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Review paper

EPIGENETICS IN DISEASE ETIOPATHOGENESIS

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The term epigenetics refers to heritable changes in gene expression that are not caused by modifications in DNA sequence. Epigenetic changes are DNA methylation, histone modifications, nucleosome positioning, and non-coding RNA (including microRNA) mediated modifications. Epigenetic mechanisms are involved in malignant diseases, imprinting defects, and some hereditary diseases. Recent research explained the role of epigenetic disorders in infections, autoimmune, neurodegenerative and bone diseases, as well as in psoriasis, endometriosis, and polycystic ovary syndrome. Epigenetic modifications have a potential clinical application as diagnostic and prognostic biomarkers, and also as therapeutic targets in oncology, endocrinology, cardiology, and neuropsychiatry. Stress, anxiety, depression, emotions and many other psychological factors may affect epigenetic mechanisms. Influence of preconception parental stress exposure transmits to the next generation through epigenetic changes, as direct results of prenatal and postnatal environmental factors. Epigenetic changes identify environmental factors which affect health and cause disease onset. Milk is the sophisticated system of communication between mother and infant, operating via epigenetic mechanisms. Lifelong consumption of bovine milk causes epigenetic disorders. Recent studies provide important information about the role of bioactive dietary nutrients which modify

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epigenome in malignancy prevention and therapy. Any interruption in the balance of intestinal microbiota initiates aberrant epigenetic modifications. Epigenetic patterns act as the "molecular watches" and they play the central role in the establishment of biological rhythms. Epigenetic mechanisms can determine the result of assisted reproductive technology and genetic engineering. The extensive research about the association of epigenetics and pharmacology led to the development of pharmacoepigenetics. All these results emphasize the importance of further research which will take into account all factors that may affect epigenetic mechanisms.

Keywords: disease, epigenomics, etiopathogenesis

EPIGENETICS: DEFINITION AND SIGNIFICANCE

Term epigenetics derives from the word epigenesis (development of complex organisms from single cell) (CORTESSIS, *et al*. 2018). In 1942, Conrad Waddington defined epigenetics as the causal interactions between genes and their products that allow phenotypic expression (WADDINGTON, 1942). The more recent definition is: epigenetics is the study of mitotically stable molecular factors and processes that regulate genome activity independent of DNA sequence. It refers to heritable changes in gene expression not caused by changes in gene sequence. An epigenetic trait may be a stable heritable phenotype on the gene and protein expression level. However, specific epigenetic mechanisms may differ in stability and Environmental sensitivity, thus contributing to transgenerational adaptation via epigenetic inheritance (PROKOPUK, *et al*. 2015).

Epigenetic mechanisms maintain homeostasis and represent both etiological factors as well as therapeutic targets, contributing to adaptation to environmental changes during growth and adulthood. Some epigenetic changes pass down to future generations, even in the absence of the causal environmental factor. They are responsible for cell- and tissue-specific differences in gene expression (GUTIERREZ-ARCELUS, *et al*. 2015).

Epigenetic inheritance does not follow the Mendelian pattern. Certain epigenetic changes might be transmitted during mitosis, but they disappear in germinal epithelium. When these changes persist in germinal epithelium and embryo, they pass onto the offspring (HEARD, *et al*. 2014).

The aim of this paper is to summarize and systematize current knowledge about the role of epigenetic mechanisms in etiopathogenesis of various diseases.

EPIGENETIC GENOME ALTERATIONS

Epigenetic changes occur more frequently than alterations in DNA sequence, which are controlled by numerous DNA repair mechanisms. Aberrant epigenetic changes are potentially reversible, but sometimes they inactivate genes required for DNA damage repair.

Epigenetic modifications may be classified into four main categories: DNA methylation, histone modification, nucleosome positioning, and non-coding RNA- mediated modification.

Epigenetic change may be a consequence of the direct effect of environmental factors, it may be inherited or may result from *de novo* mutation of genes encoding chromatin regulatory factors. Epigenetic factors are interdependent. The result of epigenetic modifications is always the sum of all epigenetic factors interactions, positive and negative feedbacks (SOSHNEV, *et al*. 2016).

DNA methylation

The most studied epigenetic modification is DNA methylation. It occurs almost exclusively on the CpG dinucleotides. In general, CpG content is very low, about 1%. CpG dinucleotides tend to cluster in CpG islands- regions with at least 200 base pairs and GC percentage greater than 50%. CpG islands overlap the promoter regions in about 60% of human genes. They are generally unmethylated in cells of healthy tissues and organs. During early development and in differentiated tissues, around 6% of CpG islands undergo tissue-specific methylation. Methylation also affects CpG shores- regions located near CpG islands (spaced about 2 000 base pairs apart) (JANG, *et al*. 2017).

The most significant aspect of DNA methylation is its effect on gene expression. In general, the more methylated a gene regulatory region is, the more the gene activity will be downregulated and *vice versa* (LAI, *et al*. 2005). There are exceptions to the rule: DNA methylation can activate transcription (e.g., in exons) by enhancing transcript elongation (JONES, 2012).

CpG methylation usually inhibits gene expression by affecting promoters- via recruitment of methyl-CpG-binding domain (MBD) proteins or direct inhibition of transcription factors binding. MBD activate histone modifying and chromatin remodeling complexes on methylated DNA sequences. Unmethylated CpG islands create chromatin structure, which allows gene expression.

DNA methylation is catalyzed by the family of DNA methyltransferases (DNMTs). DNMTs catalyze the transfer of a methyl group from S-adenosyl methionine to DNA and generate 5-methylcytosine (STANKOV, *et al*. 2011). In human, there are five DNMTs: DNMT1, DNMT3a, and DNMT3b have methyltransferase activity; however, DNMT2 and DNMT3L do not. DNMT1 maintains methylation patterns; DNMT3a and DNMT3b are *de novo* methyltransferases. *De novo* methylation patterns are established during embryonic development, but *de novo* methylation is inhibited in differentiated human cells.

DNMT1 has 30- to 40-fold preference for hemimethylated DNA. Although the primary role of DNMT1 is to maintain methylation patterns, it also has *de novo* methyltransferase activity. DNMT3a and DNMT3b are mostly positioned in nucleosomes containing higher levels of methylated DNA, and they methylate regions missed by DNMT1 at the replication fork. DNMT3L is catalytically inactive. *DNMT3L* is expressed during gametogenesis when maternal genomic imprinting is established. DNMT3L stimulates the methylation activity of DNMT3a and DNMT3b (LYKO, 2018).

DNA methylation is important for chromosome integrity, since many repetitive elements contain high levels of CpG dinucleotides. This mechanism of genome integrity maintenance prevents reactivation of endoparasitic sequences (which cause translocations and gene disruption). In stem cells, non-CG methylation maintains pluripotency: level of the methylation is high in induced pluripotent stem cells and decreases during differentiation. Classical assays determine methylation level of important loci (KURDYUKOV, *et al*. 2016).

Histone modifications

Histone modifications play a crucial role in epigenetic control of gene expression. The core histones H2A, H2B, H3 and H4 form two H2A-H2B dimers and one H3-H4 tetramer within the nucleosome. Segments of 147 base pairs wrap 1.65 fold around a core of eight histone

proteins. Neighboring nucleosomes connect via a short linker-DNA. Histone H1 binds linker-DNA at the point where it enters or exits nucleosome.

Histone N-terminal tails undergo reversible posttranslational modifications (PTMs) (e.g., acetylation and methylation) which regulate DNA transcription, error correction, synthesis, replication, alternative splicing, and chromosome condensation. Histones modify at multiple sites simultaneously. A single modification is not sufficient to determine gene expression, since the combination of all modifications determines an outcome. According to the specific combination of histone modifications it has been described more than 50 different chromatin states in human cells, including promoter-associated, transcription-associated, active intergenic, large-scale repressed and repeat-associated states (ERNST, *et al*. 2010).

Active euchromatin contains high levels of acetylation and trimethylated forms of histone proteins such as H3K4, H3K36 and H3K79. Inactive heterochromatin characterizes low acetylation and high methylation levels at H3K9, H3K27, and H4K20. Histone modifications serve a predictive function for gene expression: active gene transcription is "marked" by H3K4me3, H3K27ac, H2BK5ac and H4K20mel in promoter regions, and by K3K79mel and H4K20mel in coding gene regions.

Methyltransferases, histone demethylases, and kinases have the most specific impact on single subunits of histone and their residuals. The majority of histone acetyltransferases (HAT) and histone deacetylases (HDAC) are not highly specific and catalyze modification on several residues. Most of HDACs in the human genome reset chromatin by removing acetylation at active genes. Many corepressor complexes contain subunits with HDAC activity (DELCUVE, 2012).

Adjusting the balance between HATs and HDACs led to the development of a drug family, HDAC inhibitors (HDACIs). HDACIs are classified according to their chemical structure: short-chain fatty acids (e.g., butyrate and valproic acid), hydroxamic acids (e.g., vorinostat and trichostatin A), cyclic tetrapeptides (e.g., trapoxin A), depsipeptides, and benzamides (CONTE, *et al*. 2018). A large number of HDACIs have been purified from natural sources or have been synthesized. Each of HDACI blocks the activity of one or more of the 18 HDACs.

HDACIs have a long history of use in psychiatry and neurology, mainly as mood stabilizers and antiepileptics. More recently, they have been investigated as possible treatments for neurodegenerative diseases and depressive disorders (MISZTAK, *et al*. 2018).

Nucleosome positioning

Specific DNA packaging in nucleosomes regulate all transcription phases, and thus overall gene expression. Nucleosomes represent a barrier to transcription. They block access of transcription activators and inhibitors to DNA binding sites and inhibit transcript elongation. Nucleosome-free regions at the 5' and 3' ends of genes are required for enzyme/transcription factor binding and removal. Precise positioning of the nucleosome in close proximity to transcription start sites (TSS) influences transcription initiation and regulates DNA polymerase II activity. The absence of nucleosome downstream of the TSS is in correlation with gene activation, and nucleosome obstruction of the TSS is in association with gene repression. Nucleosome positioning participates in a constitution of whole methylome (entire genome methylation profile) and regulation of recombination during meiosis (DENIZ, *et al*. 2016).

Different histone variants incorporate into chromatin independently of DNA replication. Histone variants regulate nucleosome positioning and gene expression. For example, H2A.Z protects genes from cytosine methylation. Several groups of macromolecular complexes cause nucleosome moving, destabilization, elimination, and restructuring in an ATP-dependent way. These are chromatin remodeling complexes, and they are classified into five major subfamilies: SWI/SNF, ISWI, INO80 or SWR1, CHD, and α-thalassemia mental retardation syndrome X-linked (ATRX). Members of SWI/SNF family regulate expression of genes which control cell cycle and alternative splicing (BARTHOLOMEW, 2014).

Micro RNA

Micro RNA (miRNA) represent the non-coding short RNA stretch (18-24 nucleotide long) that are endogenously stable and evolutionarily conserved. MiRNAs are important cytoplasmic posttranscriptional regulators of their messenger RNA (mRNA) targets via mRNA degradation and/or translational repression and participate in many processes, including differentiation, proliferation, apoptosis and development (CATALANOTTO, 2016).

MiRNAs complementary bind and suppress the target mRNA, thus inhibiting the expression of the gene with specific mRNA transcription product. Another mechanism of action is stimulation of target mRNA degradation. MiRNAs are powerful gene silencers, since every miRNA influences tens or hundreds of genes. At the posttranscriptional level, miRNAs affect 20-30% of all protein-coding genes. In addition, it has been estimated that more than 60% of all mRNAs might be regulated by miRNAs in humans (FRIEDMAN, *et al*. 2009).

Expression of miRNAs is dysregulated in malignant tumors. MiRNAs principally act as tumor suppressors, since the expression of miRNAs is reduced in malignant tumors compared with normal tissue. On the other hand, miRNAs preferentially occur inside fragile regions of chromosomes, increasing the possibility of chromosomal deletions, translocations and amplifications, which may reflect their oncogenic role. MiRNAs are potential diagnostic and prognostic markers, used in studies aiming to identify etiology and progression of malignancy (DETASSIS, *et al*. 2017).

EPIGENETIC MECHANISMS IN DISEASES

Imprinting defects

Genomic imprinting is allele-specific DNA methylation (ASM): allele-specific silencing of the gene inherited from one parent. The majority of autosomal genes express both maternal and paternal inherited alleles, however, some genes are imprinted. Selection of which inherited copy to silence depends on parental sex. DNA methyltransferase specifically methylate imprinting control regions (ICR) which establish and regulate imprints in male and female germinative cells (KELSEY, *et al*. 2013).

Over the past 20 years the studies produced a list of approximately 100 imprinted genes in humans, usually residing in gene clusters in chromosomal regions 7q32, 11p15, 15q11 and 20q13 (WILKINS, *et al*. 2016). For some genes, imprinting is tissue-specific and required for its normal development: imprinting affects growth, metabolism, proliferation, differentiation and malignant transformation. DNA regulation defects during imprinting establishment and maintenance may cause the deregulation of imprinted genes, which occurs in many growth and developmental disorders (PLASSCHAERT, *et al.* 2014).

Imprinting defects are usually the result of uniparental disomy (UPD), i.e. the meiotic or in case of somatic mosaicism mitotic inheritance of both homologous chromosomes (and chromosomal segment) from only one parent. When UPD occurs in imprinted chromosome region, either two active (expressed in the parent) or two inactive (silenced in the parent) genes will be inherited. The result is an incorrect dosage of imprinted genes. UPD is associated with human disorders, such as Beckwith-Wiedemann, Silver-Russell and Angelman or Prader-Willi syndrome (AS or PWS) and transient neonatal diabetes mellitus.

The major cause of AS and PWS is 15q11.2-q13 microdeletion, while in others the genetic alterations involve point mutations, translocations, paternal UPD, and imprinting defects in this region. In PWS/AS critical chromosomal region 15q11-q13, multiple paternal (including *SNRPN*) and one maternal (*UBE3A*) genes are expressed. Characteristics of AS comprise slower growth, impaired mental performance, speech disorders, ataxia, aggressive behavior and loss of consciousness. This clinical picture is a result of aberrant methylation at 15q11.2-q13 due to one of the following: deletion of the maternally inherited 15q11.2-q13 locus (which includes UBE3A), uniparental disomy of the paternal chromosome 15 or an imprinting defect of the maternal chromosome 15q11.2-q13 locus. Characteristics of PWS are neonatal hypotonia, feeding difficulties followed by hyperphagia, obesity, slow motor development, extremely impaired verbal ability, cognition defects, and hypogonadism. PWS is a result of paternal chromosomal alterations or maternal UPD. Most probably, candidate gene locus is in proximity to small nuclear ribonucleoprotein N (*SNRPN*/*SNURF*), which contain ICR necessary for epigenetic regulation, and also *MAGEL2* with mutations that are associated with PWS clinical manifestations (SOELLNER, *et al.* 2017).

Genetic complexity in imprinting diseases is a major cause of difficulties when diagnosis is relying on single-gene analysis only. Identification of differential DNA methylation at imprinted regions is necessary for the definitive diagnosis of AS and PWS. In healthy individuals the region 15q11.2-q13 is hemimethylated, containing both a methylated and unmethylated *SNRPN* allele, whereas individuals with PWS will have only one - the maternally methylated allele. Thus, in healthy individuals, the methylation (silencing) of CpG sites in the promoter region (imprinting) occurs in maternal, whereas paternal allele is unmethylated (active). In patients with AS, the *SNPRPN/SNURF* loci are hypomethylated and in PWS hypermethylated (AREF-ESHGHI, *et al.* 2017).

Epigenetic mechanisms in human hereditary diseases

Constitutive gene aberrations, coding for proteins involved in whole-epigenome regulation usually causes embryonic lethality. Hypomorphic mutations are sometimes compatible with fetal survival but lead to severe developmental disorders. Epigenome dysregulation is involved in the etiopathogenesis of Rett, ATRX, Rubinstein-Taybi, and other syndromes. These disorders involve multiple and severe defects that affect different systems and influence the development. Syndromes resulting from epigenome deregulation are characterized by neurological dysfunctions, such as neurocognitive disturbances (DE SARIO, 2009).

Epigenetic modifications in malignancy

Malignant cells have globally altered epigenetic profile. Malignant cells epigenome is characterized by changes in DNA methylation patterns, histone modification profile as well as altered enzyme expression, which have the important role in malignancy initiation and

progression. Detection of epigenetic changes that are relatively specific for neoplastic cells enabled the identification of biomarkers with diagnostic, prognostic and predictive value in numerous solid tumors and hematological malignancies (VECCHIO, *et al.* 2013).

Malignant cells genome is characterized by global loss of methylation and specific hypermethylation of the promoter regions in several genes, including the most important silencing of tumor suppressor genes. Hypermethylation blocks are founded in many types of solid tumors samples, even during the earliest stages of premalignant development, whereas their progression occurs in later stages of the disease (TIMP, *et al.* 2014).

Hypermethylation in tumor cells affects genes involved in DNA repair (*BRCA1*, *MGMT*, *MLH1*), cell cycle control ($p16^{INK}$, $p15^{INK}4b$, *RB*) and apoptosis (*TMS1*, *DAPK1*, *WIF-1*). Epigenetic silencing via DNA methylation affect very long regions of chromosomes (up to 1Mb) and it is similar to gene silencing in loss of heterozygosity phenomenon. Pathological hypermethylation in malignant cells expands on CpG shores and causes complete gene silencing. Hypomethylation of specific promoter regions causes aberrant oncogene expression and loss of imprinting at some loci (GAO, *et al.* 2016).

The most important histone modification in malignant cells is a decreased level of monoacetylated histone H4K16. Many malignant tumors express HDACs. Some miRNAs (e.g. miR-449a) regulate HDAC expression. In certain types of malignancies, such as ovarian cancer or B-cell lymphoma, translocations generate aberrant fusion proteins, mutations or deletions of HAT coding genes (RAMASSONE, *et al.* 2018).

Malignant cells are also characterized by global decrease of miRNA expression, usually as a consequence of miRNA promoter hypermethylation. Inhibition of miRNA expression (e.g., miR-124a, miR-148, miR-34b/c, and miR-9), due to promoter hypermethylation, is associated with the occurrence of metastasis. Amplification or increased expression of oncogene miRNA may initiate oncogenesis. Increased miRNA eliminate miRNA-target tumor suppressor expression and reinforce tumor progression. The reduction or deletion of a tumor suppressor miRNA occur in all phases of miRNA biogenesis and cause inappropriate miRNA-target oncoprotein expression. As a result, many features of malignancy develop, such as increased proliferation, dedifferentiation, invasiveness, angiogenesis, and decreased apoptosis (SUZUKI, *et al.* 2012).

Global epigenetic alterations in malignant cells are the consequence of dysregulated DNMT expression (including mutations in DNMT coding genes), and clonal selection of cellular populations with aberrant methylation. Hypermethylation profile is unique for each type of tumor, in which inactivation of certain genes may cause proliferative advantage and clonal selection (ZHANG, *et al.* 2017).

Epigenome analysis accelerates the development of biomarkers and epigenome modulating drugs with antitumor activity (STANKOV, 2017). Analysis of DNA methylation and histone modification profile has a potential clinical application in tumor detection and prognosis, but also in prediction of therapy response. Specific methylation profiles enable comparison of malignant and normal cells in biological liquids (blood, urine, saliva). Methylation status is usually determined via methylation-specific polymerase chain reaction (MSP), methylation-sensitive restriction enzymes digestion, bisulfite sequencing, and pyrosequencing (KURDYUKOV, *et al.* 2016).

Hypermethylation of gene coding for enzyme glutathione S-transferase (*GSTP1*) is detected in cells derived from samples of serum and urine in 80-90% patients with intraepithelial

prostatic neoplasms. The hypermethylation of *GSTP1* is rarely detected in other malignancies, including liver, breast, and kidney carcinoma (GURIOLI, *et al*. 2018).

MiRNAs also play a significant role in malignancy prognosis. As a result of the methylation, miR-34b/c shows decreased expression in twelve different types of malignancy, particularly in case of relapsed or recurrent disease (HERMEKING, 2010).

Assessment of whole genome methylation may represent a biomarker for possible outcomes in juvenile myelomonocytic leukemia. More aggressive forms of the disease are characterized by hypermethylation, thus the identification of patients with disease that may spontaneously resolute is possible. The methylation pattern is consistent in all tissues affected by disease (blood marrow, peripheral blood, and spleen) (STIEGLITZ, 2017).

One of the best markers to predict the response to therapy is hypermethylation of *MGMT* in glioma, which indicates a better response to carmustine and temozolomide, especially in primary tumors (THOMAS, *et al*. 2013).

Understanding of functional significance of methylation disorders in tumors is challenging, partially because of the absence of adequate tools for methylation pattern modification (STANKOV, *et al*. 2017). DNA demethylation, the result of inhibition by mammalian DNA methylase, is transitory and depends on replication. Plants contain enzymes, which directly remove 5-methylcytosine from DNA, and subsequently, mechanisms of DNA repair replace the missing cytosine. An enzyme, 5-methylcytosine DNA glycosidase DEMETER (DME), from plant *Arabidopsis thaliana*, reactivates silenced locus in colon cancer cells. DME establishes methylation pattern of normal tissue: it causes changes at the whole genome level, and they include both DNA methylation and demethylation. The methylome reprogramming is associated with higher sensitivity to antitumor drugs and inhibition of tumor growth (MORALES-RUIZ, 2017).

Epigenetic mechanisms in infections

Genomes of viruses and proviral genomes of retroviruses are regular targets of epigenetic regulatory mechanisms in infected cells, such as histone modification and DNA methylation. For example, transcriptional latency of human genome integrated HIV-1 is mediated by the regulators of histone acetylation and methylation as negative control of HIV-1 transcription (MATSUDA, *et al.* 2015).

Proteins coded by viral genomes affect cellular promoters by epigenetic regulatory mechanisms. This can cause epigenetic dysregulation, cell dysfunction, and eventually processes such as carcinogenesis or immunodeficiency. Dynamic changes of viral DNA methylomes of the HPV16, HPV18, and HBV viruses occur during disease progression. The DNA methylome of these viruses evolve from unmethylated to highly methylated genome during progression of carcinogenesis. This process evolves from asymptomatic healthy carriers through tissues with chronic infection and premalignant lesions to the full-blown invasive cancers. One interpretation of this phenomenon is that the DNA methylation could be a device for viral camouflage from the human immune system (FERNANDEZ, *et al*. 2009).

Helicobacter pylori contributes to cancer progression via hypermethylation of promoters in gastric mucosa (LU, *et al.* 2012). *H. pylori*-related gastritis and gastric cancer are associated with promoter methylation by tumor suppressor *miR-133a*, and it can be reversed by *H. pylori* eradication (LIM, 2018).

Host cell chromatin remodeling has a potential to generate a transcriptional memory of microbial stimulus, thus representing a mechanism that may last even after pathogen clearance.

Inflammatory exposure leaves marks on the immune system and influences future immune system responses, enabling to adapted cells to respond in a more efficient manner then naive cells to subsequent infection or stimuli (PEREIRA, *et al*. 2016). Epigenetic memory of inflammation is not limited to innate immune cells, but potentially affects all cells that may respond to inflammation signals (NOVAKOVIC , *et al*. 2017).

Epigenetic changes in autoimmune diseases

Epigenetic dysregulation occurs in many autoimmune diseases. The epigenetic mechanisms link the genes and environmental factors that contribute to immunity (WANG, *et al.* 2017). Many functions of the immune system (rearrangement of antigen-receptor, allelic exclusion, and inducible immune responses against pathogens) are epigenetically controlled.

Altered epigenetic mechanisms could promote autoimmune diseases (MOOSAVI, *et al*. 2016). Epigenetic molecular mechanisms could explain over-reactivity of immune cells (WANG, *et al*. 2017). DNA methylation and post-translational modifications of histones are potentially responsible for immune tolerance failure in autoimmune disorders.

Global DNA hypomethylation in T cells is involved in the pathogenesis of systemic lupus erythematosus (SLE). Hypomethylation of specific regulatory regions causes overexpression of autoimmune-associated genes in CD4+ T cells in SLE patients. DNA methylation of specific genes also affects T cell activation and differentiation. Global hypoacetylation of histone H3 and H4 is a characteristic of CD4+ T cells in SLE. Aberrant expression of miRNA can be observed in T cells, B cells, dendritic cells and serum in SLE (ZHANG, *et al*. 2018). Many epigenetic markers have been investigated as potential SLE biomarkers, considering their accessibility, convenience, and specificity of measurement methods as well as their ability to modulate local inflammatory processes. Complex manifestations of SLE contribute to many challenges to confirming the diagnosis, or even misdiagnosis or missed diagnosis. Thus, SLE epigenetic biomarkers may enable early diagnosis and timely treatment of SLE (ZIJUN, *et al.* 2017).

Global DNA methylation level is significantly increased in CD4+ T cells in patients with latent autoimmune diabetes of adults, accompanied by the upregulates expression level of DNMT3b. HDAC expression is aberrant in diabetes mellitus type 1 patients (STANKOV, *et al.* 2013).

In rheumatoid arthritis, changes in DNA methylation are found in synovium and synovial fibroblasts (ZHANG, *et al.* 2018).

Epigenetic basis of neurodegenerative diseases

Alzheimer disease (AD) is a multifactorial disease representing one of the principal causes of decreased quality of life among the elderly. The etiopathogenesis of AD comprises the interplay of environmental influences and individual genetic susceptibility, including the epigenetic mechanisms that contribute to translation of environmental stimuli into modifications in gene expression (WANG, *et al*. 2013). Inappropriate histone acetylation and DNA methylation contribute to the development of Alzheimer disease (FYFE, *et al*. 2018). Specific histone modifications, DNA methylation and demethylation play a key role in adult memory formation. In experimental models of contextual fear conditioning, increased *de novo* DNMT expression in the hippocampus has been detected. In addition, increased DNA methylation at the promoter of the memory-suppressor gene protein phosphatase 1 (PP1) and decreased methylation at the promoter of the plasticity-associated gene reelin are detected. Thus, epigenetic transcriptional

balance between memory activators and inhibitors is required for memory process, including the opposing actions of proteases and phosphatases in the cytoplasm as well as of transcriptional activators and repressors at gene promoters. Pharmacological interventions that increase the euchromatin-associated PTMs, such as PP1 inhibition, estrogen treatment, or the activation of glucocorticoid receptors may also increase the memory formation, opening the new avenues for preclinical and clinical studies of epigenetic drugs in Alzheimer disease (ZOVKIC, *et al.* 2013; CACABELOS, *et al*. 2015).

Epigenetic mechanisms in bone diseases

Epigenetic regulation plays a key role in physiological bone remodeling and in bone diseases (renal osteodystrophy, osteopetrosis, osteogenesis imperfecta, and bone malignancies). Since environmental factors affect epigenetic changes, they represent the potential risk factors for chronic bone diseases. Measurement of specific serum miRNAs will be used in future as epigenetic biomarkers for prediction of osteoporotic fractures increased risk (MARINI, 2016).

Chronic stress causes proinflammatory state mediated by epigenetic mechanisms. Epigenetic changes may explain the increased risk of osteoporotic lesions in socially disadvantaged individuals. Epigenetic mechanisms also explain associations between osteoporosis and physical activity, nutrition and alcohol consumption. Vitamin D is possibly involved in regulation of histone modification, DNA methylation and miRNA modulation (MICHOU, 2017).

Epigenetic modifications in kidney diseases

Global and gene-specific changes in epigenetic mechanisms have been observed in acute and chronic kidney diseases, as well as during the transition from acute to chronic kidney diseases of distinct etiologies (ischemia-reperfusion injury, urethral obstruction, diabetes, glomerulonephritis, polycystic kidney disease, and nephrotoxicity). Beneficial *in vivo* effect over preclinical kidney injury is found for demethylating agents such as 5-azacytidine, decitabine or hydralazine that activates DNA demethylation. Additional mechanisms of action involve inhibition of histone methyltransferases or inhibition of histone deacetylases by valproic acid and vorinostat (FONTECHA-BARRIUSO, *et al*. 2018).

Epigenetics and psoriasis

Epigenetic changes, such as DNA methylation, altered expression and/or activity of epigenetic modifying enzymes and histone modifications contribute to psoriasis development (MELERO, *et al.* 2018). Ongoing research projects aim to identify biomarkers of psoriasis-prone skin before disease onset (VERMA, *et al*. 2017). In addition, recent studies of pharmacological inhibitors of epigenetic modifier enzymes demonstrate their potential applicability as novel treatment modalities for psoriasis (POLLOCK, *et al*. 2017).

Epigenetic mechanisms in endometriosis and polycystic ovary syndrome

DNA methylation, histone modification, and miRNA expression are involved in the pathogenesis of endometriosis. Locally synthesized estradiol, proinflammatory cytokines, and hypoxic stress affect these epigenetic mechanisms that are interconnected and promote the development of endometriosis. Hypoxia causes global DNA hypomethylation via miRNA-148a, which downregulate DNMT1. Inflammation induces DNMT3a expression and locus-specific

hypermethylation. MiRNA-20a induced by hypoxia and miRNA302a induced by inflammatory cytokines both contribute to modulation of DNA methylation (HSIAO, *et al.* 2017).

Epigenetic mechanisms are involved in the pathogenesis of polycystic ovary syndrome. Disruption of DNA methylation and miRNA is observed in blood, serum, adipose tissue, granulosa and theca cells of patients with polycystic ovary syndrome. Altered epigenetic regulation may be the consequence of environmental factors (nutrition, obesity) during intrauterine or postnatal growth (CONCHA, *et al.* 2017).

NUTRIEPIGENETICS

Prenatal exposure to environmental factors

Prenatal exposure to environmental factors (e.g., fetal alcohol syndrome or gestational diabetes mellitus in mother), including exposure to environmental factors before conception, cause global changes in methylation of embryos. That is the first source of epigenetic inter-individual variation that contributes to disease development.

Children of mothers with gestational diabetes mellitus have increased predisposition to obesity and diabetes mellitus, later during life. Hypermethylation is especially frequent in genes involved in embryonal development, insulin signal pathways and many metabolic pathways (CHEN, *et al*. 2014).

Alcohol exposure during embryonic development causes growth retardation, specific facial dysmorphic features, and brain damage. These malformations are the consequences of global DNA hypomethylation in fetal tissues induced by acetaldehyde, which inhibits the DNMT1 (GARRO, *et al*. 1991).

Epigenetics and diet

The optimal nutrition for term newborns is a human milk (HM). Thus, HM represents more than just a food, but also a sophisticated system of communication between mother and infant. In addition, HM orchestrates early developmental programming of the infant, mediates the protection against infections, resulting in reduced mortality and morbidity. In these processes, miRNAs and HM exosomes are the most important factors (ZHOU, *et al.* 2012; ALSAWEED, *et al*. 2016). Milk exosomes have the essential role in infant nutrition (ZEMPLENI, *et al*. 2017). Milk exosomes survive intestinal degradation and then they are taken up in intestinal cells by endocytosis (LIAO, *et al*. 2017). After that, they reach infant's systemic circulation and modify gene expression in variety of tissues. Labeled bovine milk exosomes administered orally to athymic female mice is found in liver, lungs, kidneys, pancreas, spleen, ovaries, colon, and brain (MANCA, *et al.* 2017). Milk exosome endocytosis is also detected in vascular endothelial cells (KUSUMA, *et al.* 2016). Recent studies showed that miRNAs are isolated in high quantities from human and animal milk, either in free molecular form or contained in carrier vesicles (milk exosomes and fat globules) (ALSAWEED, *et al*. 2016).

Milk contains a plethora of miRNAs, with miRNA-148a as the most abundant miRNA in bovine and human milk fat exosomes and globules. It decreases DNMT1 expression and consequently increases expression of genes involved in development, such as *FOXP3*, *INS*, *IGF1*, *FTO*, and *NRF2*. Another abundant milk miRNA is miRNA-125b which suppresses the transcriptional network of p53 in intestinal cells, thus attenuating the oxidative stress and apoptosis with reduced risk of intestinal injury (MELNIK, *et al*. 2017).

The absence of specific human milk miRNAs in artificial milk disrupts the epigenetic mechanisms. Physiologically, epigenetic signaling via human milk miRNAs is limited to the breastfeeding period. Persistent miRNAs exposure due to bovine milk consumption leads to epigenetic disorders that may induce obesity or diabetes mellitus type 2. Milk miRNA-148a stimulates the appetite and adipogenesis. Commercial bovine milk miRNAs are stable under acid environments and freezing, but they are degraded by bacterial fermentation (MELNIK, *et al.* 2017).

Recent studies provide important information about the role of bioactive dietary nutrients in prevention and therapy of malignant diseases. Many bioactive nutrients modify epigenome by DNA methylation, histone modification, miRNAs, and other non-coding RNAs. Various doses of these nutrients were reported in *in vitro* studies. Additional preclinical and clinical data are needed for clinical application of these nutrients (KHAN, *et al.* 2018).

The folate in the form of S-adenosylmethionine is the single direct factor linked with the majority of biological methylation reactions. The combined effects of folate deficiency and aging decrease the gene methylation, resulting in upregulated proto-oncogenes (*C-MYC* and *C-JUN*) and cell cycle regulator (cyclin E). In addition, the expression of tumor suppressor genes (*p53*, *p15ink4b*, and *p16ink4a*) is decreased, because of promoter hypermethylation. Long-term supplementation with physiological doses of folates leads to downregulation of proto-oncogenes and upregulation of tumor suppressor genes (NAJAR, *et al.* 2018).

Epigenetics and microbiome

Human intestinal microbiome is diverse and abundant (number exceeds 10^{14} of bacteria), and each individual is populated by roughly 15% of the 1000 or more species of intestinal bacteria (MAYNARD, *et al.* 2012.). Each individual profile of intestinal microbiome is unique and determined by numerous factors including age, gender, genetics, diet, and lifestyle. A healthy microbiome is established by use of prebiotics, probiotics, and antibiotics. The health of microbiome is directly linked to the health of the host. Intestinal microbiota can be seen as the largest endocrine organ in the body (REZASOLTANI, *et al*. 2017).

Short-chain fatty acids (such as butyrate and propionate) produced as end products of fermentation of dietary fibers by the anaerobic intestinal microbiota, act as non-competitive inhibitors of histone deacetylases. Butyrate and propionate selectively inhibit HDAC1 and HDAC3 (KASUBUCHI, *et al*. 2015).

Any interruption in the balance of microbiota leads to alteration of the microbial composition and initiate the aberrant intestinal signaling pathways and epigenetic modifications. Intestinal microbiome changes are associated with numerous diseases and conditions, such as depression, anxiety, autism, obesity, diabetes mellitus type 1 and 2, Crohn's disease, inflammatory bowel diseases, colorectal cancer and liver diseases (REZASOLTANI, *et al.* 2017). A diversity of gut microbiota and FFAR3 promoter methylation level are significantly lower in patients with obesity and type 2 diabetes mellitus, in comparison to lean individuals, therefore the epigenetic regulation may be related to beneficial effects of short-chain fatty acids on host metabolism (KASUBUCHI, *et al.* 2015). More than 90% of colorectal cancers are associated with altered epigenetic regulation, whereas the genetic factors including DNA mutations account for less than 5% of cancer cases. Metabolic products and structural components of microbiota play a crucial role in colorectal cancer progression, interacting with intestinal epithelial cells of the host. Microorganisms play an essential role in biological microenvironment, and they are

suspected to be involved in pathogenesis of nearly 16% of cancers, including liver and other gastrointestinal malignancies. Notably, *H. pylori* infection contributes to gastric adenocarcinoma and MALT lymphoma development (REZASOLTANI, *et al.* 2017).

EPIGENETICS AS POSSIBLE CONNECTION BETWEEN BIOLOGICAL CLOCKS AND DISEASES

Some epigenetic markers are strictly conserved during life, and others are closely associated with certain age and environmental factors. DNA methylation pattern dynamics is highly coordinated with development phase or age, thus may be used as "molecular watches" that indicate the age of an individual. Most studies revealed that DNA methylation changes usually occur during the first years of development and then stabilize during adolescence. These changes occur in development programs primarily related to anatomical development, differentiation, morphogenesis and maturation of immune system and CNS (NAUMOVA, *et al.* 2018). In addition, age-dependent methylation status of CpGs yields an apparent DNA methylation age that correlates with biological age, thus providing information about differences in rate of decline in biological function between individuals of the same chronological age (FIELD, *et al.* 2018).

Many diseases are associated with abnormal biological rhythms (PAGANELLI, *et al.* 2018). Daily and seasonal rhythms of epigenetic enzymes have significant consequences on overall epigenetic patterns and timing of molecular processes. DNA methylation oscillates over circadian and circannual biological rhythms and regulates processes critical to daily and seasonal environmental adaptations. DNA methylation changes are involved in the circadian locomotor activity, whereas hypothalamic histone deacetylases control daily the energy balance (STEVENSON, 2017). The central regulatory gene of circadian rhythm, *CLOCK*, is coding for a protein that has histone acetyltransferase activity (DOI, *et al.* 2006).

Internal signals of biological variation in both hormonal milieu and circadian genes functioning as clocks (e.g., *BMAL1*), determine the expression of epigenetic enzymes. The output signals operate in a tissue- and cell-specific manner on epigenetic enzymes (e.g., DNMT3a and TET 1/2) and change the epigenetic patterns. Genome-wide epigenetic modifications control the timing of gene transcription and ultimately generate ultradian (<24h), circadian (~24h) and infradian rhythm (>24h) (STEVENSON, 2017).

EPIGENETIC DEFECTS AND ASSISTED REPRODUCTIVE TECHNOLOGY

Assisted reproductive technology is associated with occurrence of one or more imprinting disorders of either maternal or paternal origin. The frequency of any disorders in general population is estimated to be 2.0 per 10000 children. Expectations are that among 10000 live births following assisted reproductive technology, there will be 3.9 children with Beckwith-Wiedemann syndrome, 3.9 with Angelman syndrome, 2.2 with Prader-Willi syndrome, and 1.5 with Silver-Russell syndrome (or 11.5 cases of any of these disorders). While the frequency of these disorders may be several folds higher following assisted reproductive technology, the absolute risk is low (CORTESSIS, *et al.* 2018).

EPIGENETIC CONSEQUENCES OF GENETIC ENGINEERING

Insertion of foreign DNA into an established eukaryotic genome is an important technique in experimental medicine. The goal of genetic engineering is to generate transgenic

cells or organisms. However, foreign DNA integrated into established mammalian genome usually becomes *de novo* methylated, and consequently inactivated. This could be important in the context of genetically modified organisms. *De novo* methylation probably represents the cellular defense mechanism against the activity of foreign genes in the established genome. Insertion of foreign DNA into mammal genome also alters the recipient genome methylation patterns at sites remote from insertion locus, probably because of disturbances in overall cellular methylation pattern. These mechanisms potentially change genome-wide gene expression, which might cause cell death or tumor disease (DOERFLER, 2011).

PHARMACOEPIGENETICS

Since diet, age and gender influence epigenetics, they also affect drug response. The extensive research about the association of epigenetics and pharmacology developed a new discipline of pharmacoepigenetics. Pharmacoepigenetics is the study of the heritable non-genetic (epigenetic) bases for variability in drug response and toxicity (PEEDICAYIL, 2008; LAUSCHKE, *et al.* 2018).

Epigenetic changes are reversible modifications, which makes them attractive target for therapy. Restoring the normal epigenetic landscape is the aim of many investigations. Only two classes of epigenetic drugs reached clinical setting: DNMT inhibitors and HDAC inhibitors (PERRI, *et al.* 2017).

Inhibitors of DNA methylation cause reactivation of silenced genes, inhibition of cell proliferation, apoptosis, and enhancement of sensitivity to other cancer drugs. Epigenetic drugs targeting DNA methylation show considerable cytotoxicity. They cause global demethylation, which limits the use of these drugs for prolonged periods of time (BOJANG, *et al.* 2014).

Histone deacetylase inhibitors represent promising tools for the therapy of many human diseases. Majority of the clinical trials (~600 trials in total) using HDACIs investigate the possibility of their use in the treatment of cancer (~550 trials). Fifteen HDACIs are listed as potential drugs. Several HDACIs demonstrate antidepressant-like efficacy. The main risk associated with the use of HDACIs is lack of specificity. HDACIs (with a few exceptions) exhibit pleiotropic effects. Their global consequences (e.g. activation of oncogenes) are difficult to predict. Frequent side effects are diarrhea, nausea, and fatigue (MISZTAK, *et al.* 2018).

Cancer cells can avoid an immune attack by epigenetic silencing, such as the decreased expression of specific cell-surface molecules, essential for the immune system to recognize and eliminate cancer cells (WANG, *et al.* 2014). Chromatin-modulating drugs reverse this escape mechanism and make cancer cells responsive to immunotherapy. On the other hand, excessive accumulation of acetylated histones in immune cells may alter their function (CONTE, *et al.* 2018).

SPECIFICS OF EPIGENETIC RESEARCH

In epigenetic research many factors (such as age, gender, diet, alcohol, lifestyle, medications, pollution exposition, smoking, etc.) influence DNA methylation: sometimes it is difficult to estimate whether single factor activity is observed, or other factors may be overlooked and potentially underestimated. For that reason, it is very important to take into account all external factors like pollution or chemicals exposure.

DNA methylation profiles are tissue-specific, gene-specific, population-specific, age-specific, cancer-specific, and markedly different on inactive and active X chromosome, as well as between expressed and silenced alleles. Tissues for epigenetic analysis should be chosen

carefully, since in studies using whole blood and other tissues, the DNA methylation discrepancies may be the result of the different content of cells in a tissue (KADER, *et al.* 2018).

FUTURE CLINICAL APPLICATIONS OF EPIGENETICS

Epigenetic modifications detection could successfully be translated as biomarkers into clinical medicine, since it provides insight into functioning of genes and explains phenotypic differences between healthy and diseased individuals. The epigenetic modifications could also be used as biomarkers for determination of environmental factors affecting health and disease onset, and also as diagnostic and prognostic biomarkers in oncology, endocrinology, cardiology, and neuropsychiatry (GARCÍA-GIMÉNEZ, *et al.* 2017; METZINGER, *et al.* 2017; SANDOVAL, *et al.* 2013).

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EPIGENETIČKI FAKTORI U ETIOPATOGENEZI BOLESTI

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Izvod

Termin epigenetika se odnosi na nasledne promene ekspresije gena koje nisu izazvane izmenama DNK sekvence. Epigenetičke alteracije obuhvataju DNK metilaciju, modifikacije histona, pozicioniranje nukleozoma i modifikacije posredstvom nekodirajuće RNK (uključujući mikroRNK). Epigenetički mehanizmi učestvuju u etiopatogenezi malignih bolesti, defekata imprintinga i određenih naslednih bolesti. Novija istraživanja su objasnila ulogu poremećaja epigenetičkih mehanizama u etiopatogenezi infekcija, autoimunih, neurodegenerativnih bolesti, kao i kod bolesti kostiju, psorijazi, endometriozi i sindromu policističnih jajnika. Epigenetičke modifikacije predstavljaju potencijalne dijagnostičke i prognostičke markere, kao i terapijske mete u onkologiji, endokrinologiji, kardiologiji i neuropsihijatriji. Stres, anksioznost, depresija, emocije i mnogi drugi psihološki faktori utiču na epigenetičke mehanizme. Uticaj izloženosti stresu prenosi se na potomstvo putem epigenetičkih promena, kao direktan rezultat delovanja faktora okruženja tokom prenatalnog i postnatalnog perioda. Epigenetičke promene mogu da identifikuju faktore okruženja koji utiču na zdravlje i nastanak bolesti. Mleko je sofisticirani sistem za komunikaciju između majke i novorođenčeta koji funkcioniše putem epigenetičkih mehanizama. Konzumiranje kravljeg mleka tokom čitavog života izaziva epigenetičke poremećaje. Skorašnja istraživanja ukazuju na ulogu bioaktivnih nutrijenasa koji modifikuju epigenom u prevenciji i terapiji maligniteta. Svaki poremećaj intestinalnog mikrobioma može da inicira aberantne epigenetičke modifikacije. Regulacija epigenetičkih biomarkera ukazuje na prisustvo „molekularnih satova“ i ima centralnu ulogu u uspostavljanju bioloških ritmova. Epigenetički mehanizmi mogu da odrede rezultat asistirane reproduktivne tehnologije i genetskog inženjeringa. Brojna istraživanja o povezanosti epigenetike i farmakologije dovela su do razvoja farmakoepigenetike. Dosadašnji rezultati naglašavaju značaj daljeg istraživanja svih faktora koji mogu da utiču na epigenetičke mehanizme.

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