reversible nature of epigenetic changes is also apparent in genes in neural tissue that can be induced by medications for mental conditions, which reverts epigenetic marks into neutral states (Kubato et al., 2014, Payne, 2014; Tsankova et al. 2006; Jessberger et al., 2007; Dong et al., 2008; 2010; Wang et al., 2011). Possible restoration of methylation patterns in autism-related genomic positions by folic acid administration in autistic children is postulated (James et al., 2004; Moretti et al., 2005). Recent advances increasingly point to the possibility of genomic site-specific epigenetic drugs, which is seen in contemporary compounds such as pyrrole-imidazole polyamide (Kubato et al., 2014, Payne, 2014; Ohtsuki et al., 2009; Matsuda et al., 2011). The rise of the epigenetics stoutly contests the agreed-upon idea that the acquired nature cannot be inherited by the progeny, which is further supported by the epigenetic changes formed due to the psychiatric conditions passed into the progeny as far as the third generation via spermatogenesis (Hackett et al., 2013; Franklin et al., 2010; Sato et al., 2003). While still a postulation for humans, it is possible to restore deviations in epigenetic marks by external interventions by drugs, nutritional supplements, and environmental conditioning (Kubato et al., 2014, Payne, 2014).

It is now established that nutritional factors and environmental determinants have effects on epigenetic changes, which are most pronounced in smoking, where alterations induced by smoking are reported to have potential for malignancies that can be alleviated by dietary and lifestyle improvements. Advances in molecular methodologies enable the discovery of anticancer traits of the wide array of natural compounds (Spencer et al., 2004; Orlikova and Diederich, 2012; Stepanic et al., 2014), which mostly revealed that such traits function through chromatin remodeling to protect the integrity of the cellular epigenome (Reuter et al., 2011).

Among these compounds, polyphenols induce chromatin remodeling by affecting the gene expression of chromatin remodeling enzymes (Suzuki and Miyata, 2011). Polyphenols are potently modifying cellular processes related to survival and death by targeting numerous kinases, which are listed as MAPKs, PI3K, AKT, Aurora B, PLK1, PARP, and some epigenome modifying enzymes like HATs. Along with their diverse effects in various bio-signaling pathways, it can be suggested that polyphenols are significant representatives of what are called polypharmacological substances (Stepanic et al., 2014).

Novel findings and significant signs of progress in epigenetics research are directly related to advances in molecular methodologies, and recent years provided the characterization efforts for the epigenome by different methodologies (Fazzari and Greally, 2004; Callinan and Feinberg, 2006; Esteller, 2007; Fraga and Esteller, 2002; Laird, 2003; Martin-Subero and Siebert, 2009; Esteller, 2009). With the utilization of microarrays in epigenetics, an increasing number of studies are directed towards epigenome characterization both in normal and aberrant conditions (Martin-Subero and Siebert, 2009). In the current study, the DNA methylation data from The Cancer Genome Atlas (TCGA) Project (Zhang and Zhang, 2022), especially the 450K array data from different cancer types (Sandoval et al., 2011), were analyzed and highly informative CpG sites located in more than 150 centrosomal genes were presented. This study is one of those that helps scientists understand the epigenetic- epigenomics mechanism underlying cancers for utilizing these epigenetic modifications as novel cancer biomarkers (Zhang and Zhang, 2022).

Epigenome Characterization

Genome regulation is being better understood by the researchers with the successful utilization of epigenomic methodology in whole genomes, which invariably leads to the discovery of disease-related patterns by revealing normal patterns (Esteller, 2009; Martin-Subero and Siebert, 2009). Recently, DNA methylation patterns revealed in different healthy tissues have been investigated by sequence analysis, conservation, and effects on gene expression for correlation. Human Epigenome Project revealed two-way modes of distribution for DNA methylation in more than 2500 genes that were sequenced by bisulfite sequencing in amplicons from example human tissues (Martin-Subero and Siebert, 2009). Overall, they surfaced that 5'UTR regions with a high amount of CpG islands are unmethylated, whereas 5'UTR regions with a lower amount of CpG islands are mostly methylated, which is supported by other research (Weber et al., 2007; Smiraglia et al., 2001; Martin-Subero and Siebert, 2009). Microarray analysis on peripheral human blood revealed that approximately 4% of CpG islands located on promoters are methylated (Shen et al., 2007; Martin-Subero and Siebert, 2009). Of the limited set of genes investigated, DNA methylation patterns did not provide significant differences between gender and age groups. A striking study, which utilized chromatin immunoprecipitation on chips (ChIP-on-Chip), comparatively investigated DNA methylation patterns in promoter regions that have low, intermediate, and high density of CpG islands by correlating with the gene expression, which revealed high DNA methylation in promoter regions with a lower density of CpG islands and unmethylation in promoter regions that have a high amount of CpG islands (Weber et al., 2007; Martin-Subero and Siebert, 2009). In terms of RNA Polymerase recruitment by the promoters, researchers revealed that a high density of CpG islands in promoters (66% of the studied promoter regions) enhanced the recruitment

activity, whereas a low density of CpG islands in promoters (11% of the studied promoter regions) have redundant recruitment activity. By this account, the relationship related to methylation and expression compared between promoters with a higher and an intermediate density of CpG islands revealed a negative correlation. Of particular note is the finding of no correlation between methylation and expression in promoters having lower CpG island densities. Summarily, it is evident that methylation is in close association with the expression suppression in promoters having no significant amount of CpG island presence (Blelloch et al., 2007). Investigation of stem and differentiated cells by using CpG-specific bead array analysis revealed significant data in methylation models between embryonic stem cells and differentiated cells in 23 genes, which provided 25 discernible sets of CpG islands (Bibikova et al., 2006; Martin-Subero and Siebert, 2009).

The ChIP-on-Chip methodology also enables the genome-wide detection and characterization of histone protein modifications (Bernstein et al., 2004; Huebert et al., 2006). Various research utilizing the ChIP-on-Chip method revealed fine aspects of the so-called histone code by discovering the patterns and combinations of histone modifications associated with a given transcriptional phase or a structural stage of chromatins (Jenuwein and Allis, 2001). Certain research efforts also revealed the structural characterization of chromatin in cell lines (Bernstein et al., 2006; Bracken et al., 2006; Lee et al., 2006; Martin-Subero and Siebert, 2009). The highly conserved nature of epigenetic marks of histone proteins, even in modest amounts in orthologous genomic regions, spanning a diverse array of species strongly suggests their universality in transcriptional regulation (Bernstein et al., 2005). From this on, comparative epigenomics would be a feasible approach to reveal regulatory factors that are not included in the conserved genomic regions. The ability of the ChIP-on-Chip method to expose histone modification differences in genomic regions, especially the transcriptional start sites (TSSs), has driven the attention of researchers to the embryonic stem cells, since these cells can be changed into any of the differentiated tissues of the organism, thus forming the cell line identity (Buszczak and Spradling, 2006). As of late, Polycomb Group (PcG) proteins are in attention due to their maintenance roles in stem cell pluripotency by their ability to suppress genes responsible for cell-line differentiation (Martin-Subero and Siebert, 2009).

Derangements of the epigenetic mechanisms, mostly more than one aberration, are best attributed to cancerous cells, which are also markers for their characterization and include DNA methylation, histone modifications, and nucleosome restructuring (Esteller, 2007; Jones and Baylin, 2007; Laird, 2005). Until now, DNA methylation, especially on tumor suppressor genes, is among the most extensively studied mechanism in cancer types (Esteller, 2007), which revealed 50+ genes silenced in cancerous differentiation and differences in methylation patterns discernible in distinct cancer types. Advances in epigenomics gradually increase the precision of accessibility of cancer cell epigenomes, which are progressing to the next generation of biomarkers for cancerous cell lines. Additionally, researchers are now able to better evaluate carcinogenesis by comparing DNA methylation patterns at the genome, development, and transcriptional levels. Having the most diverse set of cell types, the hemopoietic system cancers include many distinct leukemias and lymphomas classified by morphology, immunohistochemistry, and genetics. Recently, researchers have focused on heterogeneous diseases like those seen in the hemopoietic system cancers to utilize microarray methodology to profile methylation patterns and to reveal possible epigenetic marks having diagnostic precision (Martin-Subero and Siebert, 2009).

The Profiling Whole Genome Methylation Models

Two general approaches are available for genome-wide profiling of methylation, (1) whole-genome scanning of global DNA methylation patterns; and (2) genome-wide sequence-specific methylation profiling by microarray analysis. The former can be done by conducting different instrumentation such as HPLC, HPCE, or LUMA, but they are all limited to DNA methylation (Laird, 2003; Martin-Subero and Siebert, 2009)]. The latter is a more powerful yet biased approach for profiling with the promise of discovering new marks but is biased due to its dependence on using methylation-specific enzymes to bind to CpG islands, which may not always include recognition sequences for enzyme binding. Consequently, it is limited in scope as to which CpG islands present in the possibly investigated genome. Recently, a quadruple-enzyme approach to effectively investigate 41% of total CpG islands in a given genome was published, which also recommends including unmethylated DNA fragments to better discern methylation differences between samples. Antibody elucidation of methylcytosine is a less biased alternative for identifying methylated DNA sequences but it is limited in its ability to be sequence-specific. Certain modification efforts are available for this approach to alleviate limitations, which include sodium bisulfite treatment to induce transition of unmethylated cytosines to uracils and leave methylcytosine intact. Consequently, there are already existing analysis approaches for SNPs where differential binding of oligonucleotides into methylated (C) and unmethylated (U/T) alleles can be utilized (Bibikova et al., 2006). Again, there are numerous microarray approaches for methylation profiling differing in their resolution, capacity, and sequence specificity. In addition to the aforementioned approaches, there are also epigenomic-based oligonucleotide assays that include promoter and CpG island assays, which are yielding high-density results. Recently, a combination of both genetic and epigenetic profiles is shown to be revealed through the use of SNP-ChIP coupled with methylation-specific endonucleases (Yuan et al., 2006). Lastly, high resolution of whole-genome coverage is made possible with the advent of oligonucleotide tiling arrays, which span several millions of oligonucleotides. As it can be seen, despite the development of newer microarray approaches, there is no consensus and universal integrity in the validation of obtained data from conducting any of the methods that are all different in their technical aspects, capabilities, precisions, and analysis complexity, which naturally suggest the need of developing a universal, flexible, and undisputable validation framework encompassing all approaches for epigenomic research (Martin-Subero and Siebert, 2009).

Analysis of the Genome-wide Histone Modification Patterns

ChIP is currently the standard methodology for revealing histone modifications in a given genomic region, which utilized specific antibodies for different modifications. This method summarily includes DNA cross-linking by formaldehyde treatment, chromatin trimming, and incubation with modification-specific antibodies. Once the modified histone-antibody complex is formed, chromatins are isolated with a suitable method, e.g., Protein A or Protein G coated agarose beads, and cross-links are removed to separate the DNA, and finally, DNA is extracted. Extracted DNA can be utilized in microarray analysis for a specific histone modification by sequence enrichment (Bernstein et al., 2004; Hubert et al., 2006). The main drawbacks of the ChIP are the requirement for high-specificity antibodies, high sample integrity, and excessive

sample amounts. Recent developments enabled the ChIP to be used in smaller amounts of sample cells for histone modification profiling (Martin-Subero and Siebert, 2009).

A New Frontier in Epigenomics: High-Throughput Sequencing

The next-generation sequencing (NGS) technology enables newer frontiers in epigenomics research in addition to their substantial roles in genomics. In summary, the next-generation sequencing technology enables researchers to sequence as much as 2 Gbps sequence in a single run with different techniques deployed by the sequencing platform. Initial applications of NGS in epigenomics revealed 20 distinct profiles of histone methylation marks (Mikkelsen et al., 2007). The NGS is utilized in a recent development called the 5C method (Chromosome Conformation Capture Carbon Copy) to discover DNA to regulatory factor interactions (Simonis et al., 2007). Together with the NGS, a 3D investigation of the nuclear environment revealed intricate communication networks between different parts of the chromatin landscape (Fraser and Bickmore, 2007). A particular problem arises in sodium bisulfite treatment for the definition of methylation, where normally 4 unmethylated base pairs reduce to 3 unmethylated base pairs, which results in uncertainty in sequence origin (Martin-Subero and Siebert, 2009).

Epigenomic Marks

DNA modifications, e.g., methylation and hydroxymethylation, histone protein modifications of tails, e.g., acetylation, phosphorylation, methylation, sumoylation, ubiquitination, and ncRNA-driven regulations constitute the epigenomic marks in a genome. However, reproducibility and conservation traits currently limit these conceptual marks to methylation and histone modifications (Berger et al., 2009; Bell, 2012; Tollefsboll, 2012). Reproducibility and conservation traits are essential in the preservation of cell-line identities and cell epigenomes, especially in somatic cells having the same genomes (Richards, 2006). The methylation is the attachment of a methyl group into a 5' Carbon atom of the cytosine base to produce methylcytosine, which results in the formation of CpG islands since cytosines are followed by guanines in the genome. DNA methylation is the robust epigenetic mark. Gene expression suppression by methylation is required to be removed from specific sequences, sometimes even at a genome-wide level, depending on the developmental stage of the organism. The known stages are the post-fertilization period and the germ-line cell differentiation in both genders. Especially in the post-fertilization period, global demethylation of the entire genome is present in the embryo, which is followed by the reinstallation of global DNA methylation just before the embryonic implantation, making the embryo particularly susceptible to all kinds of environmental agitation (Faulk and Dolinoy, 2011; Bell, 2012; Tollefsbol, 2012).

Epigenome-Wide Association Studies (EWAS)

Advanced microarray methodologies with their comprehensive coverages enable the discerning of the disease-associated epigenetic alterations in humans (Rakyan et al., 2011; Bibikova,

2016). Target-specific and site-specific methods focusing on candidate genes are largely replaced by the EWAS to solicit the epigenetic effect between health and disease states in a highly systematic way. A standardized experimental framework for EWAS has yet to be achieved and the fundamental principles of whole genome association analysis (GWAS) are mostly incompatible with the EWAS (Michels et al., 2013; Bibikova, 2016). EWAS typically focuses on the epigenetic state of a great number of loci of a specific tissue to associate them with a trait. Mostly, the epigenetic state in question is DNA methylation and the trait in question is diseases or environmental conditions. Both the NGS and the microarray methodologies find themselves places in EWAS, each with its drawbacks (Plongthongkum et al., 2014; Bock et al., 2010; Harris et al., 2010; Bibikova, 2016).

Future Perspectives for Epigenomics

Currently, epigenomics is a highly thrilling subject promising many breakthroughs in the field of medical biology, so much so that researchers in the field are calling for international coordination for the human epigenome project (Esteller, 2006; Garber, 2006; Jones and Martienssen, 2005; Rauscher, 2005; Bibikova, 2016; Zhang and Zhang, 2022). Similar to the human genome project, the human epigenome project would be a real scientific challenge, which may be more so compared to the human genome project due to the very nature of epigenetics. Indeed, its dynamic adaptability, highly specified mechanisms, and the inter-variability between tissues and organisms make such an endeavor one of the truly formidable scientific achievements (Martin-Subero and Siebert, 2009; Zhang and Zhang, 2022). It is said that there are initiatives aiming to characterize the epigenome by using the power of the NGS to reveal the methylation profiles of millions of CpGs. Yet, heterogeneity of the epigenetics across the individuals still makes the microarray methodologies a necessity in epigenomics research (Zhang and Zhang, 2022). Otherwise, the requirement of multiple samples to overcome the heterogeneity would make the characterization efforts unaffordable at best. On the other hand, it is possible to reveal tissue-specific consensus of cell epigenomes per sample basis by combining the microarray approaches and the NGS. Still, the precise revelation of a cellular epigenome requires an indisputable method to detect the cell-specific DNA methylation patterns of CpG islands per cell basis. Again, the need for a unified cellular analysis system covering genomics, transcriptomics, proteomics, and epigenomics is increasingly pronounced to differentiate all the possible states of a given cell, which would substantially change the way how any alterations are associated with what kind of physiological state (VanSteensel, 2005). While the majority of the studies focused on cancer, it should be stressed that epigenetics is associated with all aspects of life due to its universality in the integration of external and internal stimuli into the genome proper (Jirtle and Skinner, 2007). Advances in the field will certainly progress to genome-wide levels and reveal genomic regions that are specifically prone to epigenetic modifications by the external stimuli, which will be pivotal in deciphering how the genetic code precisely manifests itself in the phenotype (Martin-Subero and Siebert, 2009; Zhang and Zhang, 2022).

Conclusion

Even though many different epigenetic mechanisms are known and some have been studied extensively, there are still many unanswered questions that need answers. Epigenomics is more sophisticated than epigenetics as it represents the genotype-to-phenotype transitioning in a non-linear way. The first of the challenges to overcome will be deciphering the obscurity of epigenomic programming to reveal associations of both genetic and epigenetic factors to the final phenotype. Epigenomic variation is much greater than genetic variation in cells since its dynamically adaptable and cell-line-specific traits. The nature of the data obtained from epigenomic research is multifaceted, which means that it has meaning at multiple levels, and even how to collect meaningful data is a challenge itself. The complexity of the data makes the integration and analysis of findings more complicated than genomic data. Despite the presence of reviews about epigenomic programming, essential aspects are still unknown (Smith and Huang, 2012).

Various research strongly suggests polyphenols as an effective epigenetic modulator by pointing out their ability to re-establish normal epigenomic landscape in aberrant patterns seen in cancerous cells. As natural substances, polyphenols are known to affect more than one target, in this case, epigenome modulatory enzymes. Of particular note is the curcumin, which is shown to affect antagonistic enzymes. Consequently, a debate about the difference between epigenetic balance and epigenetically balanced cell concepts has arisen. Right now, there is no telling of how such polymodal mechanisms would provide and which consequences. As expected, the effect obtained from polyphenol administration depends on the concentration, administration period, and epigenomic landscape of the administered tissue, especially in cancerous cells. Polyphenols are targets of different metabolic pathways and are processed by a multitude of enzymes, which are variable depending on the concentration and the cell. Therefore, instead of preferring their natural substance forms, their most active biological form should be clarified by chemical characterization (Stepanic et al., 2014). Lastly, further research into chromosomal band structures to reveal chromosome-specific epimutation hotspots and epigenomic marks would contribute to the understanding of disease associations with the epimutations in cell epigenomes.

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